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LETTER FROM THE EDITORS

Friends and Colleagues of the Vanderbilt Neuroscience Community,

We did it. Another year has passed and we are still here. In this year's issue of *Vanderbilt Reviews Neuroscience* the journal receives a major make-over in its style and formatting, but still preserves its major purpose: showcasing the talented writing of the next class of newly minted Ph.D. candidates in the Vanderbilt Neuroscience Program. This year's volume includes a record seventeen candidate reviews spanning both systems and molecular neuroscience. They feature the roles of biogenic amine neurotransmitters and glucocorticoids in addiction and obesity, cortical multisensory processing, genetic determinants of disease states like epilepsy, Huntington's disease, and Parkinson's disease, and the role of habituation to novel faces in patients with social phobias. We also highlight a collection of first author papers published by Vanderbilt Neuroscience Students over the past calendar year. Our students continue to publish in the field's most respected journals and make scholarly contributions highly regarded by the external peer review process. Keep up the good work!

From Left: Maureen McHugo, Andrew Hardaway, Sudipta Chakraborty and Juli Krueger; not pictured - Teniel Ramikie

In the conception and design phase of this issue of the *VRN*, we joined the ranks of the blogosphere. The blog, "Vanderbilt Depolarized", features updates on our efforts to create this year's *VRN*, but also is a general resource for program updates, career development, musings about graduate training, and advice on writing manuscripts or fellowships. We encourage all students to use this as an avenue to connect and publicly share their experiences. Check out our blog at my.vanderbilt.edu/vanderbiltdepolarized/.

This year's *VRN* would not have been possible without the help of many wonderful people. Our Associate Editors, Juli Krueger, Teniel Ramikie, and Sudipta Chakraborty, have been instrumental in each step of this issue's development and were a joy to work with. The future of *VRN* remains bright under their leadership for next year. We would also like to recognize the outgoing Co-Editors-in-Chief, Caleb Doll and Mariam Coaster, and Founder/Editor-in-Chief, Chris Ciarleglio, for helping establish *VRN* and providing a network of resources to assist us in the development of this year's issue. Our sincerest thanks go to Mark Wallace and Doug McMahon, who provide vision in shaping the role of *VRN* in the experience of each Neuroscience Ph.D. student. Lastly, we would like to thank each of the 2011 qualifying class for their hard work in crafting the "meat and potatoes" of this issue and tolerating our many edits. This would not have been possible without you.

Your Co-Editors-in-Chief,

Maureen McHugo and Andrew Hardaway

MASTHEAD

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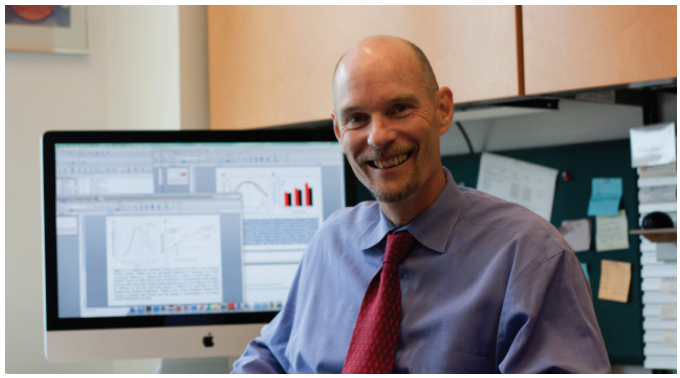
Vanderbilt Reviews Neuroscience(VRN) is an open-access journal (http://vrn.vanderbilt.edu). *VRN* is the official journal of the Vanderbilt University Neuroscience Graduate Program and the Vanderbilt Brain Institute. *VRN* is a collection of reviews submitted by Vanderbilt Neuroscience Graduate Students whilst qualifying for doctoral candidacy. The journal also offers highlights and commentary on work being done at Vanderbilt and Neuroscience laboratories around the world. *VRN* was founded in 2009 in an effort to consolidate and recognize the hard work done by each class of Ph.D. qualifiers, and is published annually by the Institute.

Review Process
All reviews submitted for doctoral qualifiication must be approved by a committee of at least four tenured or tenure-track faculty members (Phase I). All approved reviews are accepted by *VRN*.



A Message from the Director of the Vanderbilt Brain Institute

It is with a sense of deep pride that I review this fourth issue of *VRN*. Once again I am very impressed with the quality and diversity of the reviews that make up this volume, as well as with the synopses that illustrate the exceptional year our trainees have had in the realm of scholarship.

This past month has seen the VBI staff assemble the proposal for renewal of our training grant, and in gathering the materials necessary to make the strongest possible arguments for continuing (and increased) support, I was pleased to be able to point to the numerous successes that we have had over the past several years. Along with continuing our exceptional record of scholarship, with numerous papers in high profile journals authored by our trainees, we have had a remarkable record of success in garnering extramural support in the form of individual fellowship grants and foundation awards. Past students of our program are now transitioning to faculty positions at prestigious institutions such as Harvard, Yale, and Duke (and Vanderbilt of course!), and our newly minted PhDs continue to do postdoctoral fellowships in some of the world's leading neuroscience laboratories. With these accomplishments to reflect on, I feel confident that our mission to train the future leaders in the neurosciences is well on track!

Yours in science,

Mark T. Wallace

A Message from the Neuroscience Program Director of Graduate Studies

A neuroscience journal run, edited and contributed entirely by graduate students - how cool is that? Plenty, when the quality of the work published and the care with which it is assembled is as special as *VRN*. *VRN* showcases the scholarly work of our outstanding students as they assemble and synthesize their knowledge base and move toward fully implementing their dissertation research. The original articles highlight the insight and intellectual curiosity of Neuroscience graduate students at Vanderbilt and foreshadow their novel contributions to solving the mysteries of the brain. Each issue is a special pleasure to behold.

As Ever,

Douglas McMahon, PhD

An Update from the Neuroscience Student Organization President

As the Neuroscience Student Organization (NSO) president, it is my honor to highlight a few of the many achievements of our student body. While the entire scientific community has been dealing with an increasingly difficult funding environment, the Vanderbilt Brain Institute (VBI) has continued its dramatic growth - a testament to the high quality research being done by our training faculty. Since the last issue of the *VRN* was published, 17 students have successfully completed their qualifying exams and become PhD candidates. In addition, 11 new students have entered the neuroscience training program, bringing our total student body to an impressive 81 students.

To help sustain the VBI's continued growth, our faculty and administration have worked hard to bring new training and fellowship opportunities to our program. Of particular note, through a generous donation from the Dan Marino Foundation and the JB Marshall Laboratory for Neurovascular Therapeutics, the VBI helped established the Clinical Neuroscience Scholars program, which offers fellowship support to 2nd and 3rd year students(pictured right). Scholars in this program have the unique opportunity to be paired with a clinical mentor in an effort to bridge the gap between basic science research and the clinical needs of patients.

In addition to these academic highlights, I would be remiss if I did not mention the NSO's involvement in Brain Blast, the VBI's yearly community outreach event that educates kids about the brain through a series of interactive exhibits and activities. This year's Brain Blast was held at Vanderbilt's One Hundred Oaks campus in an effort to draw in a larger audience, but also to gain exposure to the Brain Matters Exhibits established by the Silvio O. Conte Center for Neuroscience Research and VBI. Under the leadership of the NSO's outreach coordinators, we set a Brain Blast attendance record, with nearly 700 eager attendees becoming burgeoning neuroscientists in a six-hour period!

As many of you are well aware, last year's neuroscience retreat was a resounding success. The NSO was committed to changing the tone of the retreat to a more laid- back atmosphere, but still have it be a venue where everyone can exchange scientific ideas and discuss their research. To highlight the day, we were lucky enough to have Dr. Tom Insel, director of the National Institute of Mental Health, talk to us about the state of psychiatric research and the challenges that await the next generation of neuroscientists. Although the bar has been set high, this year's NSO retreat committee has recruited another excellent speaker, Dr. David Eagleman from the Baylor College of Medicine, who has garnered national attention for his pioneering work integrating new discoveries in neuroscience with our legal system.

Finally, I'd like to echo the sentiments of my predecessor and urge you to make a concerted effort to get to know your fellow graduate students, whether it be at an NSO sponsored social, asking a question at our weekly neuroscience forum, or just grabbing a cup of coffee at Suzie's. At its core, science is an exchange of ideas and it's imperative that as we progress through our careers as neuroscientists we share our point of view.

Chris Muller

Neuroscience Trainees Justin Siemann, Jennifer Vega, Juli Krueger and Kale Edmiston were named to the inaugural class of Clinical Neuroscience Scholars, a program designed to bridge clinical and basic neuroscience research.

Neuroscience Outreach Activities

Perhaps one of the most important tasks we have as emerging neuroscientists is to reach out to both the scientific and lay communities. Educating the public about neuroscience research increases awareness and can inspire young members of the community to pursue a scientific career. In addition, graduate students benefit tremendously in their own careers through interaction with established and successful neuroscientists across the country. The Vanderbilt Neuroscience Student Organization is proud to support a number of outreach efforts, including an annual student-invited speaker and the educational event Brain Blast(top right).

Each year, Vanderbilt's neuroscience graduate students nominate, vote, and invite a prominent neuroscientist to give a special seminar to students and faculty. This gives students the opportunity to become further involved in the national neuroscience community, as well as learn about the most innovative science from world-renowned investigators. The 2012 student-invited speaker was Dr. Daniel Weinberger, a leader in the field of genetic contributions to schizophrenia and other neuropsychiatric diseases. Many students were able to meet with Dr. Weinberger to discuss his outstanding career and share their own research contributions, displaying the remarkably diverse and cutting-edge projects of our students. The seminar Dr. Weinberger gave was a great success, and his ground-breaking research was presented to a "standing-room only" packed lecture hall.

Brain Blast is a day-long event hosted by the Vanderbilt Neuroscience Student Organization that strives to educate children (and children-at-heart) about the vast and complex functions of the brain through a series of interactive booths. This year, Brain Blast was held at Vanderbilt's One Hundred Oaks, which gave the community additional opportunities for neuroscience education through the Brain Matters exhibit. This fun and educational event is always run entirely by volunteers, from undergraduate students interested in neuroscience to seasoned investigators from Vanderbilt's faculty. Brain Blast 2012 was a record-breaking success. Over 60 volunteers shared their love of neuroscience with over nearly 700 children and parents, the largest turn out this event has ever had. Attendees were inspired by the tour through the senses, the examination of a real human brain, extraction of DNA from strawberries, and introduction to live animal models used to investigate neuropsychiatric diseases, among other fascinating booths. Feedback from children and parents was overwhelmingly

continued on pg. 5

From Top: Families gather at 100 Oaks and the Brain Matters exhibit for this year's Brain Blast; Children pass through the cow eye dissection booth manned by Dr. Viven Casagrande; Students enjoy homebrewed beer and refreshments at the fall 2011 MTNCSfN social. (Left) 2nd year student Elaine O'Connell paints her mini pumkin at the fall social.

positive, many of whom were already excited for the return of this event in 2013.

Megan Fettig, Katie Louderback & Terry Jo Bichell

Message From Your Local SfN Chapter

Dear Members of the Vanderbilt Neuroscience Community,

I would like to take this opportunity to encourage you to join the Middle Tennessee Chapter of the Society for Neuroscience, if you haven't already. There are over 100 chapters of the Society in North America and the numbers continue to grow. The purpose of having a local chapter is to foster a sense of community within such a large organization as the SfN. Being part of a chapter increases networking opportunities, provides a forum for exchanging ideas, fosters public education about neuroscience and is a source for several travel and lecture awards. What the chapter is and what it does is determined by its membership. The possibilities are infinite and are limited only by our imagination. It is the goal of the leadership team to make the Middle TN Chapter a showcase example of what can be done through the local chapters. We aim to expand our membership and get more involvement from local institutions, to improve our outreach to the community and to catalyze stimulating scientific interactions among our members. Since its inception more than 10 years ago, the chapter has grown into a vibrant, active group with many exciting initiatives and continues to expand and gain national recognition.

We kicked off this year with a membership drive and fund-raiser, with the goal of recruiting more involvement from other local institutions. This event featured a seminar from our own Dr. David Zald followed by a chili cook-off. This was a good start, but we aim to continue growing the participation of our colleagues at other colleges and universities as well as the large pool of undergraduates right here at Vanderbilt. One of the efforts initiated last year that facilitates outreach to our neighboring institutions and to the local community is the Summer Enrichment Research Program in Education and Neuroscience Training, or SERPENT. This summer program allows an undergraduate from a local institution to come to Vanderbilt and do research over the summer. It also includes an outreach aspect since the summer student is tasked with developing a brief educational lesson/experience for middle school students. This year's SERPENT student was Caitlin Nelms from Austin Peay and she worked in Dr. Mark Wallace's lab. She developed a wonderful interactive educational tool and game that we hope to share beyond the bounds of our local chapter.

One of our signature events is the Annual Meeting, which we hold every spring. The last couple of years this event has been coupled with the Data Blitz, which is a series of very brief talks by our trainees about their research. At the end, winners are selected from among both students and postdocs and cash prizes are awarded. This is another event that we intend to expand. With the ever increasing participation of neuroscientists here at Vanderbilt as well as other middle Tennessee institutions, we hope to grow this meeting by reaching out to other local chapters and create a small, but dynamic conference that highlights the most exciting frontiers in neuroscience being pioneered by local investigators.

Although I would love to tell you more about all of our activities, from pumpkin carving socials to pizza making parties and seminars, due to space limitation, I will end by asking for your help. We can't realize the full potential of our chapter and make it into the national treasure that we aspire to without your involvement and support. Please take the opportunity to visit our website (http://www.mtncsfn.org/) and learn more about us and consider joining or making a donation.

Sincerely,

Bruce D. Carter

RESEARCH HIGHLIGHTS

Rare DAT Variant R615C in ADHD patients alters membrane microdomain localization and trafficking

In this article of The Journal of Neuroscience, senior graduate student DJ Sakrikar describes the discovery and mechanistic description of a novel gain of function mutation located in the distal C-Terminus of the presynaptic dopamine transporter (DAT) identified in an Irish cohort of attention-deficit hyperactivity disorder(ADHD) patients. Using human cell lines expressing both wildtype(615R) and 615C variants, DJ used a host of established and novel biochemical assays to systematically ascertain how this mutation impacts DAT function. 615C was found to exhibit reduced maximal DA uptake that was due to reduced total and membrane DAT expression. Surprisingly, whereas WT DAT(615R) is highly sensitive to the psychostimulant amphetamine(AMPH), a compound that reduces DA uptake and surface protein levels, 615C was insensitive. Using a novel trafficking assay, 615C displayed elevated levels of constitutive endocytosis and membrane recycling. Further biochemical assays revealed an increased association with calcium-calmodulin dependent protein kinase II(CaMKII), a signaling molecule that normally binds to the C-terminus of DAT. Pharmacological blockade of CaMKII resulted in a decrease in specific DA uptake without affecting the internalization of 615C. Further biochemical exploration of 615C biochemical phenotypes revealed a decreased association with the membrane microdomain-associated protein Flotillin-1. Congruously, direct visualization of DAT and membrane microdomains via fluorescent labeling and confocal microscopy in human cell lines demonstrated that 615C shows a decrease in microdomain association. Using membrane-permeable Tat peptides, they discovered that the distal C-Terminal fragment of 615C acts dominantly to impact the trafficking of WT 615R DATs by AMPH. Further exploration of this gain of function effect using site-directed mutagenesis of selected C-Terminal residues showed that the presence of a negatively charged amino acid at either position 615 or 613 was sufficient to produce a block in AMPH-mediated trafficking of DAT. Position T613 in combination with R615 forms a canonical phosphorylation site for multiple kinases. They hypothesized that the presence of a negative charge at either residue may mimic phosphorylation that renders the DAT insensitive to AMPH-mediated trafficking. These studies are the first to identify a human variation in DAT with alterations in protein-protein interactions and membrane microdomain association. They also validate the importance of screening for rare functionally penetrant variations in human genes in trying to understand the complex neurobiology that underlies normal DA clearance or other synaptic phenomena. They also imply that nonallelic deficits in DAT membrane trafficking may be a common molecular phenotype in the etiology of ADHD. Two of the most common drugs for treating ADHD, methylphenidate and amphetamine, target DAT directly but act through distinct mechanisms. Indeed, the impact of 615C on DAT trafficking and localization may inform new directions for targeted therapies in ADHD patient cohorts.

Sakrikar *et al. J. Neuroscience*. 2012 April 18;32 (16): 5385-5397.

Neural correlates of multisensory perceptual learning

Understanding our world depends on properly processing sensory information. An external event is typically processed through multiple sensory cues, and in order to accurately perceive such an event, the brain must be able to combine these cues. One of the most salient cues used to determine which signals should be combined is their temporal coincidence: the closer two signals are in time the more likely they will be integrated. Albert Powers, a recent graduate from our Neuroscience Ph.D. program, and colleagues revealed in a prior study (see Powers *et al.* 2011) that this temporal principle is plastic. Specifically, perceptual training can increase temporal precision between modalities; nevertheless what neural correlates elicit such plasticity is still unknown. Several regions of the brain have been previously identified to be sensitive to synchrony of multisensory stimuli. The current study sought to build upon this knowledge hypothesizing that these candidate regions differentially engage during the presentation of audiovisual stimulus pairs before and after training. In order to test this hypothesis 11 subjects underwent a two-interval forced-choice simultaneity judgment (SJ) paradigm inside and outside of a functional resonance imaging (fMRI) scanner before and after training. As previously reported in Powers et al 2011, a significant increase in temporal precision with training was observed behaviorally. Seeking out the neural correlates to such a perceptual plasticity, fMRI scans were taken while participants completed the audiovisual SJ task. In agreement with prior studies, candidate regions sensitive to synchrony perception included the posterior superior temporal sulcus (pSTS), the insula, the posterior parietal cortex, the lateral occipital cor-

tex, areas of auditory and visual cortex as well as the superior cerebellum. Of particular interest here are the pSTS and regions of auditory and visual cortex as they showed training induced effects indicated by significant BOLD decreases at two stimulus onset asynchronies (0ms and 300ms SOA) not caused by repeated stimulation of said areas and limited to only correct trials. Instead BOLD decreases here presumably indicate more efficient sensory processing thought to be a result of a reduction of recruited neurons during the multisensory task. Functional coupling analysis can give insight into network dynamics needed to process crossmodal stimuli. In the current study, connectivity analysis illustrated increased resting state coupling after training between pSTS and a set of brain regions: the right superior temporal gyrus, the right parahippocampal gyrus, the inferior parietal sulcus, the left premotor cortex, the superior colliculus, and the superior cerebellum. Moreover, modeling analysis also illustrated greater coupling among pSTS and auditory and visual cortices following training with an inherent switch from predominantly feedfoward mechanisms to a network characterized by enhanced feedback processing as well as enhanced connections between visual and auditory cortices during multisensory conditions suggesting more efficient transfer of information between these regions. In summary, the current study sheds light on the underlying neural correlates to changes in temporal binding of stimuli, and gives first evidence for a brain network responsible for the multisensory temporal processing.

Powers *et al. J. Neuroscience.* 2012 May 2;32(18) 6263-6274.

The teen brain on cocaine: how adolescent drug use correlates with long-term behavioral consequences

Cocaine is a highly addictive psychostimulant whose strong potential for abuse has permanent behavioral and neurobiological ramifications. Moreover, cocaine abuse amongst adolescents remains a critical public health concern particularly given the deleterious effects of psychostimulants on the developing brain. Furthermore, many studies have suggested that early drug-induced brain changes may act as the molecular underpinnings for permanent behavioral deficits that manifest long after cessation of drug use. Given these devastating outcomes of chronic adolescent cocaine use it is increasingly imperative to further understand the biological mechanisms that underlie its harmful effects.

In a recent Biological Psychiatry report, graduate student Stephanie Sillivan and colleagues provide significant insight into the biological mechanism of cocaine abuse by demonstrating, with anatomical focus, a strong correlation between the molecular effects of early chronic cocaine use and its consequential behavioral deficits. Quite importantly, this study focused on the amygdala, a critical limbic structure that mediates fear learning, as well as, anxiety-related behaviors and likely subserves the increased incautious actions exhibited by cocaine users.

In this study, fear learning and anxiety-related behavior in adult male rats (P70) were assessed following a binge cocaine treatment paradigm which involved increasing doses of cocaine administered during the human equivalent of early adolescence to young adulthood (P35-P46). Thereafter, elevated plus maze and contextual fear conditioning tests revealed decreased anxiety and contextual fear responses, respectively, in adult rats subjected to binge cocaine treatment as compared to their vehicle treated counterparts. Furthermore, using a similar drug-treatment paradigm, the hole board exploration task also revealed increased novelty seeking and exploratory behaviors in cocaine treated rats as compared to controls.

Interestingly, these cocaine-induced alterations of amygdalar-mediated behaviors were strongly supported by microarray and quantitative polymerase chain reaction (qPCR) studies. These studies convincingly demonstrate dynamic gene expression changes, within the amygdala, following the final binge cocaine treatment administered at P46. Importantly, these early gene expression changes were observed within Wnt signaling pathways critically involved in axonal guidance and synaptic remodeling. Moreover, these expression changes were complemented by additional immunoblotting experiments demonstrating alterations in phosphorylated glycogen synthase kinase 3 beta (GSK3β) levels (a Wnt signaling-related kinase) immediately following the binge cocaine paradigm but returning to normal levels approximately 22 hours later.

Collectively, these data strongly demonstrate that adolescent cocaine abuse has long-lasting, deleterious behavioral consequences that may be explained by cocaine-induced expression changes of critical genes. Of even greater importance is both the role these genes play in appropriate brain development, as well as the immense sensitivity of the adolescent brain particularly given the transient nature of these expression changes. As such, it is increasingly important that teenage cocaine abuse is vigorously addressed, as

choices teens make today, though fleeting, may still have an effect on their future. Now, more than ever, we have the evidence to prove this.

Sillivan *et al. Biological Psychiatry*. 2011 Sep 15;70(6):583-92.

Conflict in the literature: error related signals in macaque monkeys help resolve debate

Error detection is an essential component of learning: we cannot hope to improve future behavior without first identifying our mistakes. Accordingly, there is a large literature aimed at identifying the mechanisms by which our brain detects and corrects errors. Much of this work comes from human FMRI and event-related potential (ERP) studies and has focused on the role of the dorsal anterior cingulate cortex (dACC) in so-called performance monitoring and conflict detection. According to this body of work, the dACC is recruited during situations in which there is a conflict between response options or when an error is committed. However, studies in non-human primates conflict with this account and instead identify other medial prefrontal regions as the locus of performance monitoring. There are considerable variations in the tasks, methodology and species used to understand how the brain functions under conflict and error conditions.

Godlove and colleagues sought to provide some resolution to this ongoing debate by examining error related brain activity in non-human primates using methods with well-studied direct correlates in humans. They recorded event-related potentials (ERPs) while non-human primates performed the stop-signal task. The stop-signal task is a behavioral measure used to examine inhibition and error performance. ERPs measure the electrophysiological response to a stimulus using electrodes that are placed on the scalp in humans or implanted into the skull of non-human primates. The authors trained two monkeys in a version of the stop-signal task in which they made saccades to a target stimulus on most trials (no-stop trials). On stop trials, a signal was presented to the monkey indicating that the response should be withheld. This manipulation allowed the researchers to compare activity on trials in which the monkeys produced the same motor response (a saccade) but under different conditions (correct saccade on no-stop trials, erroneous saccade on stop trials). In humans, errors are associated with two robust ERP components, the error related negativity and positivity. The authors demonstrate that non-human primates display signals of error-related negativity and positivity directly comparable to those found in humans. The authors outline three critical future lines of research in this domain: identifying the brain regions which give rise to the error related ERPs, examining neurochemical modulation of the error related ERPs and providing a cohesive account of the relationship between error related ERPs and cognitive theories of performance monitoring. The current work is but a first step in understanding the precise meaning of error processing and cognitive function.

Godlove *et al. J. Neuroscience*. 2011 Nov. 2; 31(44), 15640–15649.

RESEARCH BRIEFS

Persistent dose-dependent changes in brain structure in young adults with low-to-moderate alcohol exposure in utero

Prenatal alcohol exposure is the leading preventable cause of cognitive impairment in the United States. Moderate-to-heavy alcohol exposure in utero has been linked to deficits in IQ, attention, verbal learning, number processing, and memory. Heavy prenatal alcohol exposure has also been associated with volume and/or structural changes in specific brain regions including the corpus callosum, caudate nucleus, hippocampus, and cerebellum. In the present study, Kristen Eckstrand, a 3rd year Neuroscience Ph.D. candidate(review on *pg 43*), and colleagues sought to investigate the effects of lower doses of alcohol exposure on brain volume. Here, 11 young adults exposed to alcohol in utero and 9 control adults underwent magnetic resonance imaging (MRI). Voxel-based morphometry and region-of-interest analyses revealed no global changes in gray or white matter volume with low-to-moderate alcohol exposure; however, local gray matter volume decreases were observed in the left cingulate gyrus, bilaterally in the middle frontal gyri, the right middle temporal gyrus, and the right caudate nucleus. Gray matter volume reductions in these areas, for which volume changes are implicated after moderate-to-heavy prenatal alcohol exposure, were further identified to be dose –dependent (the higher the in utero exposure the greater the volume reduction). These results suggest that even low doses of alcohol exposure can lead to significant changes in brain development lasting well into young adulthood.

Eckstrand *et al. Alcholism, Clinical and Experimental Research*. 2012 May 17.

Subnuclear development of the zebrafish habenular nuclei requires ER translocon function

Compared to vertebrates, anatomical asymmetry is more pronounced in the dorsal diencephalon of non-mammalian vertebrates, including zebrafish. Asymmetry of the dorsal habenular nuclei (Dh) in zebrafish exhibits distinctive features, and lateralization of the Dh can be identified using gene expression. As such, Doll and colleagues used a genetic screen to find c163 mutants that have altered kctd12.1 expression, a gene with higher expression in the left lateral Dh (LsDh) than the right that can be used as a lateralization marker in wildtype (WT) larvae. Mutants for this gene have more neurons in the LsDh (especially the right LsDh) than the medial habenular nuclei(MsDh), with no dependency on the parapineal organ, a structure that has been previously reported to influence the size of the left habenular nuclei. These LsDh neurons are produced more rapidly and for a longer time compared to WT larvae. The screen also resulted in the identification of a nonsense mutation in the sec61a-like1 (sec61al1) gene, which encodes for a pore-forming subunit of the endoplasmic reticulum (ER). This mutation results in increased neurogenesis in the habenular nuclei, independent of any changes in the expression of the Nodal pathway (normally correlated with initiation of neurogenesis). These mutants also show altered apical-based polarity of habenular progenitors, which the authors hypothesize causes excessive neurogenic divisions in c163 mutants. This study has identified the sec61al1 mutant as a great model to investigate how progenitor cell polarity and the rate of neurogenesis may be connected to the generation of left-right (L-R) asymmetry in the brain.

Doll *et al. Developmental Biology*. 2011 Dec 1;360 (1): 44-57.

Light and melatonin schedule neuronal differentiation in the habenular nuclei

Light exposure and melatonin both play important roles in the timing and stimulation of cell division in zebrafish, but their influences on neurogenesis remain poorly understood. Using the zebrafish model system, the authors find that constant darkness (DD) vs. a 14-hour light: 10-hour dark cycle (LD) postpones neuronal development by delaying the expression of kctd12.1, kctd12.2, and cadps2, genes that are specific to the habenular nuclei in embryos. However, reversing the photoperiod of light exposure (DL) does not advance the gene expression, indicating that their expression does not correlate with the second dark phase. Moreover, the authors found that in DD embryos, the right number of habenular progenitors are specified, but remain in that state for a long time before exiting. DD embryos also show delayed production of high melatonin levels, but melatonin is sufficient to rescue habenular development in embryos exposed to constant darkness. Interestingly, melatonin receptors are G-protein coupled receptors (GPCRs) that signal through the MEK pathway; pharmacological inhibition of MEK induces delayed habenular development similar to DD embryos. In conditions of constant light (LL) exposure, though melatonin production is greatly reduced, habenular nuclei development is timely and can even be considered to be slightly advanced. However, the delayed habenular neurogenesis seen in DD embryos results in a reduction in total neuropil (axons from the forebrain and dendrites from habenular neurons) compared to LD embryos, hypothesized to be from decreased dendritogenesis by habenular neurons. This study highlights the importance of studying melatonin in terms of its influence on proper brain development.

de Borsetti & Dean *et al. Developmental Biology.* 2011 Oct. 1; 358(1):251-261.

Chemoarchitecture of layer 4 isocortex in the american water shrew (*Sorex palustris*)

The juvenile cortex of small mammals, such as water shrews (*Sorex palustris*), allows for proper identification of characteristic cortical modules designated to a variety of sensory areas. In particular, microelectrode recordings and staining with the metabolic enzyme cytochrome oxidase (CO) reveal distinctive subdivisions within the chemoarchitecture of the shrew isocortex that correspond to key sensory regions. Interestingly, Leitch and colleagues discovered that the shrew isocortex was dominated by large somatosensory areas (S1 and S2 regions), while the visual V1 region only occupied about 3% of the total isocortical surface. This suggested that the water shrew does not rely heavily on vision for vital skills such as hunting and navigation. Similarly, the water shrew also contained a very small auditory region. The researchers also found that the water shrew possesses a pronounced trigeminal nerve that is devoted to somatosensation processes from facial areas, suggesting that perhaps water shrew vibrissae are vital in relaying tactile information to the cortex. Lastly, the study found conservation in the organization and positioning of the cortical areas delineated by the microelectrode recordings and CO staining. In fact, the water shrew shares such features with moles in the Talpidae family, supporting the idea of a close taxonomic relationship between the Soricidae and Talpidae families.

Leitch *et al. Brain, Behavior and Evolution*. 2011 Oct. 7; 78 (4): 261-271.

Confirmation of an epilepsy modifier locus on mouse chromosome 11 and candidate gene analysis by RNA-Seq

Epilepsy is one of the most common neurological disorders affecting 1% of the global population. Several forms of epilepsy are triggered by mutations in voltage-gated sodium channels and these forms can be modeled in mice using homologous recombination of mutant alleles of these channels found in human epilepsy patients. These "knock-in" mice are now important tools in studying epilepsy disease etiology, preclinical testing of new pharmacotherapies, and in identifying novel genetic regulators of disease penetrance and expressivity. In this study, Nicole Hawkins generated interval-specific congenic(ISC) lines to fine map a previously identified modifier of epilepsy(Moe) to a 4 Mb interval on Chromosome 11(11q). Using next generation sequence of mouse whole brain mRNA, they identified single nucleotide polymorphisms(SNPs) and gene expression differences of genes within the mapped region. In males, changes in Hlf, hepatic leukemic factor, mRNA expression that is located within the male-specific mapping interval strongly suggest that Hlf is a genetic modifier of epilepsy. In females, changes in mRNA expression of Cacn1ag splice isoforms, a gene located in a female specific interval on 11q , are consistent with its role as a genetic modifier of epilepsy. These studies show, for the first time, that two specific genetic modifiers can influence the severity of a monogenic form of epilepsy. Future studies that manipulate the expression or function of these two genes may help reveal whether they represent effective druggable targets for the treatment of epilepsy.

Hawkins et al. *Genes, Brain and Behavior* 2012 Mar. 28; 11:452-460

Gene transcripts associated with BMI in the motor cortex and caudate nucleus of calorie restricted rhesus monkeys

The global obesity epidemic has given rise to the urgent need to explore its far reaching negative health effects. Negative health indexes of obesity, such as high body mass index (BMI) and insulin resistance, have been correlated with aberrant changes in specific brain regions. A number of brain regions, such as the brain's motor region, presents itself as an attractive study target given the association between decreased obesity and physical activity. This interesting association begs the following question: could BMI changes be associated with molecular alterations in motor regions of the brain? Senior graduate student, Amanda Mitchell, convincingly addressed this question in her recent report published in *Genomics* earlier this year. Using rhesus monkeys that were fed a high fat diet, she found distinct BMI-associated gene expression alterations, such as, the upregulation of apoptotic- and insulin-related genes in the motor cortex and caudate nucleus respectively—with the motor cortex also showing decreased protein phosphorylation of the survival kinase, pERK1/2. Furthermore, these changes persisted despite two months of calorie restriction at the end of the study. Amanda's results strongly indicate that a high BMI may cause sustained molecular changes associated with decreased brain resiliency and neuronal survival in motor regions of the brain. As such, an active lifestyle coupled with a low BMI may play a key role in maintaining brain resiliency.

Mitchell *et al. Genomics* 2012 March; 99(3): 144-151

Structural pathology of fear pathways in Williams Syndrome

Williams Syndrome is a rare neurodevelopmental disorder characterized by hypersociability and exaggerated fears to nonsocial things such as thunderstorms or high places. Avery and colleagues used Diffusion Tensor Imaging (DTI) to investigate structural pathology underlying nonsocial fear in Williams Syndrome. Previous FMRI studies of individuals with Williams Syndrome have identified functional abnormalities in brain regions important for fear expression and regulation, including the amygdala, orbitofrontal cortex and subgenual cingulate cortex respectively. fMRI is limited in its ability to identify causal factors in such functional deficits.

One possible cause of the functional abnormalities in limbic regions observed in Williams Syndrome is pathology in white matter tracts connecting the amygdala with prefrontal areas important for emotion regulation. DTI is an anatomical neuroimaging technique used to identify white matter tracts in the brain based on the limited ability of water to diffuse consistently in all directions along myelinated fiber bundles. Avery *et al.* used probabilistic tractography with DTI to map pathways connecting the amygdala to orbitofrontal and subgenual cingulate cortices in a group of Williams Syndrome subjects and control subjects recruited for having high nonsocial fears. Interestingly, the authors found that Williams Syndrome subjects had decreased white matter integrity in pathways connecting the amygdala with

the both the orbitofrontal and subgenual cingulate cortices. Structural deficits in pathways connecting the prefrontal cortex and amygdala may thereby compromise regulation of the response to potential threats. These findings identify a potential mechanism for elevated levels of nonsocial fear in Williams Syndrome.

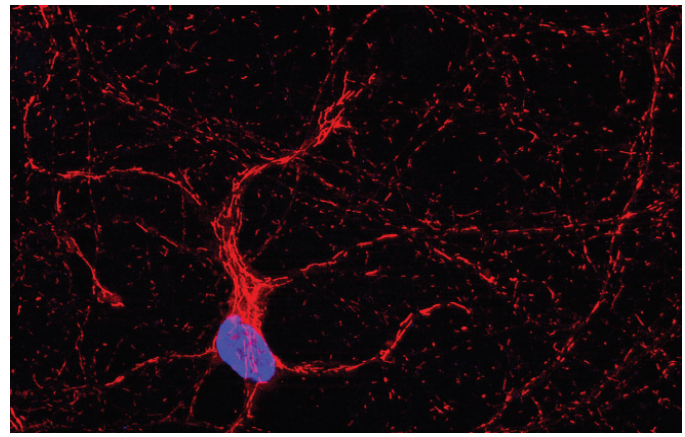
Avery *et al. NeuroImage.* 2012 Jan. 16;59(2), 887–894.

Watch your step! Neural correlates of physical risk assessment

Risk is an unavoidable part of human existence and there is considerable variability in the types of behavior that confer risk. For example, some risk seekers gamble, others climb mountains. However, most studies on the neural basis of risk calculation involve some sort of financial decision-making. Risk magnitude (high vs. low) and the numerical format in which a risk is expressed are critical factors in risky decision-making. For example, most people are more likely to treat risks more seriously when expressed in a frequency format (3/10) than in a probability format (0.3).

Coaster *et al.* characterized the brain regions involved in assessing physical risks of differing severity and format levels using fMRI. Processing of high vs. low harm scenarios was associated with increased activity in the bed nucleus of the stria terminalis and a set of language related regions. This effect was amplified when participants viewed scenarios as a frequency rather than a probability. When subjects evaluated scenarios of high vs. low risk, the authors observed increased activation in the pre-supplementary motor area and ventrolateral prefrontal cortex, as well as in the caudate/ventral striatum (both regions found to be engaged in studies of risky financial decision-making). Additional prefrontal regions were recruited when high-risk scenarios were expressed in probability rather than frequency format. This study highlights the importance of examining human decision-making in different contexts and underscores the complexity of the neural machinery involved in evaluating potential risks.

Coaster *et al. Cognitive, Affective, & Behavioral Neuroscience*. 2011 Dec.;11(4), 494–507.



On the Cover

Neurons are populated with hundreds of individual mitochondrion which dynamically interact and undergo fission, fusion, synthesis and degradation at an incredibly rapid rate. These events are key to integrating biochemical cues from other organelles, surface proteins and nuclear signaling. Moreover, alterations in the signaling molecules which control each of these events have been linked with neuronal dysfunction in response to both acute and chronic stress. The Cover Art (above) depicts dissociated cortical neurons grown in culture for three weeks and stained with MitoTracker Orange (red), a potentiometric, live cell dye that accumulates in mitochondria based on membrane potential. The nuclear marker DAPI (blue) was used as a counter stain. MitoTracker staining demonstrates diversity of mitochondrial morphology and temporal location with high numbers of mitochondria in the neuronal soma and rod like staining in processes indicative of healthy mitochondria. Under conditions of physical, genetic or environmental stress, mitochondria retreat to the soma, fuse or are removed. By tracking organelles in real time, we seek to understand the molecules which determine mitochondrial fate and overall neuronal health.

Amy Palubinsky

CANDIDATE
REVIEWS

Slow To Warm Up: Delayed Neural Habituation To Faces And Risk For Social Phobia

Suzanne Avery

Abstract

Social phobia is a chronic and disabling anxiety disorder that affects approximately 1 in 10 people in their lifetime. Individuals with social phobia experience intense anxiety and fear in situations where they are exposed to unfamiliar people or possible scrutiny by others, leading to social discomfort and isolation. Neuroimaging studies have revealed that novel faces consistently elicit heightened amygdalar and hippocampal activity in social phobia patients. Heightened amygdala activity to novel faces is also associated with inhibited temperament, a strong risk factor for social phobia, suggesting that heightened neural activity to social novelty may be a biological marker of risk. Preliminary findings suggest that slow habituation to novelty may underlie the heightened neural activity seen in inhibited individuals and patients with social phobia, with this prolonged neural response contributing to feelings of unfamiliarity, memory impairments, and increased social fear.

Introduction

Anxiety disorders are the most commonly occurring class of psychiatric disorders, with approximately 1 in 4 people affected by one or more anxiety disorders within their lifetime[1]. Due to their prevalence, the economic cost associated with anxiety disorders is estimated at a staggering $42 billion annually[2]. Social phobia is the second most common type of anxiety disorder[1] and a significant contributor to disability and economic burden[3]. Social phobia, also known as social anxiety disorder, is characterized by a persistent, intense, and chronic fear of being watched or judged by others that interferes with work, school, and other activities[4]. Individuals with social phobia are typically thought of as shy and quiet, and may show physical discomfort (blushing, lack of eye contact) when interacting with others. Although some individuals with social phobia desire the company of others, they often avoid social situations due to their fear of being judged. The burden of social phobia is often underestimated[5] because individuals with social phobia often do not seek treatment. However, impairments associated with social phobia can range from mild to severe. Individuals with mild social phobia often have fears of public speaking; however, less than 5% of individuals with social phobia meet criteria for the diagnosis based exclusively on public speaking fears[6-8]. Instead, the vast majority of individuals with social phobia experience significant fears in *most* social situations, resulting in reduced educational attainment[9;10], low occupational and financial status[10-12], and reduced quality of life[3;11-13]. Social phobia has a typical onset in adolescence[1;2;8;12;14], is highly persistent throughout the entire life course[2], and has high comorbidity with other psychiatric illness[5;12;15;16].

Understanding risk factors related to the development of social phobia could ultimately have important implications for prevention and treatment. The etiology of social phobia appears to be dependent on multiple factors, including neurobiological and developmental risk factors. This review characterizes the most prominent neurobiological findings in social phobia, and the neurobiological and developmental factors most strongly linked to risk for social phobia.

Neurobiology of social phobia

Individuals with social phobia experience intense fear of evaluation in most social situations. Social stimuli which are only mildly aversive or threatening to most people—such as seeing a negative facial expression—can cause emotional distress and anxiety in social phobia patients[17]. Accordingly, a majority of studies[18] have used mildly threatening/aversive social stimuli—such as angry, fearful, or critical faces, or anticipation of public speaking—to probe for altered neural function in social phobia. These studies have identified the amygdala and the hippocampus as two prominent brain regions which show abnormal activity in social phobia.

The amygdala is critically important in the detection of environmental threat[19] and in the expression of fear and anxiety[20;21]. Monkeys with amygdala lesions show a striking lack of fear to environmental and social threat[22;23], and human patients with bilateral amygdala damage have difficulty recognizing fearful expressions[24]. Consistent with the role of the amygdala in threat detection, functional neuroimaging studies have found increased amygdala activity in

social phobia patients in response to various types of social threat, such as viewing of threatening faces[25-27] or anticipation of public speaking[28;29]. The degree of amygdala activity in social phobia patients is correlated with the severity of social anxiety symptoms, but not trait anxiety symptoms, indicating that the amount of amygdala activity in response to social threat may be a relatively specific marker of social phobia severity[25]. Following successful anxiety treatment, social phobia patients show reduced amygdala activity in response to social threat[30;31], indicating that heightened amygdala activity is crucial in the expression of anxiety symptoms. These findings clearly support the role of the amygdala in the detection of social threat, and indicate that hyperactivity of the amygdala in response to negative or threatening social stimuli may at least partially underlie social phobia symptoms.

Interestingly, neutral faces also elicit increased amygdala activity in social phobia patients relative to controls[32;33], although to a lesser extent than threatening faces. Neutral expressions are more emotionally ambiguous than other facial expressions[34], and there is preliminary evidence that individuals with social phobia tend to view neutral expressions as slightly threatening[35], perhaps due to their ambiguity. Therefore, increased amygdala activity to neutral faces could reflect a difference in perception of social threat. Alternatively, increased amygdala activity to neutral faces may reflect generally heightened face or novelty processing in social phobia. The amygdala has a well-defined role in face detection and contains neurons which preferentially respond to faces regardless of emotional valence[36;37]. Some studies have demonstrated heightened amygdala activity to happy faces in social phobia patients[38;39], supporting the notion that social phobia patients may show heightened amygdala activity to all faces, regardless of valence. The amygdala is also critically involved in novelty detection and contains neurons which respond only to the first presentation of a stimulus[36;37;40]. Because social phobia studies often don't control for novelty effects, abnormal novelty processing cannot be ruled out as a contributor to increased amygdala activity. Therefore, it is possible that the amygdala is hyperactive not only to social threat, but also social novelty.

One region which may modulate the amygdala during social threat and social novelty processing is the hippocampus. The hippocampus provides contextual information to the amygdala through dense, reciprocal connections[41], and has been associated with the overgeneralization of anxiety[42-44]. Neural processing of the surrounding environment appears to involve a complex interaction between the amygdala and the hippocampus, with the amygdala influencing memory-related plasticity in the hippocampus[45], and the hippocampus playing a modulatory role over the amygdala during negative face viewing[46;47]. In social phobia patients relative to controls, social threat is associated with increased activity in the hippocampus and parahippocampal gyrus[27;48;49], and attenuated hippocampal activity following successful social anxiety treatment[31]. Similar to the amygdala, the hippocampus and entorhinal cortex play a role in both face detection and novelty detection[36;40], responding most strongly to the first presentation of a face regardless of valence[50]. Therefore, it is possible that increased hippocampal activity to face stimuli in social phobia patients may be at least partially related to hyperactive processing of faces or novelty. No previous studies have specifically examined hippocampal activity in response to neutral faces in social phobia, so it is unknown whether the hippocampus, similarly to the amygdala, shows increased activity to neutral social images. However, these findings suggest that the hippocampus not only participates in face and novelty detection, but also plays an important modulatory role over the amygdala during social threat detection and may be critical in the expression of anxiety in social phobia.

While amygdalar and hippocampal function are important in the neurobiology of social phobia, much less is known about whether these regions contribute to risk for the disorder. If amygdalar and hippocampal function are disrupted early in the progression of the disorder—prior to the onset of significant anxiety symptoms—these regions may serve as early biological markers of risk and help guide early prevention and treatment. Some preliminary evidence suggests that this may be the case; for example, amygdala dysfunction has been found in individuals with inhibited temperament[51-53], a group at significantly increased risk for development of social phobia.

Inhibited temperament as a risk factor for social phobia

While the lifetime prevalence of social phobia in the general population is estimated to be 12%[1], approximately 40% of individuals with inhibited temperament will develop social phobia in their lifetime[54-56]. Because of this substantially increased risk, investigation of the neurobiology underlying inhibited temperament may provide valuable insight into neural risk factors for social phobia. Temperament refers to stable, biologically-based individual differences in emotion, cognition, and behavior that are measureable during the first years of life[57]. Inhibited temperament is the predisposition to react to environmental novelty, such as new people, places, and events, as potentially threatening[58;59]. Approximately 15% of individuals are born with an

inhibited temperament[60], which biases them to react to novelty with fear and wariness. Inhibited children are thought of as "slow to warm up" in new social situations[61] and often withdraw from unfamiliar peers[62]. Investigation of the neurobiology underlying inhibited temperament may provide valuable insight into a specific risk pathway for social phobia.

Because inhibited temperament is associated with avoidance of novelty, often new people, several studies have investigated neural responses to social novelty in inhibited temperament. A seminal study by Schwartz and colleagues used neutral face stimuli to explore amygdala novelty processing in individuals with inhibited temperament. In this study, young adults who had been identified as inhibited at 2 years of age had heightened amygdala activity to novel faces compared to uninhibited individuals, but showed similar amygdala activity as uninhibited individuals to familiar faces[51]. In a later study, Blackford and colleagues showed that inhibited individuals, compared to uninhibited individuals, also have a faster and longer amygdala response to novel neutral faces[52]. These studies indicate that novelty processing in inhibited individuals is associated with exaggerated amygdala activity, which may drive the behavioral avoidance of novelty exhibited by these individuals. Although the study by Schwartz and colleagues included a small number of inhibited individuals who also had social phobia, there were no significant effects of diagnosis on amygdala reactivity to novelty[51]. Because social phobia patients and individuals with inhibited temperament showed similar amygdala response to novelty, Schwartz and colleagues proposed that amygdala hyperactivity in social phobia patients may be influenced by, or perhaps due to, differences in novelty processing that are similar to differences seen in individuals with inhibited temperament[51].

Although social threat tasks are commonly used in social phobia studies, these tasks are infrequently used in studies of inhibited temperament. Only two studies to date have investigated amygdala activity in response to threatening social stimuli in inhibited individuals, and these studies have yielded ambiguous findings. In the first study, Perez-Edgar and colleagues found that inhibited individuals showed increased amygdala activity, relative to controls, in response to threatening faces[65]. However, this increased amygdala activity was found when subjects were required to attend to the emotion of the face, but was not found during passive viewing of threat faces. In contrast, a passive viewing study by Clauss and colleagues found that inhibited individuals showed greater amygdala activity than uninhibited individuals when threatening faces were expected, but not when threatening faces were unexpected[66]. These studies indicate that amygdala activity is increased in individuals with inhibited temperament in response to threatening faces, although attention and expectation may each play a modulatory role. Because both studies were relatively small, further exploration of amygdala response to threatening faces is warranted.

The hippocampus has received much less attention than the amygdala in human studies of inhibited temperament. However, animal lesion studies have demonstrated an important hippocampal role in behavioral inhibition and social interaction. Rats with ventral hippocampal lesions show decreased behavioral inhibition in both novel and potentially dangerous environments, and engage in more social interaction than non-lesioned controls[67], consistent with an anxiolytic effect. Similarly, non-human primates with hippocampal lesions show increased exploration of novel objects and significantly fewer fear behaviors than controls when interacting with novel objects[68]. Additional findings in non-human primates indicate that increased hippocampal function during exposure to a novel environment is predictive of behavioral inhibition[69] and is a key neural signature of anxious temperament[70]. Importantly, hippocampal lesions produce slightly different behavioral phenotypes than amygdala lesions, indicating that the hippocampus has a unique role in the production of anxiety-related behaviors[67;68]. These findings suggest that the hippocampus may play an important role in the neurobiology of inhibited temperament, although investigation of hippocampal function in humans is needed.

In summary, the amygdala and hippocampus show heightened activity in response to novel faces in healthy individuals[71], consistent with their role in both face detection and novelty detection. However, individuals with inhibited temperament show abnormally heightened amygdala activity in response to novel faces[51;53;72], suggesting that abnormally heightened amygdala activity may contribute to increased fear or avoidance of novelty. The mechanisms underlying abnormally heightened amygdala activity and avoidance of novelty in inhibited temperament are not well understood, although altered habituation has been proposed to play a key role[53;72].

Habituation to novelty in inhibited temperament

At its simplest, habituation represents a decreased response to repeated presentations of a stimulus[73]. Novelty detection is a critical first step in the evaluation of potential threats (or rewards) in the environment. However, humans must continually process vast amounts of incoming sensory

information. Therefore, rapid habituation to novel stimuli which are neither threatening nor rewarding is crucial for effective navigation of our constantly changing environment. Neuronal habituation signals safety and familiarity[36;37;74-76], while a failure to rapidly habituate to novelty may trigger feelings of unfamiliarity[36;37;74-76], potentially leading to increased fear and anxiety in novel situations. Although habituation is a fundamental process, individual differences in habituation appear as early as infancy[77;78], potentially providing a neural mechanism for the increased reactivity to novelty observed in behaviorally inhibited infants.

The amygdala and hippocampus are both critically involved in the detection of novelty[36;37;40;50;51;74;75;79-81] and rapidly habituate to repeated exposure[36;37;40;50;74;75;79;81] in healthy individuals. Interestingly, both of these regions show slow habituation to novelty in individuals with inhibited temperament[53;72]. Blackford and colleagues showed that during initial viewing of novel, neutral faces, uninhibited and inhibited individuals had a similar increase in amygdalar and hippocampal activity; however, with repeated presentations of the same neutral faces, uninhibited individuals showed a quick decline to baseline, while inhibited individuals showed sustained amygdalar and hippocampal response after approximately 1 minute of face viewing (Figure 1)[72]. Single-unit recording studies have shown that habituation usually occurs rapidly, with the greatest decrease in response observed between the first and second stimulus repetition[36;37], providing a critical neuronal code for familiarity[36;37;74-76]. Additionally, slow habituation of the amygdala to novel faces, similar to that observed in inhibited temperament, has been associated with more severe social impairment in autism[82]. Therefore, prolonged neural response to novelty is likely to contribute to decreased feelings of familiarity in novel situations[36;37;74-76], and may result in anxiety and uncertainty in novel situations and increased novelty avoidance in inhibited individuals. Preliminary findings in social phobia are less clear. In a single study which investigated habituation in social phobia, social phobia patients showed an altered pattern of amygdala habituation to novel emotional faces, although group differences in the rate of habituation were not found[83]. However, social phobia patients in this study were required to make a gender selection for each face, while habituation studies in inhibited temperament have used passive viewing of faces. Task demands may significantly alter amygdala activity[84-86].

Delayed habituation is associated with reduced ability to discriminate between novel and recently-seen faces[74] in healthy adults, suggesting that altered habituation may affect conscious memory. Habituation is one of

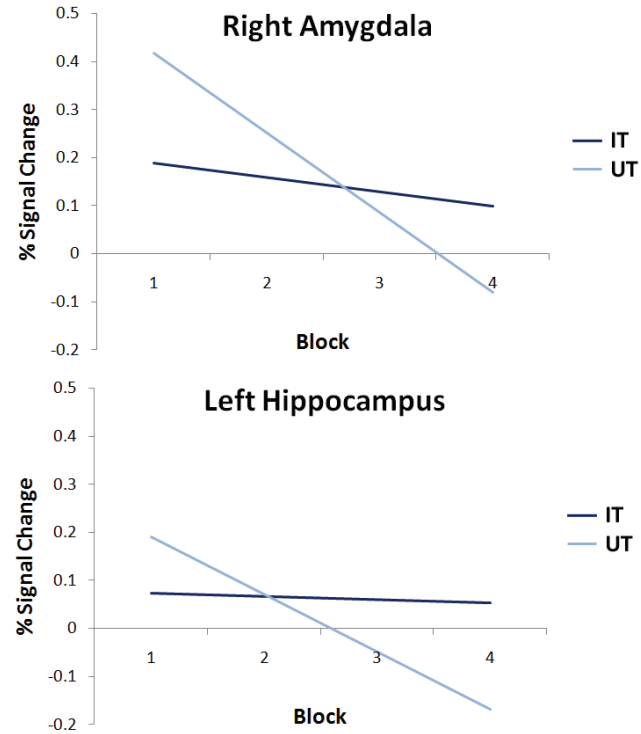


Figure 1. Linear regression of blood-oxygen-level dependent activity by region. Individuals with uninhibited temperament (UT) show a linear decrease in amygdala and hippocampal activity across repeated blocks of neutral faces, indicating habituation to face stimuli. In contrast, individuals with inhibited temperament (IT) do not show habituation of neural activity over repeated blocks of neutral faces. Note: blocks consist of repeated presentations of face stimuli. Each block of face stimuli is 18 seconds long.

the simplest forms of learning and memory in the brain and has been demonstrated in higher order processing regions involved in recognition memory, such as the amygdala and hippocampus[87;88]. This has led to speculation that habituation in the amygdala and hippocampus may be a critical component of short-term recognition memory[74]. This notion is supported by several lines of evidence in both healthy and clinical populations. In healthy individuals, poor working memory for emotional faces is associated with increased amygdala activity during the encoding of emotional faces[89], suggesting that abnormally elevated amygdala activity may disrupt or impair memory formation. Slow habituation in the amygdala, hippocampus, parahippocampal cortex, and perirhinal cortex has also been correlated with poor recognition and episodic memory in patients with Alzheimer's disease[90]. Similarly, slow habituation in the medial temporal lobe has been reported in schizophrenia[91], a psychiatric

disorder associated with memory impairments[92]. Behavioral studies have shown memory impairments for recently familiarized faces in inhibited temperament[93] and social phobia[94], although no studies to date have examined habituation in relation to memory impairments in either of these groups. Although normal amygdala[95] and hippocampal[96] activity is associated with memory improvement in healthy individuals, these findings suggest that abnormally prolonged amygdala and hippocampal activity may negatively affect short-term memory for social stimuli.

Conclusions

Preliminary evidence suggests that the increased amygdala and hippocampal responses to novelty in inhibited individuals may reflect slowed neural habituation in these brain regions. Slow amygdala and hippocampal habituation may have several consequences including increased wariness of novelty, increased fear and anxiety, and reduced declarative memory function in inhibited individuals, contributing to risk for development of social phobia. Future studies should systematically investigate habituation rate in inhibited temperament and social phobia in relation to novelty avoidance, state-based anxiety, and memory ability, in order to understand how habituation contributes to each of these factors.

References

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005;62:593-602.
2. Kessler RC, Ruscio AM, Shear K, Wittchen HU. Epidemiology of Anxiety Disorders. *Current Topics in Behavioral Neurosciences* 2010;2:21-35.
3. Saarni SI, Suvisaari J, Sintonen H et al. Impact of psychiatric disorders on health-related quality of life: general population survey. *Br J Psychiatry* 2007;190:326-332.
4. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, 4 DSM-IV.*. Washington, DC: American Psychiatric Association, 1994.
5. Wittchen HU, Stein MB, Kessler RC. Social fears and social phobia in a community sample of adolescents and young adults: prevalence, risk factors and co-morbidity. *Psychol Med* 1999;29:309-323.
6. Burstein M, He JP, Kattan G, Albano AM, Avenevoli S, Merikangas KR. Social phobia and subtypes in the national comorbidity survey-adolescent supplement: prevalence, correlates, and comorbidity. *J Am Acad Child Adolesc Psychiatry* 2011;50:870-880.
7. Stein MB, Walker JR, Forde DR. Public-speaking fears in a community sample. Prevalence, impact on functioning, and diagnostic classification. *Arch Gen Psychiatry* 1996;53:169-174.
8. Kessler RC, Stein MB, Berglund P. Social phobia subtypes in the National Comorbidity Survey. *Am J Psychiatry* 1998;155:613-619.
9. Liebowitz MR, Gorman JM, Fyer AJ, Klein DF. Social phobia. Review of a neglected anxiety disorder. *Arch Gen Psychiatry* 1985;42:729-736.
10. Schneier FR, Heckelman LR, Garfinkel R et al. Functional impairment in social phobia. *J Clin Psychiatry* 1994;55:322-331.
11. Patel A, Knapp M, Henderson J, Baldwin D. The economic consequences of social phobia. *J Affect Disord* 2002;68:221-233.
12. Wittchen HU, Fehm L. Epidemiology and natural course of social fears and social phobia. *Acta Psychiatr Scand Suppl* 2003;4-18.
13. Ruscio AM, Brown TA, Chiu WT, Sareen J, Stein MB, Kessler RC. Social fears and social phobia in the USA: results from the National Comorbidity Survey Replication. *Psychol Med* 2008;38:15-28.
14. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005;62:617-627.
15. Beesdo K, Knappe S, Pine DS. Anxiety and anxiety disorders in children and adolescents: developmental issues and implications for DSM-V. *Psychiatr Clin North Am* 2009;32:483-524.
16. Beesdo K, Bittner A, Pine DS et al. Incidence of social anxiety disorder and the consistent risk for secondary depression in the first three decades of life. *Arch Gen Psychiatry* 2007;64:903-912.
17. Stein MB, Stein DJ. Social anxiety disorder. *Lancet* 2008;371:1115-1125.
18. Freitas-Ferrari MC, Hallak JE, Trzesniak C et al. Neuroimaging in social anxiety disorder: a systematic review of the literature. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:565-580.
19. Ohman A. The role of the amygdala in human fear: automatic detection of threat. *Psychoneuroendocrinology* 2005;30:953-958.
20. Lang PJ, Davis M, Ohman A. Fear and anxiety: animal models and human cognitive psychophysiology. *J Affect Disord* 2000;61:137-159.
21. Davis M. Neurobiology of fear responses: the role of the amygdala. *J Neuropsychiatry Clin Neurosci* 1997;9:382-402.
22. Amaral DG. The amygdala, social behavior, and danger detection. *Ann N Y Acad Sci* 2003;1000:337-347.
23. Kluver H, Bucy PC. Preliminary analysis of functions of the temproal lobes in monkeys. *Archives of Neurology and Psychiatry* 1939;42:979-1000.
24. Adolphs R, Tranel D, Hamann S et al. Recognition of facial emotion in nine individuals with bilateral amygdala damage. *Neuropsychologia* 1999;37:1111-1117.
25. Phan KL, Fitzgerald DA, Nathan PJ, Tancer ME. Association between amygdala hyperactivity to harsh faces and severity of social anxiety in generalized social phobia. *Biol Psychiatry* 2006;59:424-429.
26. Blair K, Shaywitz J, Smith BW et al. Response to emotional expressions in generalized social phobia and generalized anxiety disorder: evidence for separate disorders. *Am J Psychiatry* 2008;165:1193-1202.
27. Stein MB, Goldin PR, Sareen J, Zorrilla LT, Brown GG. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry* 2002;59:1027-1034.
28. Tillfors M, Furmark T, Marteinsdottir I et al. Cerebral blood flow in subjects with social phobia during stressful speaking tasks: a PET study. *Am J Psychiatry* 2001;158:1220-1226.
29. Lorberbaum JP, Kose S, Johnson MR et al. Neural correlates

of speech anticipatory anxiety in generalized social phobia. *Neuroreport* 2004;15:2701-2705.
30. Furmark T, Tillfors M, Marteinsdottir I et al. Common changes in cerebral blood flow in patients with social phobia treated with citalopram or cognitive-behavioral therapy. *Arch Gen Psychiatry* 2002;59:425-433.
31. Furmark T, Appel L, Michelgard A et al. Cerebral blood flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram, or placebo. *Biol Psychiatry* 2005;58:132-142.
32. Birbaumer N, Grodd W, Diedrich O et al. fMRI reveals amygdala activation to human faces in social phobics. *Neuroreport* 1998;9:1223-1226.
33. Cooney RE, Atlas LY, Joormann J, Eugene F, Gotlib IH. Amygdala activation in the processing of neutral faces in social anxiety disorder: is neutral really neutral? *Psychiatry Res* 2006;148:55-59.
34. Massaro DW, Egan PB. Perceiving affect from the voice and the face. *Psychonomic Bulletin and Review* 1996;3:215-221.
35. Winton EC, Clark DM, Edelmann RJ. Social anxiety, fear of negative evaluation and the detection of negative emotion in others. *Behav Res Ther* 1995;33:193-196.
36. Fried I, MacDonald KA, Wilson CL. Single neuron activity in human hippocampus and amygdala during recognition of faces and objects. *Neuron* 1997;18:753-765.
37. Wilson FA, Rolls ET. The effects of stimulus novelty and familiarity on neuronal activity in the amygdala of monkeys performing recognition memory tasks. *Exp Brain Res* 1993;93:367-382.
38. Straube T, Mentzel HJ, Miltner WH. Common and distinct brain activation to threat and safety signals in social phobia. *Neuropsychobiology* 2005;52:163-168.
39. Gentili C, Gobbini MI, Ricciardi E et al. Differential modulation of neural activity throughout the distributed neural system for face perception in patients with Social Phobia and healthy subjects. *Brain Res Bull* 2008;77:286-292.
40. Rutishauser U, Mamelak AN, Schuman EM. Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex. *Neuron* 2006;49:805-813.
41. Stefanacci L, Suzuki WA, Amaral DG. Organization of connections between the amygdaloid complex and the perirhinal and parahippocampal cortices in macaque monkeys. *J Comp Neurol* 1996;375:552-582.
42. Cannistraro PA, Rauch SL. Neural circuitry of anxiety: evidence from structural and functional neuroimaging studies. *Psychopharmacol Bull* 2003;37:8-25.
43. Gray J. *The neuropsychology of anxiety: An enquiry into the functions of the septohippocampal system*. Oxford, UK: Clarendon Press, 1982.
44. Gray JA. The Neuropsychology of Anxiety - An Inquiry Into the Functions of the Septo-Hippocampal System. *Behavioral and Brain Sciences* 1982;5:469-484.
45. Akirav I, Richter-Levin G. Priming stimulation in the basolateral amygdala modulates synaptic plasticity in the rat dentate gyrus. *Neurosci Lett* 1999;270:83-86.
46. Iidaka T, Omori M, Murata T et al. Neural interaction of the amygdala with the prefrontal and temporal cortices in the processing of facial expressions as revealed by fMRI. *J Cogn Neurosci* 2001;13:1035-1047.
47. Guyer AE, Monk CS, McClure-Tone EB et al. A developmental examination of amygdala response to facial expressions. *J Cogn Neurosci* 2008;20:1565-1582.
48. Straube T, Kolassa IT, Glauer M, Mentzel HJ, Miltner WH. Effect of task conditions on brain responses to threatening faces in social phobics: an event-related functional magnetic resonance imaging study. *Biol Psychiatry* 2004;56:921-930.
49. Tillfors M, Furmark T, Marteinsdottir I, Fredrikson M. Cerebral blood flow during anticipation of public speaking in social phobia: a PET study. *Biol Psychiatry* 2002;52:1113-1119.
50. Fischer H, Wright CI, Whalen PJ, McInerney SC, Shin LM, Rauch SL. Brain habituation during repeated exposure to fearful and neutral faces: a functional MRI study. *Brain Res Bull* 2003;59:387-392.
51. Schwartz CE, Wright CI, Shin LM, Kagan J, Rauch SL. Inhibited and uninhibited infants "grown up": adult amygdalar response to novelty. Science 2003;300:1952-1953.
52. Blackford JU, Avery SN, Shelton RC, Zald DH. Amygdala temporal dynamics: temperamental differences in the timing of amygdala response to familiar and novel faces. *BMC Neurosci* 2009;10:145.
53. Blackford JU, Avery SN, Cowan RL, Shelton RC, Zald DH. Sustained amygdala response to both novel and newly familiar faces characterizes inhibited temperament. *Soc Cogn Affect Neurosci* 2010.
54. Hayward C, Killen JD, Kraemer HC, Taylor CB. Linking self-reported childhood behavioral inhibition to adolescent social phobia. *J Am Acad Child Adolesc Psychiatry* 1998;37:1308-1316.
55. Chronis-Tuscano A, Degnan KA, Pine DS et al. Stable early maternal report of behavioral inhibition predicts lifetime social anxiety disorder in adolescence. *J Am Acad Child Adolesc Psychiatry* 2009;48:928-935.
56. Essex MJ, Klein MH, Slattery MJ, Goldsmith HH, Kalin NH. Early risk factors and developmental pathways to chronic high inhibition and social anxiety disorder in adolescence. *Am J Psychiatry* 2010;167:40-46.
57. Zentner M, Bates J. Child Temperament: An Integrative Review of Concepts, Research Programs, and Measures. *European Journal of Developmental Science* 2008;2:7-37.
58. Garcia-Coll C, Kagan J, Reznick JS. Behavioral inhibition in young children. *Child Development* 1984;55:1005-1019.
59. Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. *Child Dev* 1987;58:1459-1473.
60. Kagan J, Reznick JS, Snidman N, Gibbons J, Johnson MO. Childhood derivatives of inhibition and lack of inhibition to the unfamiliar. *Child Dev* 1988;59:1580-1589.
61. Kagan J, Reznick JS, Snidman N. Biological bases of childhood shyness. *Science* 1988;240:167-171.
62. Fox NA, Rubin KH, Calkins SD et al. Frontal activation asymmetry and social competence at four years of age. *Child Dev* 1995;66:1770-1784.
63. Caspi A, Moffitt TE, Newman DL, Silva PA. Behavioral observations at age 3 years predict adult psychiatric disorders. Longitudinal evidence from a birth cohort. *Arch Gen Psychiatry* 1996;53:1033-1039.
64. Schwartz CE, Kunwar PS, Greve DN, Kagan J, Snidman NC, Bloch RB. A phenotype of early infancy predicts reactivity of the amygdala in male adults. *Mol Psychiatry* 2011.
65. Perez-Edgar K, Roberson-Nay R, Hardin MG et al. Attention alters neural responses to evocative faces in behaviorally inhibited adolescents. *Neuroimage* 2007;35:1538-1546.
66. Clauss JA, Cowan RL, Blackford JU. Expectation and tem-

perament moderate amygdala and dorsal anterior cingulate cortex responses to fear faces. *Cogn Affect Behav Neurosci* 2011;11:13-21.
67. Bannerman DM, Rawlins JN, McHugh SB et al. Regional dissociations within the hippocampus--memory and anxiety. *Neurosci Biobehav Rev* 2004;28:273-283.
68. Bliss-Moreau E, Toscano JE, Bauman MD, Mason WA, Amaral DG. Neonatal amygdala or hippocampus lesions influence responsiveness to objects. *Dev Psychobiol* 2010;52:487-503.
69. Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kalin NH. Trait-like brain activity during adolescence predicts anxious temperament in primates. PLoS One 2008;3:e2570.
70. Oler JA, Fox AS, Shelton SE et al. Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. *Nature* 2010;466:864-868.
71. Balderston NL, Schultz DH, Helmstetter FJ. The human amygdala plays a stimulus specific role in the detection of novelty. *Neuroimage* 2011;55:1889-1898.
72. Blackford JU, Allen A, Cowan RL, Avery SN. Failure of the amygdala and hippocampus to habituate to novel faces is associated with inhibited temperament in young adults. *Social Cognitive and Affective Neuroscience* 2011;Under Review..
73. Rankin CH, Abrams T, Barry RJ et al. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol Learn Mem* 2009;92:135-138.
74. Gonsalves BD, Kahn I, Curran T, Norman KA, Wagner AD. Memory strength and repetition suppression: multimodal imaging of medial temporal cortical contributions to recognition. Neuron 2005;47:751-761.
75. Wright CI, Fischer H, Whalen PJ, McInerney SC, Shin LM, Rauch SL. Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. *Neuroreport* 2001;12:379-383.
76. Dubois S, Rossion B, Schiltz C et al. Effect of familiarity on the processing of human faces. *Neuroimage* 1999;9:278-289.
77. Bushnell IW. Discrimination of faces by young infants. *J Exp Child Psychol* 1982;33:298-308.
78. Snyder KA, Keil A. Repetition suppression of induced gamma activity predicts enhanced orienting toward a novel stimulus in 6-month-old infants. *J Cogn Neurosci* 2008;20:2137-2152.
79. Breiter HC, Etcoff NL, Whalen PJ et al. Response and habituation of the human amygdala during visual processing of facial expression. Neuron 1996;17:875-887.
80. Blackford JU, Buckholtz JW, Avery SN, Zald DH. A unique role for the human amygdala in novelty detection. *Neuroimage* 2010;50:1188-1193.
81. Yamaguchi S, Hale LA, D'Esposito M, Knight RT. Rapid prefrontal-hippocampal habituation to novel events. *J Neurosci* 2004;24:5356-5363.
82. Kleinhans NM, Johnson LC, Richards T et al. Reduced neural habituation in the amygdala and social impairments in autism spectrum disorders. *Am J Psychiatry* 2009;166:467-475.
83. Campbell DW, Sareen J, Paulus MP, Goldin PR, Stein MB, Reiss JP. Time-varying amygdala response to emotional faces in generalized social phobia. *Biol Psychiatry* 2007;62:455-463.
84. Lieberman MD, Inagaki TK, Tabibnia G, Crockett MJ. Subjective responses to emotional stimuli during labeling, reappraisal, and distraction. *Emotion* 2011;11:468-480.
85. Costafreda SG, Brammer MJ, David AS, Fu CH. Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PET and fMRI studies. *Brain Res Rev* 2008;58:57-70.
86. Zald DH. The human amygdala and the emotional evaluation of sensory stimuli. *Brain Res Brain Res Rev* 2003;41:88-123.
87. Xiang JZ, Brown MW. Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. *Neuropharmacology* 1998;37:657-676.
88. Fahy FL, Riches IP, Brown MW. Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. *Exp Brain Res* 1993;96:457-472.
89. Neta M, Whalen PJ. Individual differences in neural activity during a facial expression vs. identity working memory task. *Neuroimage* 2011;56:1685-1692.
90. Pihlajamaki M, O'Keefe K, O'Brien J, Blacker D, Sperling RA. Failure of repetition suppression and memory encoding in aging and Alzheimer's disease. *Brain Imaging Behav* 2011;5:36-44.
91. Holt DJ, Weiss AP, Rauch SL et al. Sustained activation of the hippocampus in response to fearful faces in schizophrenia. *Biol Psychiatry* 2005;57:1011-1019.
92. Kuperberg G, Heckers S. Schizophrenia and cognitive function. *Curr Opin Neurobiol* 2000;10:205-210.
93. Avery SN, Blackford JU. Memory for novel faces in inhibited temperament. Unpublished raw data.. 2011.
94. Perez-Lopez JR, Woody SR. Memory for facial expressions in social phobia. *Behav Res Ther* 2001;39:967-975.
95. Phelps EA, Anderson AK. Emotional memory: what does the amygdala do? *Curr Biol* 1997;7:R311-R314.
96. Squire LR, Wixted JT, Clark RE. Recognition memory and the medial temporal lobe: a new perspective. *Nat Rev Neurosci* 2007;8:872-883.

Gene-Environment Interactions in Huntington's Disease

Terry Jo Bichell

Abstract

Referring to the debate about nature versus nurture, neuropsychologist Dr. Donald Hebb is said to have asked, "Which contributes more to the area of a rectangle, its length or its width?"

Huntington's Disease (HD) is a neurodegenerative disease characterized by selective loss of striatal GABAergic medium spiny neurons (MSNs). Despite the inverse relationship between CAG repeat number and age of onset, repeat number only accounts for 60% of the variability in HD. Interestingly, the residual variability is caused by other genetic and/or environmental factors, including single nucleotide polymorphisms, heavy metal toxicity, environmental enrichment, exercise, and diet that modify synaptic activity and neuroprotective functions. Importantly, heavy metals such as manganese (Mn) can accumulate in the striatum, which is the most vulnerable brain region in HD following excessive exposure. Unfortunately, the precise function of Mn in the striatum as a positive or negative modifier of age of onset, disease progression, and clinical symptomology is unknown. Our laboratory has previously reported a striatal specific gene-environment interaction between the mutant Huntingtin gene (HTT) and Mn in both in vitro and in vivo models of HD. Importantly, mutant HTT expression confers resistance against Mn toxicity partly by decreasing net Mn uptake and storage capabilities compared to wildtype. Thus, reduced physiological Mn levels in the brain may profoundly affect Mn-dependent neuronal enzyme function, downstream signaling pathways, and explain some of the aforementioned variability observed in HD. In essence, understanding the relationship between mutant HTT and Mn transport dynamics may elucidate additional in vivo functions of the huntingtin protein (HTT) and contribute to therapeutic interventions in HD.

Keywords

Huntington's
Metals
Manganese
BDNF
Environment

Huntington's Disease: Onset, Genetics, Protein, and Models

In the 1980's, researchers studying an isolated community in Venezuela with a very high incidence of HD pinpointed the genetic cause to an increase in CAG repeats in exon 1 of the huntingtin gene (HTT). This excess of CAG repeats leads to an expansion in the polyglutamine (polyQ) tract in the huntingtin protein[1]. Despite the inverse relationship between prognosis and number of CAG repeats, CAG repeats only explain 60% of the large variability in age of onset, disease progression, and susceptibility. In addition, subsequent sibling studies have demonstrated that modifier genes explained only 13% more of this variance[2,3,4,5]. Furthermore, cases of identical twins discordant for onset and symptoms reveal the influence of other environmental factors on the phenotypic variability observed in HD patients[6,27] (Figure 1). Environmental influences have not been demonstrated to accelerate the pace of HD, but many factors described below (including enriched environments, exercise and diets) have been demonstrated to increase endogenous brain derived neurotrophic factor (BDNF) levels, with a concomitant delay in disease progression. Pollutants and heavy metals, such as copper (Cu), iron (Fe), and manganese (Mn), have been suggested to influence the pathology of many neurodegenerative diseases, via alterations in vesicular transport, mitochondrial dysfunction, protein aggregation, and induction of oxidative stress. The pace of neurodegeneration may be due to several mechanisms, including diminished neurotrophic support and deranged essential metal ion homeostasis in vivo (Figure 2).

Huntington's Disease is an autosomal dominant neurodegenerative disorder with a median age of onset at 39[8]. Although it has been two decades since the identification of the HD-associated mutation, there is growing debate about whether HD symptoms are caused by a: (i) loss of function of normal HTT; (ii) toxic gain of function in mutant HTT[9]; or both (i) and (ii)[10]. HTT knockout mouse models cannot survive past embryonic day 7[11,12],but the essential role for HTT in embryological development remains unclear[13]. Moreover, mouse models of HD develop disease[14]

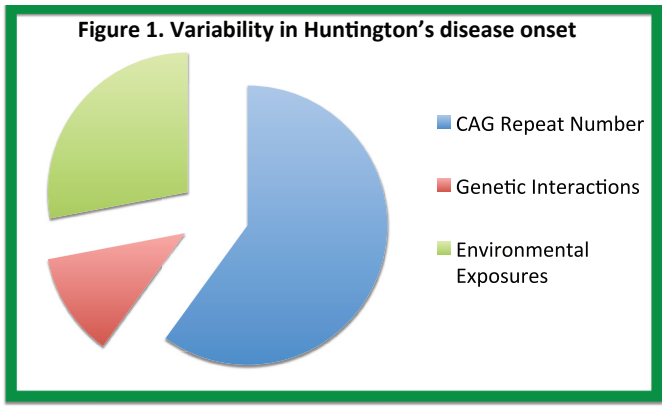


Figure 1: Proportion of the variability in Huntington's disease onset explained by CAG repeat number and modifier genes, with the remainder attributed to environmental influence.

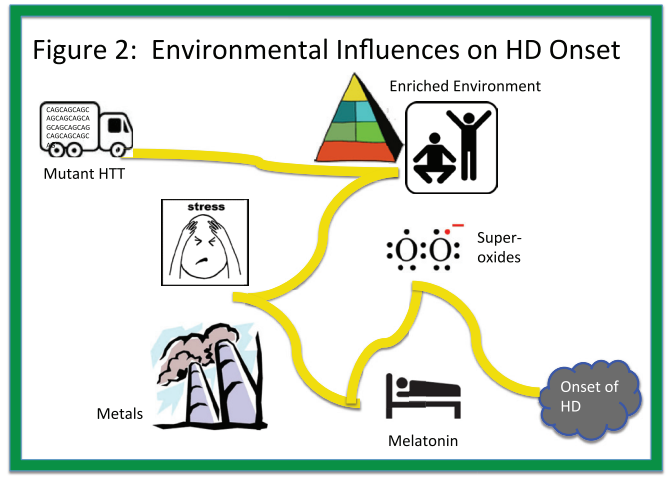


Figure 2: Selected purported environmental influences on age of HD onset, both positive and negative: enriched environment, stress, heavy metals, melatonin, and mitochondrial insults.

while normal HTT is still functional, which suggests that excess mutant HTT is the cause of HD neurodegeneration. However, a careful study of homozygous human patients controlled for CAG repeat number on both alleles demonstrated worsened disease progression compared to heterozygotes[15]. The results from this study suggest that loss of function of normal HTT may still play a role in disease pathology.

Although all of the functions of wildtype HTT are unknown, it has been shown to be involved in crucial neuronal pathways, including apoptosis, transcriptional regulation, axonal transport, and as a scaffold for protein-protein interactions[16]. The ubiquitously expressed HTT protein is large (348-kDa), with over 50 identified binding partners[9] and its unwieldy size has delayed a determination of its crystal structure. The polyQ tract is located in the first exon at the N-terminal of HTT and appears to have evolved relatively recently, adoptimg several different structures (helix, coil, loop) that enable it to function as a key regulator of binding interactions[17,18]. HTT is post-translationally modified by phosphorylation[19], sumoylation[20], ubiquitination[21], acetylation[22,23], palmitoylation[24] and is cleaved in a several different ways by caspases and calpain[25,26]. The N-terminal fragment is the main site of these modifications and the source of HD pathology, including aggregate formation and mitochondrial dysfunction[14,27].

There are at least nine commonly used mouse models of HD, including both transgenics and knock-ins (reviewed in Zuccato et al[14]). The R6/1 and R6/2 transgenic mice carry a fragment of exon 1 from the 5' end of human HTT with 113 and 144 CAG repeats, respectively[28]. The R6/2 mouse has a pronounced HD phenotype, developing weight loss, aggregates, brain atrophy, and motor symptoms by 12 weeks. The YAC128 mouse has a transgene expressing the full-length human HTT gene with 128 repeats[29]. Though it lives a normal lifespan and has increased weight, it develops motor symptoms and has increased n-methyl-D-aspartate (NMDA), AMPA, and metabotropic glutamate receptor (mGluR) binding, and reduced striatal and cortical volume. The BACHD transgenic mouse also expresses the full-length HTT gene and develops inclusions and brain atrophy, but demonstrates fewer motor symptoms than the YAC128[30]. Other models with knock-in polyQ sequences inserted into the mouse htt gene do not develop a robust phenotype[31,32], but do show some neuronal defects and motor abnormalities.

HD is described as having three phases, both in humans and mouse models: (i) pre-manifest, in which the gene mutation has been identified but there are no signs and symptoms; (ii) prodromal, during which there are cognitive and emotional signs, but no loss of function; and (iii) manifest, in which motor symptoms become obvious and there is sharp functional decline[33]. During the prodromal phase, there is loss of cortical mass followed by loss of striatal GABAergic MSNs, which suggests that disease processes in cortical neurons may lead to the subsequent excitotoxic postsynaptic deterioration of MSN's[34]. In fact, decortication in an HD mouse model has been reported to ameliorate HD symptomology[35]. Currently, there are at least nine diseases that are known to be caused by excess CAG repeats, all of which are neurodegenerative. Each of these nine diseases have neuronal loss restricted to specific brain regions[36] and

similar intracellular manifestations, which include alterations in metal processing, protein misfolding, inclusions and aggregates[37]. Protein misfolding and cellular metal mishandling is also present in non-CAG repeat neurodegenerative diseases, such as Alzheimer's Disease (AD), and Parkinson's Disease (PD), both of which have a greater environmental basis than genetic attribution[37]. Environmental factors such as environmental enrichment (EE), exercise, diet, and exposures to xenobiotics have been reported to worsen or ameliorate disease processes in all of these neurodegenerative diseases[38].

Environmental Influences in HD: Lifestyle Effects

Stimulation: Research mice are usually kept in small boxes with bedding, food and water. Under these standardized conditions, mice expressing full length or fragments of the mutant HTT protein develop motor and cognitive disease[28]. However, when allowed access to exercise wheels, stimulating toys and novel objects, their healthy phase is prolonged[39, 40,41]. Exercise alone prolongs the premanifest phase, as does EE alone[42]. Both exercise and EE have been shown to increase levels of striatal BDNF[43] as well as neurogenesis in wildtype mice[44]. This addition to the reserve pool of healthy neurons may explain the protective effects observed in neurodegenerative diseases in general[45]. With HD in particular, BDNF gene transcription[46], as well as serum and cortical BDNF levels are reduced in patients[47-48] compared to healthy controls. However, BDNF protein levels are increased via EE even in the HD murine model[49].

The general function of BDNF is to promote neurogenesis and neuronal survival[50] through binding to the tyrosine kinase B receptor (TrkB), thereby phosphorylating and activating neuroprotective pathways[50,51]. Striatal neurons express TrkB to receive BDNF transported from the cortex or substantia nigra, but have substantially less BDNF when compared to other brain regions[52]. One of the recognized roles of HTT is in the interaction with Huntingtin-associated protein 1 (HAP1) to facilitate transport of BDNF along cortical axons to synapses on MSN's[53]. In addition, wildtype HTT regulates transcription of BDNF by suppressing a key site in the BDNF promoter[54,55]. Emerging evidence has shown that increasing BDNF protein levels protects post-synaptic MSN's even in the presence of mutant HTT[56]. Furthermore, overexpression of cortical BDNF transcription ameliorates symptoms in HD model mice[57] and protects mitochondria[58]. Increasing BDNF in the brain, either directly or indirectly, has been suggested to improve the symptoms observed in HD, AD, PD and Amylotrophic Lateral Sclerosis (ALS)[14] as well.

Diet: There is also a role for diet in delaying the inevitable genetic destiny of HD. Glucose metabolism is altered in HD, with early weight gain followed by hyperglycemia and severe weight loss[59,60,61]. Leptin levels are normal in premanifest human patients[62], but levels do not increase appropriately with BMI[61,63], and leptin is high in murine models compared to wildtype[64,65]. The R6/2 mouse develops metabolic and motor symptoms similar to what is observed in HD human patients[66] . Treating these mice with dietary supplements of essential fatty acids (linoleic and a-linoleic acids) reduced motor signs such as foot clasping and locomotor deficits, but did not correct weight loss or reduction in dopamine receptors[67]. A randomized placebo-controlled double-blind study of fatty acid supplementation in humans with HD also showed a significant improvement compared to placebo[68]. Interestingly, restriction of a-linoleic acid reduces BDNF in a striatal specific manner in wildtype mice[69]. Other dietary manipulations such as dietary restriction (DR) (fasting on alternate days), have been shown to be neuroprotective in wildtype animals[70], delaying locomotor dysfunction, reducing oxidative stress, restoring BDNF levels and glucose metabolism, and increasing lifespan in mice[65]. The DR model has also been shown to increase longevity in C. elegans[71,72]. Ironically, both DR and fatty acid supplementation increases BDNF, which may be the protective mechanism of dietary manipulation in HD.

Oxidative Stress: In addition to the factors that prolong the premanifest phase, oxidative stress and mitochondrial insults[73] from either genetic and/or environmental factors hasten HD pathology. HD post-mortem tissue exhibits severe reductions in mitochondrial complexes II – IV in the striatum with no effect in the blood[74]. Furthermore, PET studies have revealed abnormalities in energy metabolism prior to striatal loss[75]. Systemic treatment with the complex II inhibitor, 3-nitropropionic acid (3NP), causes HD-like abnormal motor behavior[76], striatal-specific neurodegeneration[77] and reduced phosphorylation of DARPP-32[78] (a protein encoded by a modifier gene reported to affect HD onset). Interestingly, pre-treatment with BDNF protects neurons from the effects of 3NP[58].

Mitochondria are abnormal in HD with alterations in enzymatic complexes[27] and calcium (Ca^{2+}) kinetics in HD models[75]. In the YAC128 mouse, mutant HTT interacts with the NR2B subunit of the NMDA receptor, enhancing Ca^{2+} influx and increasing excitotoxicity in striatal MSN's, which carry the NR2B subunit longer in adulthood than most other neurons[27]. Mitochondria are both a source and target of reactive oxygen species[79], and oxidative stress further hastens pathology, increasing apoptosis

and aggregation in cultured cells expressing mutant HTT[80]. Overexpression of the mitochondrial enzyme, superoxide dismutase 1 (SOD1), which binds Cu/Zn, reverses oxidative stress in cultured murine cells[80]. Systemic supplementation with mitochondrial components, such as creatine and ubiquinone, also known as coenzymeQ10 (CoQ10), improved HD symptomology in both HD animal models and human clinical trials[81,82] . Perhaps diets highly enriched with creatine and CoQ10 may contribute to delay in onset of HD, while exposure to toxins that target or accumulate in mitochondria may hasten onset.

Environmental Influences: Metals

Pollutants and heavy metals, expecially Cu, Fe, Mg and Mn, have been implicated in many neurodegenerative diseases[27,83]. The brain appears to be more vulnerable to the toxic effects of metals than other organs, and the striatum appears to be especially vulnerable to mitochondrial toxins[84]. The presence of mutant HTT on mitochondrial membranes causes mitochondria to be even less resilient to excitotoxic insults than other tissues. Heavy metals selectively accumulate in specific brain regions; specifically, Mn^{2+} accumulates in the thalamus and substantia nigra, Fe^{2+} in globus pallidus and Cu^{2+} in the striatum and thalamus following excessive exposures in rats[85]. Emerging studies have demonstrated accumulation of Cu^{2+} and Fe^{2+} in HD[86] and decreased serum ferritin in the striatum of HD[87]. Cu^{2+} interacts with wildtype HTT and decreases its solubility[88]. Furthermore, nuclear inclusions of fragments of the mutant polyQ protein are associated with Fe-dependent oxidation[89]

HTT has been associated with both endocytic and microtubule-mediated vesicle transport, mechanisms which transport both BDNF and various metals into cells and organelles. HTT is closely associated with vesicles and endosomes[90,91,92,93], microtubles[91,94], and directly with the plasma membrane[95]. HTT interacts via HAP1 with an integral member of the microtubule transport system, the dynactin subunit p150Glued[96,97], and co-fractionates with the transferrin receptor (TfR)[98,99]. Knockdown of HTT expression in zebrafish causes increased transferrin receptor 1 transcription in the presence of hypochromic blood. Interestingly, this phenotype is reversed upon administration of bioavailable iron, demonstrating a functional role of normal HTT to make endocytosed iron accessible[100]. The authors theorize that the function of normal HTT must be related to the release of iron from endocytic vesicles.

Manganese: Overexposure to Mn causes preferential accumulation in the mitochondria of the brain and liver[101], especially in the basal ganglia of rats[102] and humans[103], the region also most affected in both HD and PD. Mn also appears to selectively accumulate in the mitochondria of these regions[101] and causes apoptosis from mitochondrial cytochrome c release[104,105] in a caspase-dependent pathway[106]. Subtoxic Mn exposure causes greater susceptibility to 1-methyl-4-phenylpyridinium (MPP), a mitochondrial toxin which targets nigral dopaminergic neurons and is used to create a common PD model. This MPP+ related apoptosis can be reversed by n-acetyl creatine[107]. In HD, it is possible that mutant HTT causes normal levels of bioactive agents to become neurotoxic to selected populations of neurons.

Overexposure to Mn increases risk of a Parkinsonian phenotype referred to as manganism[108,109]. This condition is similar to HD in that it is a progressive neurodegenerative condition which primarily affects the basal ganglia motor pathways. However, there is a loss of nigrostriatal dopaminergic pathways in PD, while there is deterioration of the striatal GABAergic MSN's in HD, and motor symptoms differ[110]. Surprisingly, in HD models, studies utilizing immortalized striatal cells and striatum of knock-in mouse models of HD have demonstrated a resistance to the toxic effects of Mn[111-113]. Emerging evidence from our laboratory aimed at examining Mn transport dynamics in the immortalized striatal cell line model of HD has revealed a significant decrease in instantaneous Mn uptake and storage capablilites in mutant HTT cells compared to wildtype following Mn exposure, though efflux rate appears to be equal in both[114]. It is possible that mutant HTT interacts with constituents of the neuronal Mn transport system and dysregulates Mn kinetics. This gene-environment interaction between mutant HTT and Mn may serve to explain how xenobiotics influence genetic functions. A reduction of Mn in neurons would alter the normal neuronal and glial functions of proteins that cannot function without sufficient Mn, and their byproducts would be reduced in HD. A review of studies on manganoproteins shows either directly or indirectly that they are all reduced in the presence of mutant HTT.

Manganoproteins: Enzymes which require Mn^{2+} to function include glutamine synthetase (GLN), superoxide dismutase 2 (SOD2), arginase 1 and 2 (ARG1, ARG2), pyruvate carboxylase (PC), and selected serine/threonine phosphatase (PPMs)[115].

GLN: The function of GLN is to convert glutamate to glutamine in astrocytes, and a reduction in its activity could partially explain the neurotoxic destruction of MSNs[103,116]. Indeed, Mn accumulates in astrocytes[117], and glial dysfunc-

tion has been shown to play a large a role in the pathogenesis of HD[118]. Interestingly, the glutamate-glutamine cycle is dysregulated in HD[119], as well as reduced GLN is found in HD animal models and patients[120,121].

SOD2: SOD2 is a mitochondrial protein that requires four Mn ions per tetramer, and it acts to convert toxic superoxide into harmless hydrogen peroxide and O_2[122]. A reduction in SOD2 worsens the severity of HD symptoms as confirmed in HD brain tissue[123].

ARG1 and ARG2: Arginase is part of the urea cycle, responsible for converting arginine into either ornithine and urea in somatic tissues or ornithine and nitric oxide in neurons. Arginase requires two Mn ions to function[124]. ARG1 has been found to prevent neuronal death in trophic factor-deprived cell cultures[125]. Patients with HD have an abnormal growth hormone response to arginine infusion[126], and an HD mouse model fed with diets high in arginine has an earlier onset[127]. However, there have been no studies directly examining arginase levels in HD to date.

PC: PC also depends on magnesium and thiamine pyrophosphate as cofactors. PC is essential in the urea cycle and in the metabolism of glucose, cleaving pyruvic acid into acetaldehyde and CO_2[128,129]. There have not been studies of PC in HD, but imaging studies confirm severe hypometabolism of glucose in basal ganglia, even in premanifest HD[130].

PPM: Finally, a subset of the serine/threonine phosphatases require both Mn^{2+} and Mg^{2+} ions to perform the vital function of dephosphorylation at the serine and threonine sites, which is a crucial regulatory step for myriad neuronal proteins[131]. There have not yet been published studies of PPMs in HD, however there has been a reported reduction in gene expression of PPMs after 3NP treatment[78].

Importantly, there is either direct or indirect evidence of reduced activity of all of these manganoproteins in HD models.

Transporters: The mechanism by which expression of mutant HTT reduces neuronal Mn uptake is unknown. Mn has been shown to be transported across neuronal cell membranes by at least 9 known metal transporters. Surprisingly, alterations in many of these transporters have been definitively linked to other neurodegenerative disorders[132], but not specifically to HD[115]. Among these transporters, PARK9 and HIP14 might be especially interesting in the context of HD. Wild type PARK9 (also known as ATP13a2) has been shown to prevent Mn toxicity in neuronal cells and yeast, but mutations in this gene cause Kufor-Rakeb syndrome, a form of parkinsonism[133,134]. Recent studies investigating the function of the metal transporters HIP14 and HIP14L has revealed the necessity for HIP14 to palmitoylate Htt for proper function of the protein[24,135] In addition, the expanded mutant polyQ tract reduces its palmitoylation[136]. In fact, mice with disrupted expression of HIP14 show reduced palmitoylation and display many of the manifestations of HD[137].

Conclusion

There is no evidence yet to show that Mn treatment or exposure will worsen or improve the condition of HD, but the molecular interactions between Mn and HTT may help explain the both the function of the gene and the role of metals in neurological processes. The expanded polyQ series on mutant HTT has an impact on myriad region-specific neuronal functions, including transcriptional regulation, protein interactions, neurogenesis, cell death, and glucose metabolism[138]. A lack or excess of Mn may potentially be involved with many neurodegenerative disorders, including PD, AD, ALS, HD and prion diseases that cause severe progressive encephalophathies in humans[139]. Recently, the prion protein was shown to bind Mn as well as Cu[140]. Moreover, cells expressing abnormal prion proteins are also resistant to Mn toxicity[141]. All of these neurodegenerative diseases share alterations in protein aggregation, mitochondrial damage, and oxidative stress, and all are improved with interventions that increase BDNF. In addition, it is possible that mutant HTT dysregulates both intracellular neurotrophin and metal transport dynamics, and impairs downstream signaling cascades that have been implicated in HD and other neurodegenerative diseases.

References

1. Wexler, N. S. et al. Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci USA 101, 3498–3503 (2004).
2. Rubinsztein, D. C. et al. Phenotypic characterization of individuals with 30-40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36-39 repeats. Am. J. Hum. Genet. 59, 16–22 (1996).
3. Kaltenbach, L. S. et al. Huntingtin Interacting Proteins Are Genetic Modifiers of Neurodegeneration. PLoS Genet 3, e82 (2007).
4. Gusella, J. Huntington's disease: the case for genetic modifiers. Genome Med (2009).
5. Taherzadeh-Fard, E., Saft, C., Wieczorek, S., Epplen, J. T. & Arning, L. Age at onset in Huntington's disease: replication study on the associations of ADORA2A, HAP1 and OGG1. Neurogenetics 11, 435–439 (2010).
6. McNeil, S. M. et al. Reduced penetrance of the Huntington's disease mutation. Hum Mol Genet 6, 775–779 (1997).
7. Friedman, J. H., Trieschmann, M. E., Myers, R. H. & Fernandez, H. H. Monozygotic twins discordant for Huntington disease

after 7 years. Arch Neurol 62, 995–997 (2005).
8. Novak, M. J. U. & Tabrizi, S. J. Huntington's Disease: Clinical Presentation and Treatment. International Review of Neurobiology 98, 297–323 (Elsevier Inc.: 2011).
9. Cattaneo, E., Zuccato, C. & Tartari, M. Normal huntingtin function: an alternative approach to Huntington's disease. Nat Rev Neurosci 6, 919–930 (2005).
10. Cattaneo, E. et al. Loss of normal huntingtin function: new developments in Huntington's disease research. Trends Neurosci 24, 182–188 (2001).
11. Nasir, J. et al. Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell 81, 811–823 (1995).
12. Zeitlin, S., Liu, J. P., Chapman, D. L., Papaioannou, V. E. & Efstratiadis, A. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. Nat Genet 11, 155–163 (1995).
13. Reiner, A., Dragatsis, I., Zeitlin, S. & Goldowitz, D. Wild-type huntingtin plays a role in brain development and neuronal survival. Mol. Neurobiol. 28, 259–276 (2003).
14. Zuccato, C., Valenza, M. & Cattaneo, E. Molecular Mechanisms and Potential Therapeutical Targets in Huntington's Disease. Physiological Reviews 90, 905–981 (2010).
15. Squitieri, F. Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. Brain 126, 946–955 (2003).
16. Lo, D. Neurobiology of Huntington's Disease: Applications to Drug Discovery. (2011).
17. Harjes, P. & Wanker, E. E. The hunt for huntingtin function: interaction partners tell many different stories. Trends in Biochemical Sciences 28, 425–433 (2003).
18. Kim, M. W., Chelliah, Y., Kim, S. W., Otwinowski, Z. & Bezprozvanny, I. Secondary Structure of Huntingtin Amino-Terminal Region. Structure 17, 1205–1212 (2009).
19. Warby, S. C. et al. Phosphorylation of huntingtin reduces the accumulation of its nuclear fragments. Molecular and Cellular Neuroscience 40, 121–127 (2009).
20. Steffan, J. S. SUMO Modification of Huntingtin and Huntington's Disease Pathology. Science 304, 100–104 (2004).
21. Kalchman, M. A. et al. Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. J Biol Chem 271, 19385–19394 (1996).
22. Steffan, J. S. et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature 413, 739–743 (2001).
23. Jeong, H. et al. Acetylation Targets Mutant Huntingtin to Autophagosomes for Degradation. Cell 137, 60–72 (2009).
24. Yanai, A. et al. Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. Nat Neurosci 9, 824–831 (2006).
25. Jones, L. & Hughes, A. Pathogenic Mechanisms in Huntington's Disease. International Review of Neurobiology 98, 373–418 (Elsevier Inc.: 2011).
26. Ratovitski, T. et al. Mutant huntingtin N-terminal fragments of specific size mediate aggregation and toxicity in neuronal cells. J Biol Chem 284, 10855–10867 (2009).
27. Cowan, C. M. & Raymond, L. A. Selective Neuronal Degeneration in Huntington's Disease. Current Topics in Developmental Biology 75, 25–71 (2006).
28. Mangiarini, L., Sathasivam, K., Seller, M. & Cozens, B. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell (1996).
29. Slow, E. J. et al. Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. Proc Natl Acad Sci USA 102, 11402–11407 (2005).
30. Gray, M. et al. Full-Length Human Mutant Huntingtin with a Stable Polyglutamine Repeat Can Elicit Progressive and Selective Neuropathogenesis in BACHD Mice. Journal of Neuroscience 28, 6182–6195 (2008).
31. Menalled, L. B. et al. Early motor dysfunction and striosomal distribution of huntingtin microaggregates in Huntington's disease knock-in mice. Journal of Neuroscience 22, 8266–8276 (2002).
32. Lin, C. & Tallaksen-Greene, S. Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10, 137–144 (2001).
33. Lo, D., Hughes, R. & Hersch, S. Biomarkers to Enable the Development of Neuroprotective Therapies for Huntington's Disease. (2011).
34. Stack, E. C. et al. Neuroprotective Effects of Synaptic Modulation in Huntington's Disease R6/2 Mice. J Neurosci 27, 12908–12915 (2007).
35. Kim, J., Bordiuk, O. L. & Ferrante, R. J. Experimental Models of HD and Reflection on Therapeutic Strategies. International Review of Neurobiology 98, 419–481 (Elsevier Inc.: 2011).
36. Orr, H. T. & Zoghbi, H. Y. Trinucleotide Repeat Disorders. Annu Rev Neurosci 30, 575–621 (2007).
37. Wolfe, K. J. & Cyr, D. M. Amyloid in neurodegenerative diseases: Friend or foe? Seminars in Cell and Developmental Biology 22, 476–481 (2011).
38. Spires, T. L. & Hannan, A. J. Nature, nurture and neurology: gene-environment interactions in neurodegenerative disease. FEBS Journal 272, 2347–2361 (2005).
39. van Dellen, A., Blakemore, C., Deacon, R. & York, D. Delaying the onset of Huntington's in mice. Nature (2000).
40. Carter, R. J., Hunt, M. J. & Morton, A. J. Environmental stimulation increases survival in mice transgenic for exon 1 of the Huntington's disease gene. Mov. Disord. 15, 925–937 (2000).
41. Hockly, E. et al. Environmental enrichment slows disease progression in R6/2 Huntington's disease mice. Ann Neurol. 51, 235–242 (2002).
42. Wood, N. I., Glynn, D. & Morton, A. J. "Brain training" improves cognitive performance and survival in a transgenic mouse model of Huntington's disease. Neurobiol Dis 42, 427–437 (2011).
43. Turner, C. A. & Lewis, M. H. Environmental enrichment: effects on stereotyped behavior and neurotrophin levels. Physiol. Behav. 80, 259–266 (2003).
44. van Praag, H., Kempermann, G. & Gage, F. H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2, 266–270 (1999).
45. Laviola, G., Hannan, A. J., Macrì, S., Solinas, M. & Jaber, M. Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol Dis 31, 159–168 (2008).
46. Zuccato, C. Loss of Huntingtin-Mediated BDNF Gene Transcription in Huntington's Disease. Science 293, 493–498 (2001).
47. Ciammola, A. et al. Low brain-derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. Am. J. Med.

Genet. 144B, 574–577 (2007).
48. Zuccato, C. et al. RESEARCH ARTICLE: Systematic Assessment of BDNF and Its Receptor Levels in Human Cortices Affected by Huntington's Disease. Brain Pathology 18, 225–238 (2007).
49. Spires, T. L. Environmental Enrichment Rescues Protein Deficits in a Mouse Model of Huntington's Disease, Indicating a Possible Disease Mechanism. J Neurosci 24, 2270–2276 (2004).
50. Huang, E. J. & Reichardt, L. F. Neurotrophins: Roles in Neuronal Development and Function. Annu Rev Neurosci 24, 677–736 (2001).
51. Saylor, A. J. & McGinty, J. F. An intrastriatal brain-derived neurotrophic factor infusion restores striatal gene expression in Bdnf heterozygous mice. Brain Struct Funct 215, 97–104 (2010).
52. Altar, C. A. et al. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 389, 856–860 (1997).
53. Gauthier, L. R. et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 118, 127–138 (2004).Groundbreaking exploration of the role of HTT in vesicular transport.
54. Shimojo, M. Huntingtin Regulates RE1-silencing Transcription Factor/Neuron-restrictive Silencer Factor (REST/NRSF) Nuclear Trafficking Indirectly through a Complex with REST/ …. Journal of Biological Chemistry (2008).
55. Zuccato, C. et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35, 76–83 (2003).
56. Canals, J. M. Brain-Derived Neurotrophic Factor Regulates the Onset and Severity of Motor Dysfunction Associated with Enkephalinergic Neuronal Degeneration in Huntington's Disease. J Neurosci 24, 7727–7739 (2004).
57. Gharami, K., Xie, Y., An, J. J., Tonegawa, S. & Xu, B. Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice. Journal of Neurochemistry 105, 369–379 (2008).
58. Wu, C.-L., Hwang, C.-S., Chen, S.-D., Yin, J.-H. & Yang, D.-I. Neuroprotective mechanisms of brain-derived neurotrophic factor against 3-nitropropionic acid toxicity: therapeutic implications for Huntington's disease. Annals of the New York Academy of Sciences 1201, 8–12 (2010).
59. Hurlbert, M. S. et al. Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. Diabetes 48, 649–651 (1999).
60. Farrer, L., Yu, P. & Opitz, J. Anthropometric discrimination among affected, at risk, and not at risk individuals in families with Huntington disease. American journal of Medical Genetics 21, 307–316 (1985).
61. Pratley, R. E., Salbe, A. D., Ravussin, E. & Caviness, J. N. Higher sedentary energy expenditure in patients with Huntington's disease. Ann Neurol. 47, 64–70 (2000).
62. Goodman, A. O. G. & Barker, R. A. Body composition in premanifest Huntington's disease reveals lower bone density compared to controls. PLoS Curr 3, RRN1214 (2011).
63. Ahmad Aziz, N. et al. Leptin secretion rate increases with higher CAG repeat number in Huntington's disease patients. Clinical Endocrinology 73, 206–211 (2009).
64. Hult, S. et al. Mutant Huntingtin Causes Metabolic Imbalance by Disruption of Hypothalamic Neurocircuits. Cell Metabolism 13, 428–439 (2011).
65. Duan, W., Guo, Z., Jiang, H. & Ware, M. Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. Proceedings of the National Academy of Sciences 100, 2911–2916 (2003).
66. Bjorkqvist, M. Progressive alterations in the hypothalamic-pituitary-adrenal axis in the R6/2 transgenic mouse model of Huntington's disease. Hum Mol Genet 15, 1713–1721 (2006).
67. Clifford, J., Drago, J., Natoli, A. & Wong, J. Essential fatty acids given from conception prevent topographies of motor deficit in a transgenic model of Huntington's disease. Neuroscience (2002).
68. Vaddadi, K. S., Soosai, E., Chiu, E. & Dingjan, P. A randomised, placebo-controlled, double blind study of treatment of Huntington's disease with unsaturated fatty acids. Neuroreport 13, 29–33 (2002).
69. Miyazawa, D., Yasui, Y., Yamada, K., Ohara, N. & Okuyama, H. Regional differences of the mouse brain in response to an α-linolenic acid-restricted diet: Neurotrophin content and protein kinase activity. Life Sciences 87, 490–494 (2010).
70. Weindruch, R. The Retardation of Aging by Caloric Restriction: Studies in Rodents and Primates. Toxicologic Pathology 24, 742–745 (1996).
71. Steinkraus, K. A. et al. Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in Caenorhabditis elegans. Aging Cell 7, 394–404 (2008).
72. Martinez-Finley, E. J., Avila, D. S., Chakraborty, S. & Aschner, M. Insights from Caenorhabditis elegans on the role of metals in neurodegenerative diseases. Metallomics 3, 271 (2011).
73. Lin, M. T. & Beal, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443, 787–795 (2006).
74. Browne, S. E. et al. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol. 41, 646–653 (1997).
75. Damiano, M., Galvan, L., Déglon, N. & Brouillet, E. Mitochondria in Huntington's disease. BBA - Molecular Basis of Disease 1802, 52–61 (2010).
76. Beal, M. F. et al. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci 13, 4181–4192 (1993).
77. Brouillet, E. et al. Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. Journal of Neurochemistry 70, 794–805 (1998).
78. Napolitano, M. et al. Inhibition of mitochondrial complex II alters striatal expression of genes involved in glutamatergic and dopaminergic signaling: possible implications for Huntington's disease. Neurobiol Dis 15, 407–414 (2004).
79. Beal, M. F. Mitochondria take center stage in aging and neurodegeneration. Ann Neurol. 58, 495–505 (2005).
80. Goswami, A. et al. Oxidative stress promotes mutant huntingtin aggregation and mutant huntingtin-dependent cell death by mimicking proteasomal malfunction. Biochemical and Biophysical Research Communications 342, 184–190 (2006).
81. Yang, L. et al. Combination therapy with Coenzyme Q 10and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's Diseases. Journal of Neurochemistry 109, 1427–1439 (2009).
82. Dedeoglu, A. et al. Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. Journal of Neurochemistry 85, 1359–1367 (2003).

83. Migliore, L. & Coppedè, F. Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 667, 82–97 (2009).
84. Beal, M. F. Bioenergetic approaches for neuroprotection in Parkinson's disease. Ann Neurol. 53, S39–S48 (2003).
85. Tarohda, T., Yamamoto, M. & Amamo, R. Regional distribution of manganese, iron, copper, and zinc in the rat brain during development. Anal Bioanal Chem 380, 240–246 (2004).
86. Dexter, D. T. et al. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 114 (Pt 4), 1953–1975 (1991).
87. Bonilla, E. et al. Serum ferritin deficiency in Huntington's disease patients. Neurosci Lett 129, 22–24 (1991).
88. Fox, J. H. et al. Mechanisms of Copper Ion Mediated Huntington's Disease Progression. PLoS ONE 2, e334 (2007).
89. Firdaus, W. J. J. et al. Huntingtin inclusion bodies are iron-dependent centers of oxidative events. FEBS Journal 273, 5428–5441 (2006).
90. Tao, T. & Tartakoff, A. M. Nuclear relocation of normal huntingtin. Traffic 2, 385–394 (2001).
91. Gutekunst, C. A. et al. Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. Proc Natl Acad Sci USA 92, 8710–8714 (1995).
92. DiFiglia, M. et al. Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron 14, 1075–1081 (1995).
93. Velier, J. et al. Wild-type and mutant huntingtins function in vesicle trafficking in the secretory and endocytic pathways. Exp Neurol 152, 34–40 (1998).
94. Hoffner, G. & Kahlem, P. Perinuclear localization of huntingtin as a consequence of its binding to microtubules through an interaction with β-tubulin: relevance to Huntington's disease. J Cell Sci (2002).
95. Kegel, K. B. et al. Huntingtin associates with acidic phospholipids at the plasma membrane. J Biol Chem 280, 36464–36473 (2005).
96. Engelender, S. et al. Huntingtin-associated protein 1 (HAP1) interacts with the p150Glued subunit of dynactin. Hum Mol Genet 6, 2205–2212 (1997).
97. Li, S. H., Gutekunst, C. A., Hersch, S. M. & Li, X. J. Interaction of huntingtin-associated protein with dynactin P150Glued. J Neurosci 18, 1261–1269 (1998).
98. Qin, Z. H. Huntingtin Bodies Sequester Vesicle-Associated Proteins by a Polyproline-Dependent Interaction. Journal of Neuroscience 24, 269–281 (2004).
99. Hilditch-Maguire, P. et al. Huntingtin: an iron-regulated protein essential for normal nuclear and perinuclear organelles. Hum Mol Genet 9, 2789–2797 (2000).
100. Lumsden, A. L., Henshall, T. L., Dayan, S., Lardelli, M. T. & Richards, R. I. Huntingtin-deficient zebrafish exhibit defects in iron utilization and development. Hum Mol Genet 16, 1905–1920 (2007).
101. Gavin, C. E., Gunter, K. K. & Gunter, T. E. Manganese and calcium efflux kinetics in brain mitochondria. Relevance to manganese toxicity. Biochem. J. 266, 329–334 (1990).
102. Prohaska, J. Functions of trace elements in brain metabolism. Physiological Reviews (1987).
103. Bowman, A. B., Kwakye, G. F., Herrero Hernández, E. & Aschner, M. Role of manganese in neurodegenerative diseases. Journal of Trace Elements in Medicine and Biology (2011).doi:10.1016/j.jtemb.2011.08.144
104. Prabhakaran, K., Chapman, G. D. & Gunasekar, P. G. BNIP3 up-regulation and mitochondrial dysfunction in manganese-induced neurotoxicity. Neurotoxicology 30, 414–422 (2009).
105. Choo, Y. S. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 13, 1407–1420 (2004).
106. Tamm, C., Sabri, F. & Ceccatelli, S. Mitochondrial-Mediated Apoptosis in Neural Stem Cells Exposed to Manganese. Toxicol Sci 101, 310–320 (2008).
107. Wang, R.-G. & Zhu, X.-Z. Subtoxic concentration of manganese synergistically potentiates 1-methyl-4-phenylpyridinium-induced neurotoxicity in PC12 cells. Brain Res 961, 131–138 (2003).
108. Gorrell, J. & DiMonte, D. The role of the environment in Parkinson's disease. Environmental Health … (1996).
109. Racette, B. et al. Welding-related parkinsonism: clinical features, treatment, and pathophysiology. Neurology 56, 8–13 (2001).
110. Saxena, S. & Caroni, P. Selective Neuronal Vulnerability in Neurodegenerative Diseases: from Stressor Thresholds to Degeneration. Neuron 71, 35–48 (2011).
111. Williams, B. B. et al. Disease-toxicant screen reveals a neuroprotective interaction between Huntington's disease and manganese exposure. Journal of Neurochemistry 112, 227–237 (2010).
112. Williams, B. B. et al. Altered Manganese Homeostasis and Manganese Toxicity in a Huntington's Disease Striatal Cell Model Are Not Explained by Defects in the Iron Transport System. Toxicol Sci 117, 169–179 (2010).
113. Madison, J. L., Wegrzynowicz, M., Aschner, M. & Bowman, A. B. Gender and manganese exposure interactions on mouse striatal neuron morphology. Neurotoxicology 1–11 (2011).doi:10.1016/j.neuro.2011.05.007
114. Kwakye, G. Development of a Novel High Throughput Assay: Impaired Manganese Transport Kinetics and Homeostasis in Huntington's Disease. Dissertation, Vanderbilt University (2011).
115. Bowman, A. B., Kwakye, G. F., Hernández, E. H. & Aschner, M. Role of manganese in neurodegenerative diseases. Journal of Trace Elements in Medicine and Biology 1–13 (2011).doi:10.1016/j.jtemb.2011.08.144
116. Maciejewski, P. K. & Rothman, D. L. Proposed cycles for functional glutamate trafficking in synaptic neurotransmission. Neurochem. Int. 52, 809–825 (2008).
117. Milatovic, D. et al. Manganese Induces Oxidative Impairment in Cultured Rat Astrocytes. Toxicol Sci 98, 198–205 (2007).
118. Lobsiger, C. S. & Cleveland, D. W. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. Nat Neurosci 10, 1355–1360 (2007).
119. Behrens, P. F., Franz, P., Woodman, B., Lindenberg, K. S. & Landwehrmeyer, G. B. Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. Brain 125, 1908–1922 (2002).
120. Butterworth, J. Changes in nine enzyme markers for neurons, glia, and endothelial cells in agonal state and Huntington's disease caudate nucleus. Journal of Neurochemistry (1986).
121. Carter, C. J. Loss of glutamine synthetase activity in the brain in Huntington's disease. Lancet 1, 782–783 (1981).
122. Zelko, I. N., Mariani, T. J. & Folz, R. J. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1),

Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic. Biol. Med. 33, 337–349 (2002).
123. Kim, J. et al. Mitochondrial loss, dysfunction and altered dynamics in Huntington's disease. Hum Mol Genet 19, 3919–3935 (2010).
124. Ash, D. Structure and function of arginases. J. Nutr. (2004).
125. Estevez, A. G. Arginase 1 Regulation of Nitric Oxide Production Is Key to Survival of Trophic Factor-Deprived Motor Neurons. J Neurosci 26, 8512–8516 (2006).
126. Leopold, N. A. & Podolsky, S. Exaggerated growth hormone response to arginine infusion in Huntington's disease. J. Clin. Endocrinol. Metab. 41, 160–163 (1975).
127. Deckel, A. W. et al. Dietary arginine alters time of symptom onset in Huntington's disease transgenic mice. Brain Res 875, 187–195 (2000).
128. Scrutton, M. C., Utter, M. F. & Mildvan, A. S. Pyruvate carboxylase. VI. The presence of tightly bound manganese. J Biol Chem 241, 3480–3487 (1966).
129. Mildvan, A. S., Scrutton, M. C. & Utter, M. F. Pyruvate carboxylase. VII. A possible role for tightly bound manganese. J Biol Chem 241, 3488–3498 (1966).
130. Ciarmiello, A. et al. Brain white-matter volume loss and glucose hypometabolism precede the clinical symptoms of Huntington's disease. J. Nucl. Med. 47, 215–222 (2006).
131. Wozniak, E., Ołdziej, S. & Ciarkowski, J. Molecular modeling of the catalytic domain of serine/threonine phosphatase-1 with the Zn2+ and Mn2+ di-nuclear ion centers in the active site. Comput. Chem. 24, 381–390 (2000).
132. Ugolino, J., Fang, S., Kubisch, C. & Monteiro, M. J. Mutant Atp13a2 proteins involved in parkinsonism are degraded by ER-associated degradation and sensitize cells to ER-stress induced cell death. Hum Mol Genet 20, 3565–3577 (2011).
133. Tan, J. et al. Regulation of Intracellular Manganese Homeostasis by Kufor-Rakeb Syndrome-associated ATP13A2 Protein. Journal of Biological Chemistry 286, 29654–29662 (2011).
134. Gitler, A. D. et al. α-Synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nat Genet 41, 308–315 (2009). Description of the role of a novel Mn transporter in reducing a-synuclein toxicity and Mn toxicity in yeast, C. elegans and primary rodent neuronal cultures.
135. Goytain, A., Hines, R. M. & Quamme, G. A. Huntingtin-interacting Proteins, HIP14 and HIP14L, Mediate Dual Functions, Palmitoyl Acyltransferase and Mg2+ Transport. Journal of Biological Chemistry 283, 33365–33374 (2008). Experimental evidence for the interaction of HTT and HIP14 in palmitoylation and metal transport and localization to the golgi.
136. Ohyama, T. et al. Huntingtin-interacting protein 14, a palmitoyl transferase required for exocytosis and targeting of CSP to synaptic vesicles. The Journal of Cell Biology 179, 1481–1496 (2007).
137. Singaraja, R. R. et al. Altered palmitoylation and neuropathological deficits in mice lacking HIP14. Hum Mol Genet 20, 3899–3909 (2011). Description of HD-like phenotype in HIP14 -/- mouse and changes in palmitoylated HIP14 substrates.
138. van Dellen, A. & Hannan, A. J. Genetic and environmental factors in the pathogenesis of Huntington's disease. Neurogenetics 5, 9–17 (2004).
139. Belay, E. D. & Schonberger, L. B. THE PUBLIC HEALTH IMPACT OF PRION DISEASES 1. Annu. Rev. Public. Health. 26, 191–212 (2005).
140. Brazier, M., Davies, P., Player, E. & Marken, F. Manganese binding to the prion protein. Journal of Biological … (2008).
141. Martin, D. P. et al. Infectious prion protein alters manganese transport and neurotoxicity in a cell culture model of prion disease. Neurotoxicology 1–9 (2011).doi:10.1016/j.neuro.2011.07.008

Catecholamine Transporters: Differential Regulation by Insulin

Olga Dadalko

Abstract

Accumulating evidence supports growing appreciation for the prevalence of comorbidity of metabolic disorders and mental illness. Historically, pancreatic hormone insulin is considered to be one of the most important metabolic regulators in the body. Recently, it has also been shown that insulin signaling pathway is implicated in brain catecholamine homeostasis, perturbations of which manifest in many psychiatric disorders. Synaptic control of catecholamine neurotransmission is accomplished by an intricately regulated system of catecholamine reuptake, facilitated by dopamine (DA) and norepinephrine (NE) transporters (DAT and NET, respectively). Despite structural homology and functional similarity of DAT and NET, their dynamic regulation is transporter specific and cell context dependent. Thus, metabolic insulin signaling has been demonstrated to differentially regulate DAT and NET trafficking to control brain catecholamine neurotransmission.

Introduction

A sophisticated system of chemical neurotransmission between neurons enables the brain to control our physiology and behavior. Complex dynamics of the fast neuronal communication is modulated by slow-acting monoaminergic system[1]. Of particular interest here is catecholamine neurotransmission, which is essential for many brain functions such as learning, memory, attention, reward, mood, and stress[2,3]. Catecholamine signaling fidelity is maintained by transporter proteins, DAT and NET, which govern duration and magnitude of dopamine and norepinephrine neurotransmission by actively translocating catecholamines from the extracellular space into presynaptic neurons[4–6]. The essential role of DAT and NET is demonstrated by the adverse health consequences resulting from the polymorphisms in the human DAT and NET genes[7,8]. Also, transgenic mouse models lacking DAT or NET reveal phenotypes of aberrant brain physiology and severe behavioral alterations[9,10].

DAT and NET are expressed in their respective catecholaminergic neurons, which project throughout the brain from a few midbrain nuclei. The four major DA projections include the nigrostriatal, mesocortical, mesolimbic, and tuberoinfundibular pathways, while the locus coeruleus (LC) NE neurons innervate all brain regions[11]. DAT and NET belong to the solute carrier 6 (SLC6) gene family, which constitutes Na^+/Cl^- -dependent neurotransmitter-sodium symporters. These transporters utilize secondary active transport by coupling neurotransmitter reuptake with sodium gradient across the cellular plasma membrane. Cloning of NET[4] and DAT[6,12] revealed a high level of amino acid sequence homology between transmembrane domains and intracellular loops of the two transporter proteins. The predicted topological model of catecholamine transporters was later confirmed by high-resolution X-ray crystallographic structure of the bacterial leucine transporter (LeuT), a prokaryotic homolog of the SLC6 family that is structurally and functionally related to monoamine transporters[13]. Structural similarity between DAT and NET proteins may explain why the transporters are "promiscuous" for each other's neurotransmitters[3,5]. However, despite the fact that DAT and NET may substitute for each other in fulfilling their function[14], regulation of the two proteins is transporter-specific and depends on regional and cellular contexts. While highlighting general principles that control transporters' function, this review will specifically focus on how insulin signaling pathway exerts differential regulation of DAT and NET.

Potential mechanisms of transporter regulation

Transporter activity can be regulated by two distinct mechanisms: modulation of the intrinsic molecular properties and control of protein expression on the plasma membrane[2]. We will briefly discuss both potential mechanisms.

1) Transcription, translation, and anterograde trafficking to the plasma membrane are the fundamental processes that modulate transporter function[15]. However, regulatory checkpoints guiding these processes for transporters are not well understood. Only two NET and DAT transcription factors have been discovered: Phox2 and Nurr1. Overexpression of Phox2 and Nurr1 have been shown to elevate

mRNA and protein levels of NET[16] and DAT[17], respectively. Nonetheless, neither the mechanism, nor the upstream molecular regulators of Phox2 and Nurr1 have been identified.

Moreover, translation and anterograde trafficking of the transporters are also not fully understood. During protein synthesis, transporters are co-translationally translocated through the endoplasmic reticulum (ER) membrane[18]. Upon formation of oligomers in the ER, they are transported to the *cis*-Golgi network by COP (coatomer) II vesicles[19]. Oligomer formation was found to be essential for the ability of the transporters to exit the ER[20]. In order to move from the Golgi to the cell surface, both DAT and NET require N-glycosylation in the second extracellular loop[15]. Therefore, the mechanisms guiding transporters oligomerization and glycosylation indirectly regulate DAT and NET cell surface expression.

Thus, *de novo* protein synthesis, its half-life, as well as the rate of initial insertion of the transporters into the plasma membrane are essential processes controlling transporter function. Unfortunately, molecular machinery responsible for quality control of NET and DAT production as well as the mechanisms that guide sorting of the transporter proteins at the ER/Golgi interface and that govern anterograde transporter trafficking are not completely understood yet.

2) Immediate control over transporter function is maintained within the neuronal bouton *via* intraterminal trafficking and intrinsic protein modifications of NET and DAT. Initially, the transporters were thought to be the stagnant monitors of synaptic neurotransmitter concentration. Transporter conformation was thought to be the only determinant of the transient reuptake rate[2]. Understanding of the transporter regulation mechanism was propelled to a new level when cortical NET membrane expression was shown to be dependent on the extracellular norepinephrine concentration. This result suggested that neurons could control the rate of neurotransmitter reuptake by regulating the concentration of transporter proteins on the plasma membrane[21]. This regulatory method is slower than the rapid "on-site" modification of the intrinsic protein structure. However, transporters exhibit the slow kinetics of substrate translocation (approximately one substrate molecule per second per transporter)[3]. Thus, speedy intraterminal transporter trafficking to and from the plasma membrane in response to immediate external stimuli (such as changes in extracellular neurotransmitter concentration) is a plausible method to control the rate of catecholamine reuptake.

Endocytosis as a means of transporter function regulation

The transporter membrane availability is supported by local protein trafficking to and from the plasma membrane *via* exocytic and endocytic processes[20,22–24]. The process of endocytic recycling is guided by a number of different molecular mediators that maintain specificity of endosomal compartments and control the process of endosomal maturation. Endosomal regulators define the fate of the cargo – whether the endocytosed proteins recycle back to the membrane or undergo lysosomal degradation. Endosomal differentiation, mediated in large by Rab GTPases, allows for temporal and spatial segregation of the recycled cargo[25]. Rab GTPases provide organelle identity markers and serve as multifaceted organizers of nearly all membrane trafficking processes in eukaryotic cells. The array of proteins associated with Rab GTPases (such as guanine-nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), GDP dissociation inhibitors (GDIs), and GDI displacement factors (GDFs)) help to maintain the multi-level regulation system that allows precise control over the movement and longevity of endocytosed proteins[26].

Recent efforts have identified a few Rab GTPases involved in NET and DAT trafficking[23,24,27,28]. As excellent endosomal identity markers, Rab GTPases can reveal which endosomal pathway is utilized during transporters intraterminal redistribution. Various Rab GTPases are differentially involved in early and late recycling endosomes, in mature endosomes, and in lysosomal compartments[25,26]. Unraveling the sequence in which Rab proteins co-localize with translocating transporters will help understanding the timeline of trafficking events, as well as the fate of the transporter proteins during those events. Thus, analysis of NET anterograde transport allowed to exclude the possibility of NET segregation to either small or large dense core vesicles[23]. This suggested that endosomes could be involved in NET trafficking. Indeed, studies conducted in the superior cervical ganglion (SCG) nerve terminals showed co-localization of NET with Rab4 and Rab11 (recycling endosome markers)[24]. Based on the special case of amphetamine (AMPH)[a] induced NET trafficking, this seminal research provides grounds for further investigations aiming to understand the mechanisms behind intraterminal transporter trafficking.

SCG neurons elaborate profuse noradrenergic fibers in culture and present large terminals extending laterally from axonal membranes[23]. This makes SCG a convenient endogenous experimental model to study NET function.

a. **Amphetamine (AMPH)** - sympathomimetic drug inducing monoamine release from the nerve terminals into the extracellular milieu.

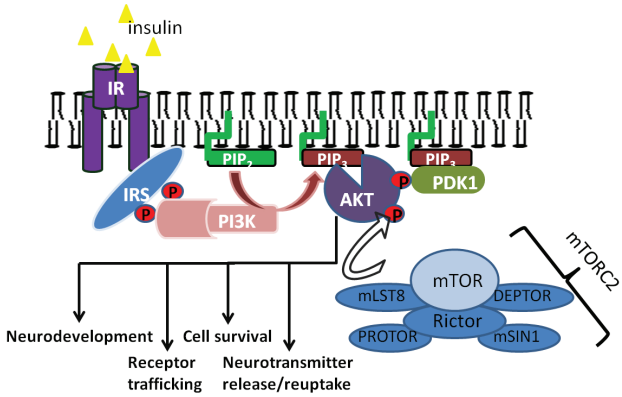


Figure 1: Insulin signaling pathway in the brain

Absence of similar experiment-friendly natural dopaminergic model system forces researchers to study DAT trafficking in heterologous expression systems. Studying constitutive and PKC-induced DAT internalization revealed co-localization of DAT with Rab11 and Rab5, respectively[28]. Interestingly, Rab5 may be substituted for Rab7 during the course of endosome maturation. This switch is a well-known trigger for endosome fusion with a lysosome, signifying a degrading pathway[25]. However, it has not been determined whether DAT trafficking undertakes this molecular route.

In vitro evidence: insulin enhances DAT function and reduces NET function

Metabolic hormone insulin was shown to influence a broad spectrum of cellular function in the nervous system *via* PI3K (phosphatidylinositol 3-kinase) / Akt signaling pathway[29] (Figure 1). Importantly, catecholamine transporters function was also found to be dependent on the integrity of the PI3K-Akt pathway, the main molecular players of which are briefly described here. Upon ligand binding, insulin receptor (IR) is autophosphorylated on its intracellular tyrosine residues, an essential step in the activation cascade. Activated IR is a tyrosine kinase (RTK), which binds and phosphorylates scaffold protein insulin receptor substrate (IRS). The downstream cascade is generated through signaling complexes that are assembled around the tyrosine-phosphorylated IRS[30]. PI3K is a lipid kinase that gets recruited to the activated IRS and converts phosphatidyl-inositol into phosphoinositide phosphates PIP_2 and PIP_3. PIP_2 and PIP_3 are "docking" lipids that trigger activation of serine/threonine kinases including 3-phosphoinoitide-dependent protein kinase-1 (PDK1) and Akt (also known as protein kinase B (PKB)) by recruiting them to the plasma membrane. Membrane-localized Akt is subsequently activated by phosphorylation at two key residues – Thr308 (by PDK1)[29], and Ser473 (by mammalian target of rapamycin complex 2 (mTORC2))[31]. Phosphorylated Akt is involved in multiple cellular functions, including metabolism, cell stress, cell-cycle, apoptosis, as well as regulation of protein synthesis and trafficking[32].

In vitro studies demonstrate that PI3K-Akt signaling differentially influences trafficking of catecholamine transporters. In case of the DAT, inhibition of the insulin signaling pathway was shown to rapidly decrease DAT function. Particularly, broad-spectrum tyrosine kinase inhibitors reduced DAT transport-associated currents, decreased DAT surface expression, and diminished DA uptake into DAT expressing *Xenopus* oocytes[33]. Brief application of PI3K inhibitors resulted in clathrin-mediated dynamin-dependent DAT endocytosis[34]. The effect was reversed with acute insulin treatment. Utilizing DAT-mediated DA releasing properties of AMPH[35,36], researchers were able to show that insulin signaling is required to maintain DAT cell surface expression, since application of PI3K inhibitors resulted in dramatic reduction of AMPH-induced DAT-mediated DA efflux in heterologous cells and dopaminergic neurons[37]. Later, *in vivo* studies confirmed these results[38].

Continuing to unravel the mechanism, researchers turned their attention to Akt, a serine/threonine protein kinase at the center of metabolic insulin signaling[32]. Expression of the dominant-negative Akt mutant or application of pharmacological Akt inhibitors induced a decrease in cell-surface expression of DAT, whereas a constitutively active form of Akt inhibited AMPH-induced DAT internalization[39]. Importantly, DAT trafficking effect was observed within minutes after stimulus application[34,37,39]. These data do not eliminate the possibility of intrinsic transporter modifications, which could occur prior to trafficking events. Further research is needed to understand whether insulin signaling has a direct role in the mechanism of maintaining DAT on the plasma membrane. Another important question is whether cytosol-redistributed DAT is capable of returning to the surface, i.e., which endosomal pathway – recycling or degrading - is employed during inhibition of insulin signaling.

In contrast with the DAT phenotype, decrease of the NET function was caused by activation of the insulin signaling pathway. Insulin application inhibited tritiated NE uptake in dissociated NET-expressing brain cells, whole brain synaptosomes, and in acute brain slices[40–43]. The mechanism behind such NET downregulation remains unknown. Interestingly, a later study conducted in the SK-N-SH cells (a human neuroblastoma cell line) demonstrated a contradicting result of elevated NET function upon insulin

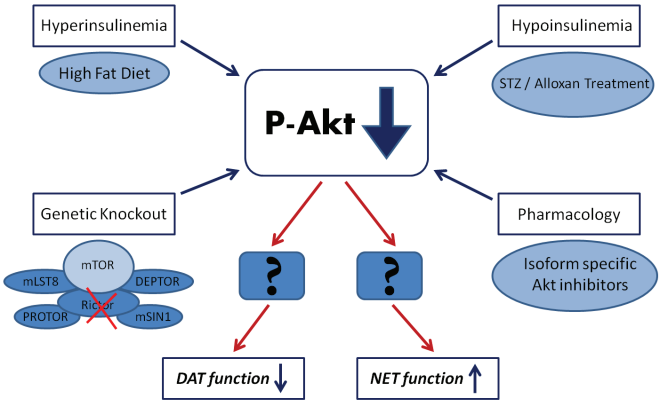


Figure 2: Multiple ways lead to disrupted insulin signaling and aberrant Akt activity

treatment[44]. Perhaps, a detailed analysis of the differences within the molecular machineries of the systems used will help deducing the occurrence of opposing results described above. This may bring us closer to understanding how insulin causes the opposite dynamics of two structurally and functionally similar transporters: DAT and NET.

Insulin signaling regulates the transporter function *in vivo*

a) Insulin in the brain: direct dependence on the peripheral insulin tone. The notion of insulin presence in the brain was controversial until 1967, when the use of sensitive radioimmunoassay techniques demonstrated not only that insulin is present in the cerebro-spinal fluid (CSF), but also that CSF levels are increased with peripheral insulin infusion[45]. Furthermore, IR is abundantly expressed in the brain, including dopaminergic and noradrenergic neurons[46]. Despite the ongoing debate on the source of brain insulin, the majority of evidence demonstrates that CNS insulin concentration depends on the fidelity of the active saturable transport of pancreatic insulin past the blood brain barrier[47–49]. Indeed, alterations in the plasma insulin concentration are mirrored by the changes in the CSF insulin level[50]. Human positron emission tomography (PET) studies showed attenuated neuronal activity evoked by a peripheral insulin injection in non-diabetic subjects with insulin resistance[51]. Such tight correlation between peripheral and central insulin tone supports the fact that alteration in plasma insulin level is capable of disrupting insulin signaling in the brain, which will consequently cause disturbance in catecholamine transporter function.

b) Pathophysiological alterations in the insulin signaling pathway and the animal models mimicking these alterations. Disruption in insulin signaling is commonly caused by persistent pathological alterations in the plasma insulin level known as hypo- or hyperinsulinemia. Both conditions result in inhibition of Akt phosphorylation[52,53] (Figure 2). In case of hypoinsulinemia, the lack of IR ligand shuts down the PI3K-Akt signaling cascade. In response to chronic hyperinsulinemia, cells develop insulin resistance by increasing degradation of IRS proteins[30,52] (Figure 1). Consequently, both hypo- and hyperinsulinemia disrupt Akt activity, leading to alteration of multiple intracellular functions, including transcription, protein synthesis and trafficking.

How can we induce alterations in peripheral insulin level in order to study its influence on the brain? In humans, hypoinsulinemia – a feature of type I diabetes mellitus – results from disrupted production of insulin by the pancreatic β cells[54]. To mimic this disease in an animal model, rodents are injected with streptozotocin (STZ) or alloxan – drugs that selectively destroy the pancreatic β cells. Hyperinsulinemia is a hallmark of an array of metabolic disturbances in humans, such as metabolic syndrome, obesity, and type II diabetes mellitus, all of which feature various levels of insulin resistance. An animal model of hyperinsulinemia may be created by feeding rodents with high fat diet. Both hypo- and hyperinsulinemic animal models are used to study how the disturbance in the insulin signaling pathway influences cellular physiology. Molecularly, both models converge on the downregulation of the Akt phosphorylation and activity (Figure 2). As discussed above, peripheral and central insulin tone are tightly interconnected, making hypo- and hyperinsulinemic animal models a good platform to study how disrupted insulin signaling is implicated in neurophysiology.

Perturbed insulin signaling *in vivo* causes aberrant DAT and NET cell surface expression

a) Disrupted insulin signaling causes DAT function downregulation. The evidence that insulin signaling may regulate catecholamine homeostasis was initially obtained from the STZ or alloxan-treated hypoinsulinemic rodents. AMPH exerts its psychostimulant action via DAT-mediated DA efflux; thus, the effect of AMPH is highly dependent on the DAT plasma membrane availability. Alloxan-treated rats demonstrated diminished locomotor activity and stereotyped behavior following AMPH administration. Importantly, the attenuated behavior was reversed by subsequent insulin treatment[55]. Such reduced response to AMPH in hypoinsulinemic animals suggested that basal insulin signaling is critical for appropriate DAT cell surface expression. Subsequent research demonstrated the ability of insulin to specifically regulate DAT plasma membrane availability. The direct assessment of striatal DAT plasma membrane expression in STZ-pretreated hypoinsulinemic rats showed reduced surface DAT[38]. *In vivo* chronoamperometric recordings in hypoinsulinemic animals demonstrated decreased striatal DA clearance, which signified of the reduced DAT cell surface

expression[56,57]. Importantly, acute insulin application rescued this phenotype. Moreover, high fat-fed insulin resistant rats were found to exhibit downregulation of the striatal DAT function that was rescued *via* restoration of the nigrostriatal Akt phosphorylation by the recombinant viral vector expression technology[58]. These findings demonstrated the plasticity of the system and showed that insulin acts rapidly *via* the PI3K/Akt pathway to regulate DAT function. However, it was also demonstrated that DAT mRNA in the ventral tegmental area (VTA) and substantia nigra (SN) regions was reduced in STZ-treated rats compared to control animals[59]. Such multifaceted evidence underlines the level of complexity, as well as the diversity of the mechanisms involved in insulin regulation of DAT. Further studies will allow deducing what external factors lead to the divergence in regulation: whether it occurs at the level of transcription or at the level of transient intraterminal protein trafficking.

*b) Disrupted insulin signaling causes NET function upregulation***.** As mentioned before, alterations in the insulin signaling pathway cause opposing dynamics for NET and DAT function. Using *in vivo* microdialysis, Shimizu et al showed reduced hypothalamic extracellular NE content in freely moving hypoinsulinemic rats[60]. In line with this finding, an increase in NE tissue content (an assessment of the intracellular neurotransmitter concentration) in the hypothalamus was also found in hypoinsulinemic animals[61]. With no significant changes in NE metabolites, these data supports the fact that altered NE reuptake could be the driving force of such an imbalance between intra- and intercellular concentration of the neurotransmitter. Recently published evidence demonstrated that the hypoinsulinemic condition induced NET trafficking *via* the Akt signaling pathway. In particular, STZ-treated mice showed enhanced NE brain tissue content levels, increased NE clearance, and elevated NET cell surface expression, a phenotype that was recapitulated by pharmacological Akt inhibition[53]. An excellent illustration of the insulin signaling influence on NET function *via* the PI3K/Akt pathway was provided in a study which analyzed cortical NE homeostasis in a genetic mouse model with attenuated ability to phosphorylate Akt in neurons. Mice with aberrant neuronal Akt function exhibited increase in total and cell surface NET expression[14]. Earlier investigations demonstrated an increase in NET mRNA in the LC of STZ-treated rats[59]. Similarly to the DAT story, NET regulation by insulin may depend on other factors and, thus, occurs at different stages of the protein life time. Further research is needed in order to pinpoint influencing factors and understand the mechanism.

Concluding remarks

Catecholamine neurotransmission is essential for normal brain physiology. Given the importance of transporters in maintaining brain catecholamine homeostasis, substantial effort must be invested to enhance our knowledge of NET and DAT regulation. The studies described above provide strong evidence that metabolic dysfunction, induced by impaired insulin signaling, impacts brain catecholamine neurotransmission by altering transporter function. Insulin was shown to influence brain NE and DA homeostasis by dynamic regulation of DAT and NET *via* PI3K/Akt signaling. Importantly, activation of insulin signaling causes downregulation of NET and upregulation of DAT function. Thus, two structurally and functionally homologous transporters with affinity for each other's neurotransmitters are regulated differently by the insulin signaling pathway. This could be the consequence of the divergent amino acid sequence within transporters intracellular domains. Another plausible explanation for such difference in transporter regulation may be the unique regional and cellular contexts of DAT and NET. Initial studies show that Akt, a kinase in the center of metabolic insulin signaling pathway, plays the key role in transporter function regulation. Further studies are warranted in order for us to understand the mechanisms underlying the comorbidity of metabolic disorders and mental illness. Identification of the molecular players will lead to new therapeutic approaches and, hopefully, to prevention of mental illnesses manifested by aberrant catecholamine homeostasis.

References

1. Robbins TW, ArnstenAFT (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annual review of neuroscience 32:267-287.
2. Blakely RD, Bauman AL (2000). Biogenic amine transporters: regulation in flux. Current opinion in neurobiology 10:328-336.
3. Kristensen AS, Andersen J, Jorgensen TN, Sorensen L, Eriksen J, Loland CJ, Stromgaard K, Gether U (2011). SLC6 Neurotransmitter Transporters: Structure, Function, and Regulation. Pharmacological reviews 63:585-640.
4. Pacholczyk T, Blakely RD, Amara SG (1991). Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature 350:350-354.
5. Iversen LL (1971). Role of transmitter uptake mechanisms in synaptic neurotransmission. British journal of pharmacology 41:571-591.
6. Shimada S, Kitayama S, Lin CL, Patel A, Nanthakumar E, Gregor P, Kuhar M, Uhl G (1991). Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 254:576-578.
7. Hahn MK, Mazei-Robison MS, Blakely RD (2005). Single Nucleotide Polymorphisms in the Human Norepinephrine Transporter Gene Affect Expression, Trafficking, Antidepressant Interaction, and

Protein Kinase C Regulation. Neuroscience 68:457-466.
8. Mazei-Robison MS, Bowton E, Holy M, Schmudermaier M, Freissmuth M, Sitte HH, Galli A, Blakely RD (2008). Anomalous dopamine release associated with a human dopamine transporter coding variant. The Journal of neuroscience 28:7040-7046.
9. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606-612.
10. Xu F, Gainetdinov RR, Wetsel WC, Jones SR, Bohn LM, Miller GW, Wang YM, Caron MG (2000). Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. Nature neuroscience 3, 465-471.
11. Iversen L (2000). Neurotransmitter transporters: fruitful targets for CNS drug discovery. Molecular psychiatry 5, 357-362.
12. Kilty JE, Lorang D, Amara SG (1991). Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254, 578-579.
13. Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E (2005). Crystal structure of a bacterial homologue of Na+/Cl--dependent neurotransmitter transporters. Nature 437, 215-223.
14. Siuta MA, Robertson SD, Kocalis H, Saunders C, Gresch PJ, Khatri V, Shiota C, Kennedy JP, Lindsley CW, Daws LC, Polley DB, Veenstra-Vanderweele1 J, Stanwood GD, Magnuson MA, Niswender KD, Galli A (2010). Dysregulation of the norepinephrine transporter sustains cortical hypodopaminergia and schizophrenia-like behaviors in neuronal rictor null mice. PLoS biology 8(6): e1000393.
15. Zahniser NR, Sorkin A (2009). Trafficking of dopamine transporters in psychostimulant actions. Seminars in cell & developmental biology 20, 411-417.
16. Fan Y, Huang J, Duffourc M, Kao RL, Ordway GA, Huang R, Zhu MY (2011). Transcription factor Phox2 upregulates expression of norepinephrine transporter and dopamine β-hydroxylase in adult rat brains. Neuroscience 192, 37-53.
17. Sacchetti P, Mitchell TR, Granneman JG, Bannon MJ (2001). Nurr1 enhances transcription of the human dopamine transporter gene through a novel mechanism. Journal of neurochemistry 76, 1565-1572.
18. Hersch SM, Yi H, Heilman CJ, Edwards RH, Levey AI (1997). Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. The Journal of comparative neurology 388, 211-227.
19. Sitte HH, Farhan H, Javitch JA (2004). Sodium-dependent neurotransmitter transporters: oligomerization as a determinant of transporter function and trafficking. Molecular interventions 4, 38-47.
20. Sorkina T, Doolen S, Galperin E, Zahniser NR, Sorkin A (2003). Oligomerization of dopamine transporters visualized in living cells by fluorescence resonance energy transfer microscopy. The Journal of biological chemistry 278, 28274-28283.
21. Lee CM, Javitch JA, Snyder SH (1983). Recognition sites for norepinephrine uptake: regulation by neurotransmitter. Science 220, 626-629.
22. Melikian HE, Buckley KM (1999). Membrane trafficking regulates the activity of the human dopamine transporter. The Journal of neuroscience 19, 7699-7710.
23. Matthies HJG, Han Q, Shields A, Wright J, Moore JL, Winder DG, Galli A, Blakely RD (2009). Subcellular localization of the antidepressant-sensitive norepinephrine transporter. BMC neuroscience 10, 65.
24. Matthies HJG, Moore JL, Saunders C, Matthies DS, Lapierre LA, Goldenring JR, Blakely RD, Galli A (2010). Rab11 supports amphetamine-stimulated norepinephrine transporter trafficking. The Journal of neuroscience 30, 7863-7877.
25. Huotari J, Helenius A (2011). Endosome maturation. The EMBO Journal 30, 3481-3500.
26. Stenmark H (2009). Rab GTPases as coordinators of vesicle traffic. Nature reviews. Molecular cell biology 10, 513-525.
27. Navaroli DM, Navaroli DM, Stevens ZH, Uzelac Z, Gabriel L, King MJ, Lifshitz LM, Sitte HH, Melikian HE (2011). The Plasma Membrane-Associated GTPase Rin Interacts with the Dopamine Transporter and Is Required for Protein Kinase C-Regulated Dopamine Transporter Trafficking. Journal of Neuroscience 31, 13758-13770.
28. Furman CA, Lo CB, Stokes S, Esteban JA, Gnegy ME (2009). Rab 11 regulates constitutive dopamine transporter trafficking and function in N2A neuroblastoma cells. Neuroscience letters 463, 78-81.
29. van der Heide LP, Ramakers GMJ, Smidt MP (2006). Insulin signaling in the central nervous system: learning to survive. Progress in neurobiology 79, 205-221.
30. White MF (2002). IRS proteins and the common path to diabetes. American journal of physiology. Endocrinology and metabolism 283, E413-422.
31. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-1101 (2005).
32. Franke TF (2008). PI3K/Akt: getting it right matters. Oncogene 27, 6473-6488.
33. Doolen S, Zahniser NR (2001). Protein tyrosine kinase inhibitors alter human dopamine transporter activity in Xenopus oocytes. The Journal of pharmacology and experimental therapeutics 296, 931-938.
34. Carvelli L, Moron JA, Kahlig KM, Ferrer JV, Sen N, Lechleiter JD, Leeb-Lundberg LMF, Merrill G, Lafer EM, Ballou LM, Shippenberg TS, Javitch JA, Lin RZ, Galli A (2002). PI 3-kinase regulation of dopamine uptake. Journal of neurochemistry 81, 859-869.
35. Fischer JF, Cho AK (1979). Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. The Journal of pharmacology and experimental therapeutics 208, 203-209.
36. Sulzer D, Sonders MS, Poulsen NW, Galli, A (2005). Mechanisms of neurotransmitter release by amphetamines: a review. Progress in neurobiology 75, 406-433.
37. Lute BJ, Khoshbouei H, Saunders C, Sen N, Lin RZ, Javitch JA, Galli A (2008). PI3K signaling supports amphetamine-induced dopamine efflux. Biochemical and biophysical research communications 372, 656-661.
38. Williams JM, Owens WA, Turner GH, Saunders C, Dipace C, Blakely RD, France CP, Gore JC, Daws LC, Avison MJ, Galli A (2007). Hypoinsulinemia regulates amphetamine-induced reverse transport of dopamine. PLoS biology 5(10), e274.
This paper is the first to show that the blunted behavioral effect to AMPH observed in STZ-treated hypoinsulinemic animals results from the reduction in the DAT plasma membrane expression. The authors provide the first in vivo demonstration that hypoinsulinemia reduces AMPH-induced DA efflux in the dorsal striatum of STZ-treated rats via downregulation of the PI3K signaling pathway. Importantly, the group shows that the DAT phenotype resulted from the chronic STZ-induced depletion of insulin could

be rescued via local insulin application in vivo.
39. Garcia BG, Wei Y, Moron JA, Lin RZ, Javitch JA, Galli A (2005). Akt is essential for insulin modulation of amphetamine-induced human dopamine transporter cell-surface redistribution. Molecular pharmacology 68, 102-109.
40. Boyd FT, Clarke DW, Muther TF, Raizada MK (1985). Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. The Journal of biological chemistry 260, 15880-15884.
41. Boyd FT, Clarke DW, Raizada MK (1986). Insulin inhibits specific norepinephrine uptake in neuronal cultures from rat brain. Brain research 398, 1-5.
42. Raizada MK, Shemer J, Judkins JH, Clarke DW, Masters BA, LeRoith D (1988). Insulin receptors in the brain: structural and physiological characterization. Neurochemical research 13, 297-303.
43. Figlewicz DP, Bentson K, Ocrant I (1993). The effect of insulin on norepinephrine uptake by PC12 cells. Brain research bulletin 32, 425-431.
44. Apparsundaram S, Sung U, Price RD, Blakely RD (2001). Trafficking-dependent and -independent pathways of neurotransmitter transporter regulation differentially involving p38 mitogen-activated protein kinase revealed in studies of insulin modulation of norepinephrine transport in SK-N-SH cells. The Journal of pharmacology and experimental therapeutics 299, 666-677.
45. Margolis RU, Altszuler N (1967). Insulin in the cerebrospinal fluid. Nature 215, 1375-1376.
46. Schulingkamp RJ, Pagano TC, Hung D, Raffa RB (2000). Insulin receptors and insulin action in the brain: review and clinical implications. Neuroscience and biobehavioral reviews 24, 855-872.
47. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D (1992). Insulin in the brain: a hormonal regulator of energy balance. Endocrine reviews 13, 387-414.
48. Banks WA (2004). The source of cerebral insulin. European journal of pharmacology 490, 5-12.
49. Baura GD, Foster DM, Porte DJ, Kahn SE, Bergman RN, Cobelli C, Schwartz MW (1993). Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. The Journal of clinical investigation 92, 1824-1830.
50. Schwartz MW, Sipols A, Kahn SE, Lattemann DF, Taborsky GJJ, Bergman RN, Woods SC, Porte DJ (1990). Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. The American journal of physiology 259, E378-383.
51. Anthony K, Reed LJ, Dunn JT, Bingham E, Hopkins D, Marsden PK, Amiel SA (2006). Attenuation of Insulin-Evoked Responses in Brain Networks Controlling Appetite and Reward in Insulin Resistance. Neurology 55, 2986-2992.
52. Schinner S, Scherbaum WA, Bornstein SR, Barthel A (2005). Molecular mechanisms of insulin resistance. Diabetic medicine 22, 674-682.
53. Robertson SD, Matthies HJG, Owens WA, Sathananthan V, Christianson NSB, Kennedy JP, Lindsley GW, Daws LC, Galli A (2010). Insulin reveals Akt signaling as a novel regulator of norepinephrine transporter trafficking and norepinephrine homeostasis. The Journal of neuroscience 30, 11305-11316.
This paper is the first to reveal the implication of insulin/Akt signaling in the regulation of the NET function and surface expression. The authors provide strong in vitro and in vivo evidence to support the hypothesis that insulin inhibits NE uptake via reduction of the NET surface expression, which resolves the previous debate on this matter in the literature.
54. Courtney M, Pfeifer A, Al-Hasani K, Gjernes E, Vieira A, Ben-Othman N, Collombat P (2011). In vivo conversion of adult α-cells into β-like cells: a new research avenue in the context of type 1 diabetes. Diabetes, obesity & metabolism 13, 47-52.
55. Marshall JF (1978). Further analysis of the resistance of the diabetic rat to d-amphetamine. Pharmacology, biochemistry, and behavior 8, 281-286.
This paper was the first to demonstrate that alloxan-treated hypoinsulinemic rats exhibit robust resistance to the behavioral effects of AMPH. The author observed similarity in how diabetic animals and animals with lesioned brain catecholamine neurons react to AMPH. Based on this observation, the author proposed that diabetic animals may have aberrant brain catecholaminergic signaling.
56. Owens WA, Sevak RJ, Galici R, Chang X, Javors MA, Galli A, France CP, Daws LC (2005). Deficits in dopamine clearance and locomotion in hypoinsulinemic rats unmask novel modulation of dopamine transporters by amphetamine. Insulin 1402-1410.
57. Sevak RJ, Koek W, Owens WA, Galli A, Daws LC, France CP (2008). Feeding conditions differentially affect the neurochemical and behavioral effects of dopaminergic drugs in male rats. European journal of pharmacology 592, 109-115.
58. Speed N, Saunders C, Davis AR, Owens WA, Matthies HJ, Saadat S, Kennedy JP, Vaughan RA, Neve RL, Lindsley CW, Russo SJ, Daws LC, Niswender KD, Galli A (2011). Impaired striatal Akt signaling disrupts dopamine homeostasis and increases feeding. PloS one 6, e25169.
59. Figlewicz DP, Brot MD, McCall AL, Szot P (1996). Diabetes causes differential changes in CNS noradrenergic and dopaminergic neurons in the rat: a molecular study. Brain research 736, 54-60.
This paper shows for the first time that endogenous insulin differentially regulates the catecholamine transporters: hypoinsulinemia reduced the DAT mRNA, and significantly elevated the NET mRNA in the brain of animals rendered diabetic via streptozotocin treatment. Importantly, assessment of the tyrosine hydroxylase mRNA, which was elevated in the locus coeruleus (noradrenergic site) and reduced in the substantia nigra (dopaminergic site), demonstrated the possible dependence of the insulin signaling on the brain region and cellular context.
60. Shimizu H (1991). Alteration in hypothalamic monoamine metabolism of freely moving diabetic rat. Neuroscience letters 131, 225-227.
61. Lacković Z, Salković M, Kuci Z, Relja M (1990). Effect of long-lasting diabetes mellitus on rat and human brain monoamines. Journal of neurochemistry 54, 143-147.

FGF8 Signaling In Brain Development: Ex Uno, Plures

Benjamin Jurrien Dean

Abstract

A steady stream of studies solidifies fibroblast-growth-factor-8 (fgf8) as a crucial mediator of regionalization in the developing vertebrate brain. Secreted from secondary organizers within the forebrain, midbrain and hindbrain, FGF8 is implicated in patterning, proliferation, specification, migration, differentiation and axon guidance. How can a single molecule drive this diverse array of developmental processes in tight or overlapping spatial and temporal contexts? Signal diversification arises from isoform-specific deployment of FGF8 and its receptors which in turn activate several intracellular pathways each subject to negative feedback modulation. This review will summarize the multiple roles of FGF8 and begin to pull together two decades of work on these diversifying mechanisms. In place of a more conventional understanding of FGF8 as a broadly acting morphogen, this body of work motivates a new conceptualization of FGF8 as a nimble component of several isoform-specific ligand-receptor-pathway axes which guide different aspects of vertebrate brain development.

Keywords

Body patterning
Brain development
FGF8
Alternative splicing
CNS

Introduction

Fibroblast growth factors (FGFs) are a class of growth factor proteins which play an integral role in vertebrate brain development. Of the many members of this large family, FGF8 has been a focus for its role in directing regionalization of neuroepithelium and subsequent specification of neural territories. FGF8 exerts its developmental influence as the primary ligand secreted from a handful of "secondary organizers" within the central nervous system (CNS) during and after neural tube closure[1]. The role of the secondary organizers is to define each brain region – forebrain, midbrain and hindbrain - laying a groundwork for the more particular developmental programs of each region. The two best characterized organizers are the anterior neural ridge (ANR) along the most rostral aspect of the forebrain and the midbrain-hindbrain boundary (MHB) between the developing midbrain and hindbrain. There are additional foci of expression in the dorsal diencephalon (DD) and ventral diencephalon (hypothalamus) (Figure 1A).

It has become clear that FGF8 regulates a wide variety of developmental programs in neural tissue. Newly identified roles for FGF8 are strikingly diverse and include regulation of anterior-posterior patterning, cell proliferation, cell specification, cell survival, axon guidance and hormone production (Figure 1B). These results challenge a classical conception of FGF8, and FGFs more broadly, as rather blunt and broadly acting mitogenic and morphogenic molecules and begs the question of how the FGF8 ligand can guide a remarkable variety of cellular programs in small subsets of cells within the developing CNS. What is the molecular basis for its signaling diversity?

There are clear signs that FGF8 employs a variety of methods to generate its multiplicity of functions including alternative splicing of *fgf8* and three of the four vertebrate FGF receptors (*fgfr*s)[2-6]. In addition, FGFs are capable of activating several different intracellular signaling cascades which in turn can induce a growing list of feedback inhibitors[7-8]. This paper will be concerned with reviewing and organizing the many known roles FGF8 signaling plays in CNS development. I will then discuss what is known about the diversification methods just mentioned. Ultimately, I will suggest that this exciting body of literature requires a reformulation for how we view FGF8 signaling in development – not as the effect of a master ligand, but as the function of specific ligand-receptor-pathway axes. While a modest reformulation, this mode of thought can more effectively guide future experimentation.

Many FGF8 functions

FGF8, like most FGFs, is an ER-Golgi secreted protein with strong affinity to heparin and heparan-like glycosaminoglycans (HLGAGs) of the ECM. FGF8 was first identified as a secreted molecule from an androgen--dependent mouse mammary carcinoma cell line[9]. Vertebrate species generate multiple isoforms through alternative splicing of four 5' exons (exons 1A, 1B, 1C and 1D). Exons 2 and 3 are conserved across all isoforms. To date, there are four human,

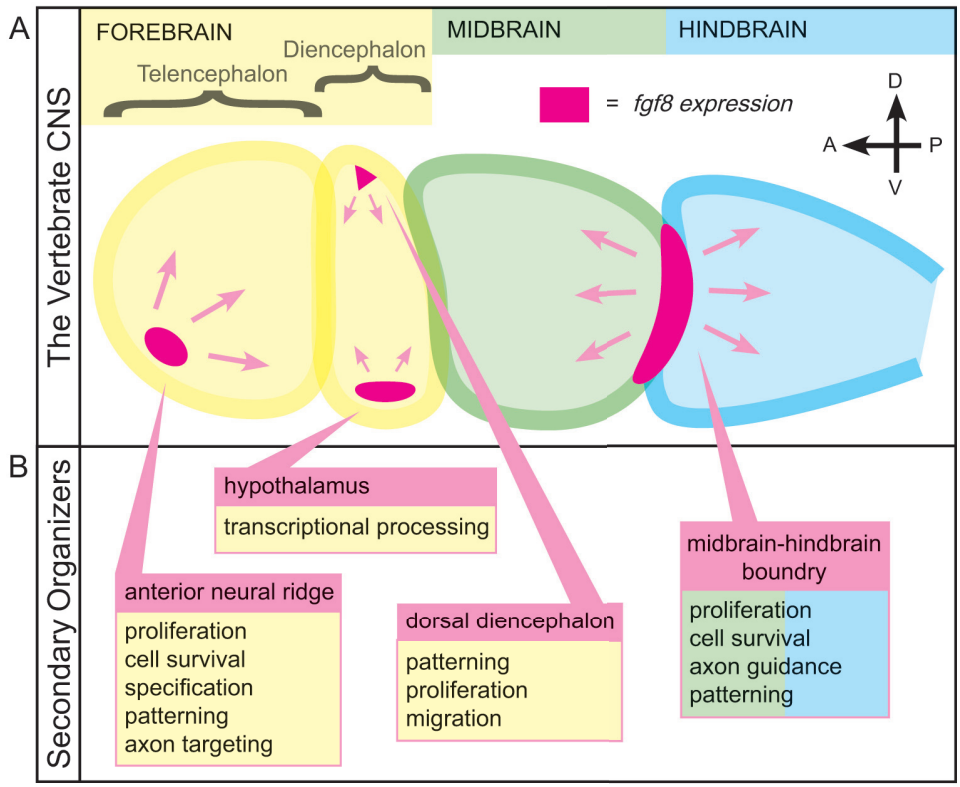


Figure 1. A schematic of the vertebrate CNS showing the three major divisions as well as the subdivisions of the forebrain. The four main secondary ogranizers expressing fgf8 are shown in pink. These expression domains arise during neurulation and persist for some time after that (A). Each organizer is labeled and the known functions of FGF8 for that organizer are listed below (B). Axes give anterior and posterior to the left and right respectively; dorsal and ventral are up and down respectively.

eight mouse, two chick and two fish isoforms[3,10-12] (Figure 2A). Once secreted, FGF8 binds to cellular membranes via a coordinated interaction between heparin/HLGAGs and one of four vertebrate FGFRs[13]. The binding interaction results in receptor dimerization and cross-phosphorylation of their intracellular domains initiating signal transduction[14].

The anterior neural ridge. In the mouse, the generation of an allelogenic mouse series has been incredibly illuminating to the study of FGF8[16].[a] Null alleles reveal the absolute requirement for FGF8 for proliferation as well as cell survival in the forebrain[7,17]. However, hypomorphs show normal proliferation and no ectopic cell death, thus no reduction in the size of the telencephalon[7]. Instead, there is a rostralization of expression of neocortical transcription factors suggesting a shift in the cortical identities of subregions[18]. The functional implications of the territorial shift within the telencephalon were explored further by *in utero* electroporation studies in mouse brain. Overexpression of *fgf8* in the ANR shifts neocortical subregional boundaries posteriorly. The addition of *fgf8* caudally leads to an ectopic S1 barrel field – an area of cortex which processes somatosensory information from the whiskers of the mouse[19]. Barrel fields normally receive thalamic inputs from the ventrobasal thalamic nuclei. In the case of *fgf8* overexpression, both endogenous and ectopic fields received thalamic innervation[20]. Together these results suggest proliferation and cell survival depend on basal levels of FGF8 while patterning events of the cortex are more dosage--dependent. How do the cells of the telencephalon as well as axon growth cones of the thalamic inputs interpret precise levels of FGF8 signal? The answer is still largely obscure.

FGF8's ability to drive specification is also evident in the ANR. The gonadotropin-releasing hormone (GnRH) neurons which drive sexual development derive from the ANR as a part of the olfactory placode. These endocrine cells then migrate to the hypothalamus. Without FGF8, GnRH precursors are not specified and no GnRH neurons populate the hypothalamus[15]. This crucial function of FGF8 underlies the pathophysiology of Kallman Syndrome – a combination of idiopathic hypogonadotropic hypogondism

a. This approach allows for the generation of an allelic series from a founder line. In this case, the founder contains an fgf8 knock-in with an intronic neomycin cassette which renders the allele hypomorphic. The construct also takes advantage of both cre and flp recombinase systems. Exons 2 and 3 are floxed and the neo cassette is frted allowing null mutations and wildtype rescues respectively to be deployed in a tissue specific manner by crossing the founder with desirable cre- or flp- transgeneic lines16.

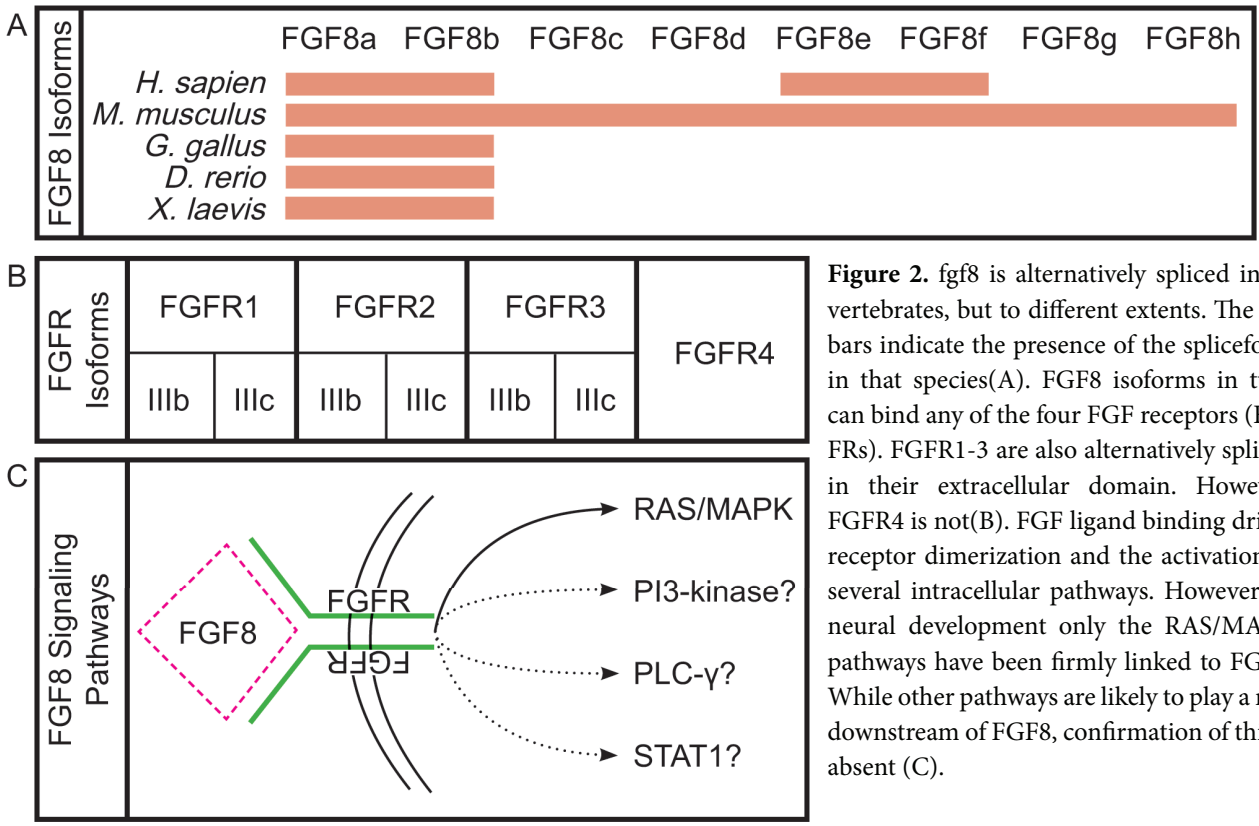


Figure 2. fgf8 is alternatively spliced in all vertebrates, but to different extents. The red bars indicate the presence of the spliceform in that species(A). FGF8 isoforms in turn can bind any of the four FGF receptors (FGFRs). FGFR1-3 are also alternatively spliced in their extracellular domain. However, FGFR4 is not(B). FGF ligand binding drives receptor dimerization and the activation of several intracellular pathways. However, in neural development only the RAS/MAPK pathways have been firmly linked to FGF8. While other pathways are likely to play a role downstream of FGF8, confirmation of this is absent (C).

and anosmia – the only known clinical outcome of human *fgf8* mutations.

The diencephalon. The multiple roles of FGF8 seen in the ANR are similar to the findings of Martinez-Ferre *et al* in the DD of mice[21]. Allelogenic analysis suggests FGF8 acts as "the master gene" for the DD. Formation of the dorsal structures (the pineal gland and habenular nuclei) relies on FGF8 in a dose-dependent manner. In this context, FGF8 regionalizes the DD by inhibiting posteriorizing Wnts, activating dorsalizing Wnts and stimulating proliferation. In contrast to the ANR, FGF8 does not contribute to cell survival, but does guide migration of some epithalamic neurons into the more ventral thalamus. So while some effects of FGF8 are the same as in the telencephalon, others are not.

To complicate the matter, the role of FGF8 in the DD of zebrafish is strikingly different. *fgf8* nulls retain intact DDs with a prominent pineal gland[22]. Martinez-Ferre *et al.* hypothesize that "master gene" *fgf8* expression in the DD began *de novo* in the vertebrate lineage allowing for the development of DD structures[21]. The presence of a pineal gland in zebrafish lacking FGF8 challenges this assertion. Instead, in the zebrafish, FGF8 is indispensable for the asymmetric migration of the parapineal, an accessory organ to the pineal[22]. This tension highlights the question of how FGF8 can direct proliferative and migratory cues differently across species or, in the case of mouse, simultaneously in tight spatial proximity.

More ventrally, oxytocin-producing cells derive from the ventricular zone before migrating to the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus[23]. Mice hypomorphic for FGF8 show a reduced number of cells in the PVN and SON positive for mature oxytocin yet a wildtype level of oxyphysin transcript, the oxytocin prohormone. In the oxytocin system, FGF8 takes on a role in regulating processing of the prohormone into the mature oxytocin molecule. This is a novel function for FGF8, but not unprecedented among other FGFs[24].

The midbrain-hindbrain boundary (MHB). The MHB is the most extensively characterized FGF8 signaling center. Also known as the isthmic organizer, it is crucial for the development of both the midbrain and hindbrain and sits along the border of these two regions (Figure 1A). As in the telencephalon, MHB-specific deletion of FGF8 leads quickly to increased cell death in both the midbrain and hindbrain

deleting the entire midbrain, the MHB and the cerebellum within the hindbrain. Interestingly, the cell death which produces the deletion occurs a full half day earlier in the midbrain than in the cerebellum[25].

Proliferation along the MHB is also FGF8 dependent. In exquisite work, high resolution microscopy reveals that FGF8 acts in a thin band along the basal aspect of the ventricular zone[26]. Here FGF8 signals through the basal processes of the neural progenitors to maintain proliferative divisions among the dividing progenitors. In the absence of FGF8, cells undergo neurogenic divisions and exit the cell cycle prematurely reducing the progenitor population.
Complementing axon targeting in the telencephalon, FGF8 directs axon outgrowth in the MHB. Midbrain dopaminergic neurons arise near the MHB and extend axons to innervate diencephalic and telencephalic targets. *In vitro* implantation of FGF8-soaked beads into whole-mouse embryo cultures formed ectopic MHBs and perturbed axon outgrowth of dopaminergic neurons leaving the endogenous MHB[27]. In this context, FGF8 was found to induce expression of the axon guidance cue *semaphorin3f* throughout the MHB. The semaphorin is then interpreted as a short-range chemorepellant by *neuropilin2* receptors on the dopaminergic axons. This result is of particular interest as the understanding of rostral-caudal axon guidance lags far behind that of dorsal-ventral guidance. It will be interesting to determine the mechanism by which FGF8 influences axons in the forebrain.

Generating signal diversity

We have seen that a single ligand, FGF8, expressed in a few secondary organizers in the developing CNS is able to execute a variety of cellular programs (Figure 1B). Work in the FGF8 field is uncovering how we go from the vague notion of a secondary organizer to a more nuanced understanding of FGF8 signaling in brain development.

Ligand splicing. fgf8 has multiple spliceforms across vertebrate species[3] (Figure 2A). These different isoforms have different transforming potentials on tumors, suggesting that if different isoforms are expressed in developing tissues they may have different effects[28]. Indeed, *in ovo* electroporation of *fgf8a* or *fgf8b* reveal that *fgf8a* transforms diencephalon into midbrain and expands midbrain, but only fgf8b can induce cerebellum in these tissues. Significantly, weaker overexpression of *fgf8b* yields an *fgf8a*-like phenotype suggesting that different effects of the isoforms are due to dosage as opposed to different molecular mechanisms[11]. However, more recent work challenges this conclusion. Overexpression of *fgf8a* via electroporation in chick does not phenocopy *fgf8b*'s transformative activity[14]. Complementing these overexpression studies, Guo *et al.* have built a genetic mouse model containing isoform-specific knockouts[29]. They find that only loss of FGF8b has any discernible effect on midbrain and cerebellar formation. Loss of FGF8a leads to no gross effect on these brain regions. This may warrant a closer look at FGF8a knockouts, but preliminarily reveals what overexpression experiments could not, fgf8a is dispensable for the bulk of MHB organizer activity.

This raises the question; can spliceforms ever play a simultaneous role? The possibility remains as crystal structure and biochemical analysis reveal a mechanism to explain the above described overexpression studies. A single phenylalanine at position 32 of the FGF8b isoform confers a significant difference in receptor binding[4]. Replacing the phenylalanine with an alanine converts the transformative ability of FGF8b to that of FGF8a, when electroporated into chick midbrains and murine midbrain explants. It will be very interesting to see if isoform-specific knockouts reveal simultaneous but unique requirements in other brain regions.

Receptor diversity. Another mechanism diversifying FGF8 signaling is the four FGF receptors and the alternative splicing of three of them[30] (Figure 2B). Indeed, FGFR1 alone mediates some FGF8 functions already discussed. FGFR1 is the FGF8 receptor for GnRH neuron specification and is crucial for some, but not all, MHB function in both fish and mouse[15, 31-32]. This indicates that various downstream effects of FGF8 at the MHB are mediated by different FGFRs. While investigating the differences between FGF8a and FGF8b, Olsen *et al.* also tested the association of FGF8 isoforms with the many receptor isoforms using *in vitro* surface plasmon resonance (SPR) to determine dissociation constants[4]. An alternative splicing event in the third immunoglobulin domain generates either a "b" or "c" isoform of FGFR1, 2 and 3. In all cases, the "c" isoform confers a greater affinity for FGF8b. This is due to a hydrophobic groove, exposed in the "c" isoform, that can more directly interact with the ligand phenylalanine at position 32 previously discussed[4]. These structural and *in vitro* results are not true in *in vivo* conditions, but nonetheless provide a possibility of alternative receptor splicing as a method to regulate ligand specificity. It remains to be seen if FGFR1 activity in the MHB is isoform-specific. It is exciting to imagine a suite of experiments combining isoform-specific knockouts of both ligand and receptors.

Signaling Pathways & negative feedback. Two additional layers of diversification in FGF8 signaling have become apparent recently, signaling cascade selectivity and negative-feedback modulators. FGFs have at least four separate signaling cascades they can activate; RAS/MAPK, PI3 kinase, PLC- and STAT1[7] (Figure 2C). There is an FGF8 isoform-specific relationship with some pathways. For example, FGF8b, but neither FGF8a nor low doses of FGF8b, activates the RAS/MAPK pathways along the MHB[33]. However, in ANR signaling, RAS/MAPK signaling persists in the absence of all FGF8 indicating that other signaling cascades are activated by FGF8 in the forebrain of zebrafish; which cascades is unclear[34].

Various cascades lead to activation of negative regulators of the FGF8 pathways; *sprouty*, *sef* and *mkp3* are principle among these[8]. Very recently, the negative regulators *Sprouty1* and *Sprouty2* have been shown to inhibit FGF8 rostralization in early cortical patterning, however, later only Sprouty2 shows a role by inhibiting the RAS/MAPK pathway in the telencephalic ventricular zone[35]. The mechanism and dependence on FGF8 of this switch are uncertain. Much more work must be done to understand which intracellular pathways are used to effect different FGF8 functions.

Conclusions and Future Directions.

We have seen studies connecting ligand isoforms to receptor isoforms, receptor isoforms to signaling pathways and signaling pathways to negative feedback regulators[4,33,35]. A clear direction forward is to begin to piece together these links to form a chain of developmental signaling. We must continue to find endogenous isoform-specific ligand-receptor pairs and begin to pare out which intracellular pathways as well as negative regulators are subsequently activated. In looking forward it may be helpful to begin to construct ligand-receptor-pathway axes as opposed to listing broad effects downstream of FGF8. The broad view does not reflect the evolutionary diversification of the components of FGF8 signaling so central to neurodevelopment. As work incrementally enriches our understanding of isoforms, cascades and feedback mechanisms, simpler axes may immerge from what otherwise appears to be a multifarious interaction network. More than just a tool to clear our heads, these hypothetical axes can guide experiments taking advantage of isoform-specific knockouts as well as signaling cascade and feedback regulator mutants.

References

1. Vieira, C., Pombero, A., García-Lopez, R., Gimeno, L., Echevarria, D., & Martínez, S. (2010). Molecular mechanisms controlling brain development: an overview of neuroepithelial secondary organizers. The International journal of developmental biology, 54(1), 7-20.

2. MacArthur, C. a, Shankar, D. B., & Shackleford, G. M. (1995). Fgf-8, activated by proviral insertion, cooperates with the Wnt-1 transgene in murine mammary tumorigenesis. Journal of virology, 69(4), 2501-7.

3. MacArthur, C. a, Lawshé, a, Xu, J., Santos-Ocampo, S., Heikinheimo, M., Chellaiah, a T., & Ornitz, D. M. (1995). FGF-8 isoforms activate receptor splice forms that are expressed in mesenchymal regions of mouse development. Development (Cambridge, England), 121(11), 3603-13.

4. Olsen, S. K., Li, J. Y. H., Bromleigh, C., Eliseenkova, A. V., Ibrahimi, O. A., Lao, Z., Zhang, F., et al. (2006). Structural basis by which alternative splicing modulates the organizer activity of FGF8 in the brain. Genes & development, 20(2), 185-98.

5. Kalyani, a J., Mujtaba, T., & Rao, M. S. (1999). Expression of EGF receptor and FGF receptor isoforms during neuroepithelial stem cell differentiation. Journal of neurobiology, 38(2), 207-24.

6. Ota, S., Tonou-Fujimori, N., & Yamasu, K. (2009). The roles of the FGF signal in zebrafish embryos analyzed using constitutive activation and dominant-negative suppression of different FGF receptors. Mechanisms of development, 126(1-2), 1-17.

7. Storm, E. E., Rubenstein, J. L. R., & Martin, G. R. (2003). Dosage of Fgf8 determines whether cell survival is positively or negatively regulated in the developing forebrain. Proceedings of the National Academy of Sciences of the United States of America, 100(4), 1757-62.

8. Echevarria, D., Belo, J. A., & Martinez, S. (2005). Modulation of Fgf8 activity during vertebrate brain development. Brain research. Brain research reviews, 49(2), 150-7.

9. Tanaka, a, Miyamoto, K., Minamino, N., Takeda, M., Sato, B., Matsuo, H., & Matsumoto, K. (1992). Cloning and characterization of an androgen-induced growth factor essential for the androgen-dependent growth of mouse mammary carcinoma cells. Proceedings of the National Academy of Sciences of the United States of America, 89(19), 8928-32.

10. Gemel, J., Gorry, M., Ehrlich, G. D., & MacArthur, C. A. (1996). Structure and sequence of human FGF8. Genomics, 35(1), 253-7.

11. Sato, T., Araki, I., & Nakamura, H. (2001). Inductive signal and tissue responsiveness defining the tectum and the cerebellum. Development (Cambridge, England), 128(13), 2461-9.

12. Inoue, F., Nagayoshi, S., Ota, S., Islam, M. E., Tonou-Fujimori, N., Odaira, Y., Kawakami, K., et al. (2006). Genomic organization, alternative splicing, and multiple regulatory regions of the zebrafish fgf8 gene. Development, growth & differentiation, 48(7), 447-62.

13. Mohammadi, M., Olsen, S. K., & Ibrahimi, O. a. (2005). Structural basis for fibroblast growth factor receptor activation. Cytokine & growth factor reviews, 16(2), 107-37.

14. Sunmonu, N. A., Li, K., & Li, J. Y. H. (2011). Numerous isoforms of Fgf8 reflect its multiple roles in the developing brain. Journal of cellular physiology, 226(7), 1722-6.

15. Chung, W. C. J., Matthews, T. A., Tata, B. K., & Tsai, P.-S. (2010). Compound deficiencies in multiple fibroblast growth factor

signalling components differentially impact the murine gonadotropin-releasing hormone system. Journal of neuroendocrinology, 22(8), 944-50.
16. Meyers, E. N., Lewandoski, M., & Martin, G. R. (1998). An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. Nature genetics, 18(2), 136-41.
17. Storm, E. E., Garel, S., Borello, U., Hebert, J. M., Martinez, S., McConnell, S. K., Martin, G. R., et al. (2006). Dose-dependent functions of Fgf8 in regulating telencephalic patterning centers. Development (Cambridge, England), 133(9), 1831-44.
18. Garel, S., Huffman, K. J., & Rubenstein, J. L. R. (2003). Molecular regionalization of the neocortex is disrupted in Fgf8 hypomorphic mutants. Development (Cambridge, England), 130(9), 1903-14.
19. Fukuchi-Shimogori, T., & Grove, E. a. (2001). Neocortex patterning by the secreted signaling molecule FGF8. Science (New York, N.Y.), 294(5544), 1071-4.
20. Shimogori, T., & Grove, E. a. (2005). Fibroblast growth factor 8 regulates neocortical guidance of area-specific thalamic innervation. The Journal of neuroscience : the official journal of the Society for Neuroscience, 25(28), 6550-60.
21. Martinez-Ferre, A., & Martinez, S. (2009). The development of the thalamic motor learning area is regulated by Fgf8 expression. The Journal of neuroscience : the official journal of the Society for Neuroscience, 29(42), 13389-400.
22. Regan, J. C., Concha, M. L., Roussigne, M., Russell, C., & Wilson, S. W. (2009). An Fgf8-dependent bistable cell migratory event establishes CNS asymmetry. Neuron, 61(1), 27-34.
23. Karim, M. a, & Sloper, J. C. (1980). Histogenesis of the supraoptic and paraventricular neurosecretory cells of the mouse hypothalamus. Journal of anatomy, 130(Pt 2), 341-7.
24. Brooks, L. R., Chung, W. C. J., & Tsai, P.-S. (2010). Abnormal hypothalamic oxytocin system in fibroblast growth factor 8-deficient mice. Endocrine, 38(2), 174-80.
25. Chi, C. L., Martinez, S., Wurst, W., & Martin, G. R. (2003). The isthmic organizer signal FGF8 is required for cell survival in the prospective midbrain and cerebellum. Development (Cambridge, England), 130(12), 2633-44.
26. Lahti, L., Saarimäki-Vire, J., Rita, H., & Partanen, J. (2010). FGF signaling gradient maintains symmetrical proliferative divisions of midbrain neuronal progenitors. Developmental biology, 349(2), 270-282.
27. Yamauchi, K., Mizushima, S., Tamada, A., Yamamoto, N., Takashima, S., & Murakami, F. (2009). FGF8 signaling regulates growth of midbrain dopaminergic axons by inducing semaphorin 3F. The Journal of neuroscience : the official journal of the Society for Neuroscience, 29(13), 4044-55.
28. MacArthur, C. a, Lawshé, a, Shankar, D. B., Heikinheimo, M., & Shackleford, G. M. (1995). FGF-8 isoforms differ in NIH3T3 cell transforming potential. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research, 6(7), 817-25.
29. Guo, Q., Li, K., Sunmonu, N. A., & Li, J. Y. H. (2010). Fgf8b-containing spliceforms, but not Fgf8a, are essential for Fgf8 function during development of the midbrain and cerebellum. Developmental biology, 338(2), 183-92.
30. Zhang, X., Ibrahimi, O. a, Olsen, S. K., Umemori, H., Mohammadi, M., & Ornitz, D. M. (2006). Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. The Journal of biological chemistry, 281(23), 15694-700.
31. Scholpp, S., Groth, C., Lohs, C., Lardelli, M., & Brand, M. (2004). Zebrafish fgfr1 is a member of the fgf8 synexpression group and is required for fgf8 signaling at the midbrain-hindbrain boundary. Development genes and evolution, 214(6), 285-95.
32. Trokovic, R., Trokovic, N., Hernesniemi, S., Pirvola, U., Vogt Weisenhorn, D. M., Rossant, J., McMahon, A. P., et al. (2003). FGFR1 is independently required in both developing mid- and hindbrain for sustained response to isthmic signals. The EMBO journal, 22(8), 1811-23.
33. Sato, T., & Nakamura, H. (2004). The Fgf8 signal causes cerebellar differentiation by activating the Ras-ERK signaling pathway. Development (Cambridge, England), 131(17), 4275-85.
34. Shinya, M., Koshida, S., Sawada, a, Kuroiwa, a, & Takeda, H. (2001). Fgf signaling through MAPK cascade is required for development of the subpallial telencephalon in zebrafish embryos. Development (Cambridge, England), 128(21), 4153-64.
35. Faedo, A., Borello, U., & Rubenstein, J. L. R. (2010). Repression of Fgf signaling by sprouty1-2 regulates cortical patterning in two distinct regions and times. The Journal of neuroscience : the official journal of the Society for Neuroscience, 30(11), 4015-23.

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Is a Picture Worth 1000 Calories: The Neuroimaging of Obesity

Kristen Eckstrand

Abstract

In healthy weight individuals, complex brain circuits interact with peripheral feeding signals to control feeding behavior, and it is thought that the dysregulation of these circuits can lead to excessive food intake and obesity. Human neuroimaging studies have shown BMI-dependent deficits in dopamine neurotransmission encoding reward, suggesting a "hypodopaminergic reward deficiency" whereby obese individuals overeat to compensate for a hypofunctioning reward circuitry. However, other imaging studies demonstrate hyperactivation of dopamine networks that positively correlate with BMI in obese individuals. Animal studies link these seemingly opposing theories, revealing that insulin promotes the intracellular trafficking and surface expression of the dopamine transporter (DAT) while inhibiting that of the norepinephrine transporter (NET). Together these transporters control dopamine levels in the striatum and cortex respectively, areas critically involved in reward, habits, and cognitive control. The purpose of this review is to integrate the molecular aspects of food overconsumption and obesity with human neuroimaging data, focusing on the role and dysregulation of dopamine in the neural circuits subserving food intake.

Keywords

Obesity
Insulin
Dopamine
Reward
Feeding behavior
Habits
Dopamine transporter
Response inhibition

An Introduction to the Obesity Epidemic

The fundamental neurocircuitry of the homeostatic feeding system and its interactions with peripheral feeding signals to modulate appetitive behavior and energy expenditure around a physiologic set point has maintained a relatively stable human body composition until only recently, when the prevalence of obesity has increased dramatically[1]. The rapid elevation in obesity over the past generation, with nearly seventy percent of the United States population meeting criteria for being overweight[2], suggests environmental factors play a key role. Current research indicates the presence and dysfunction of expanded neural circuits controlling reward, habits, and decision-making may mediate feeding behavior and subsequent overconsumption, contributing to the obesity epidemic[3-7].

Animal research has been critical for elucidating molecular aspects of obesity, with studies showing that food overconsumption is both driven and paralleled by broad changes in dopaminergic circuitry. Indeed, an overarching question in the field is how the physiologic response to food consumption augments brain dopaminergic circuits that enable the progression and maintenance of obesity. Neuroimaging is an important and novel tool for non-invasively examining the structural, molecular, and functional correlates of obesity[8]. The purpose of this review is to integrate the molecular aspects of food overconsumption and obesity with human neuroimaging data, focusing on the role and dysregulation of dopamine in the neural circuits subserving food intake.

Molecular Aspects of Dopamine in Obesity

Homeostatic Feeding, Dopamine, Reward

The hypothalamus regulates homeostatic feeding (i.e. food consumption for the purpose of maintaining energy balance; for review, see[1, 9, 10]), by responding to peripheral hormonal signals relaying information about the body's energy state[11, 12]. The anorexigenic gut peptides leptin and insulin, negative feedback adiposity signals circulating in proportion to body fat mass, indicate a positive energy balance while the orexigenic gut peptide ghrelin, whose levels inversely correlate with adiposity, signals a negative energy balance. In addition to their homeostatic action in the hypothalamus to regulate future feeding behavior, these peripheral hormonal signals also act on the mesolimbic dopamine system. Activity in mesolimbic reward circuitry (for review, see [13]), an area that is acutely activated with all drugs of abuse[14], implies that feeding signals operate outside of brain circuits subserving homeostatic feeding and that feeding itself may have rewarding properties.

Current evidence suggests that gut peptides signaling a positive energy balance function to negatively modulate midbrain dopamine (DA) neurotransmission and food

reward while those signaling a negative energy balance are positive DA modulators. For example, as determined by both electrophysiology and receptor knockout studies, leptin acts directly on the DA neurons of the ventral tegmental area (VTA) to inhibit action potential firing[15, 16] and reduce food intake[15] and reward-seeking behaviors[17, 18]. In contrast, ghrelin activates VTA DA neurons, triggering feeding[19]. New research points to a critical role for insulin in the regulation of reward circuitry. Insulin promotes the intracellular trafficking and surface expression of the dopamine transporter (DAT) via the PI3K/Akt signaling pathway, regulating the high-affinity uptake of dopamine from the mesolimbic synapse[20-23] while reducing food-intake[17]. Further, dopamine receptor (D2R) expression[24] is impaired in insulin-depleted states, suggesting a hypofunctioning of the dopamine reward system with insulin resistance. These findings together demonstrate the role of peripheral feeding signals, particularly insulin, in fine-tuning extracellular synaptic dopamine in the reward circuitry and subsequently influencing feeding behavior.

An understanding of mesolimbic dopamine's function and behavioral correlates is critical for discerning the role of dopamine dysregulation in obesity. In the mesolimbic circuitry, dopamine encodes the expectation of, motivation for, and approach behaviors seeking reward[13, 25, 26], all processes which are "hijacked" in the early stages of addiction[14, 27]. Consistent with dopamine's role in reward, dopamine levels are elevated during food seeking[28, 29], exposure to and consumption of novel food stimuli[30, 31], and daily intermittent consumption of both sugar[32-34] and fat[35, 36]. Further, it is the phasic firing of these dopamine neurons that encodes this food reward[26, 37-39]. In contrast, evoked dopamine release, basal dopamine levels[35, 40, 41], and D2R availability[24, 42] are blunted in chronic obesity. One study links these two states, demonstrating increased basal DA and DA efflux in obesity-prone young, insulin-sensitive rats in the mesolimbic reward system but decreased basal DA and DA efflux in obesity-prone, adult, insulin-resistant rats[43]. These results, combined with evidence that short-term elevations in insulin or glucose increase basal DA[44] while decreasing D2R[42, 45], provides evidence for the progressive nature of dopamine dysregulation in obesity.

According to the dopamine reward hypothesis, dopamine signaling in the mesolimbic system encodes reward and promotes reward-seeking behavior; consequently, impaired dopamine signaling will focus and drive behaviors aimed at restoring dopamine tone[6, 46]. It is hypothesized that the blunted dopamine signaling in obesity may attenuate the rewarding aspects of food, a hypodopaminergic reward deficiency syndrome (HRDS), leading obese individuals to consume increasing quantities of palatable food to achieve the same level of reward[41, 47]. A problem with this "reward deficiency" hypothesis, however, is that decreased perceived reward might be expected to suppress rather than promote excessive feeding. An alternative view is that reduced dopamine receptor availability may be a consequence, rather than a cause, of obesity due to elevated dopamine levels from excessive food intake and/or abnormal food seeking[4, 5, 28-31, 37]. Several studies have, in fact, demonstrated a hyperresponsiveness to reward in the mesolimbic circuitry in obesity[5, 48, 49] corroborating this hypothesis. Indeed, dysregulation of dopamine circuitry is a clear component of obesity, but the exact nature of the dysregulation remains undefined.

Food-Seeking, Habits, and Addiction

Despite mesolimbic dopamine having a clear role in reward and feeding behavior, studies in dopamine deficient mice (a severely hypoactive phenotype which will die of starvation without supplemented dopamine) show that viral restoration of dopamine to the nucleus accumbens does not restore feeding behavior[6, 50]. However, restoration of dopamine to the dorsal striatum, specifically the dorsolateral striatum, rescues the dopamine-deficient phenotype and induces feeding[51-53]. These results suggest a role for dopamine action outside the mesolimbic reward system in feeding behavior. In fact, it is the dorsal striatum that mediates goal-directed behaviors and habit formation such as the repeated seeking of reward-conditioned, highly salient, food stimuli[54-56].

Habits are "sequential, repetitive, motor, or cognitive behaviors elicited by external or internal triggers that, once released, can go to completion without conscious oversight"[55]. Habits begin as goal-directed behaviors, where a salient[57] stimulus is achieved through a specific action sequence, but progress to cue-mediated behaviors with repeated reward training that persist even with reward devaluation[58, 59]. This progression involves an underlying ventral-to-dorsal striatal shift[14, 55, 60] as dopamine-directed reward behaviors of the ventral striatum are replaced by dorsal striatal cue-initiated action sequences [61, 62] mediated by multiple neurotransmitters that do not appear to be under the regulatory influence of insulin. Indeed, this shift is well defined with food reward, indicating that salient foods and their cues are sufficient to initiate reward-seeking and the subsequent habitual behaviors characteristic of addiction[14, 63].

Decision-Making and Disinhibition

The progression from reward learning to habit formation relies on active oversight by the prefrontal cortex (PFC)[64], a region responsible for 'top-down' regulation of subcortical function to promote situation-appropriate and task-relevant behaviors[65]. While the complexities of PFC function are beyond the scope of this article (for review, see [66-68]), there is strong evidence for the specific role of dopamine in regulating PFC activity[69, 70] through volume transmission maintaining extrasynaptic dopamine tone[70]. Dopamine appears to improve prefrontal cortical cognitive function[71-73] by enhancing glutamatergic signaling through D1 receptor binding[74, 75], however this effect is non-linear where either too much[76] or too little[77, 78] dopamine actually impairs proper PFC function. This non-linear impairment is readily seen in measures of response inhibition, where both deficits[79-82] and elevations[83] in central dopamine produce a faster cue-driven response and/or a decreased ability to rapidly inhibit unwanted responses.

Dopamine tone is maintained in the prefrontal cortex by the norepinephrine transporter (NET)[84, 85] whose intracellular trafficking and surface expression, in contrast to the striatal dopamine transporter, is inhibited by insulin[86]. Insulin further inhibits dopamine release in the PFC[87], thus providing multiple mechanisms that would both serve to diminish cortical dopamine levels. As dopamine acts through an inverted-U response[88], even minor deviations from optimal tone can alter PFC function[70]. In the setting of impaired insulin signaling, such dysregulation may set the stage for the emergence of the habitual, cue-driven behaviors. Indeed, given that the increased availability of highly palatable food provided by the modern environment requires the continuous inhibition of cue-mediated feeding behaviors, it is easy to see how dopamine-mediated prefrontal disinhibition could unmask the established subcortical salience attributions and response patterns leading to obesity[89].

The Progression to Obesity

Here we propose a plausible molecular mechanism by which the physiologic response to food consumption promotes progressive neuroadaptations in brain dopaminergic circuits subserving reward, habits, and decision-making that further bias towards the maintenance of obesity. Insulin maintains dopamine homeostasis in reward circuitry, supporting a synaptic environment ideal for the perception of food reward. The onset of mild insulin resistance with repeated consumption of highly palatable food drives a striatal synaptic hyperdopaminergia from increased dopamine release, decreased dopamine clearance, and allostatic

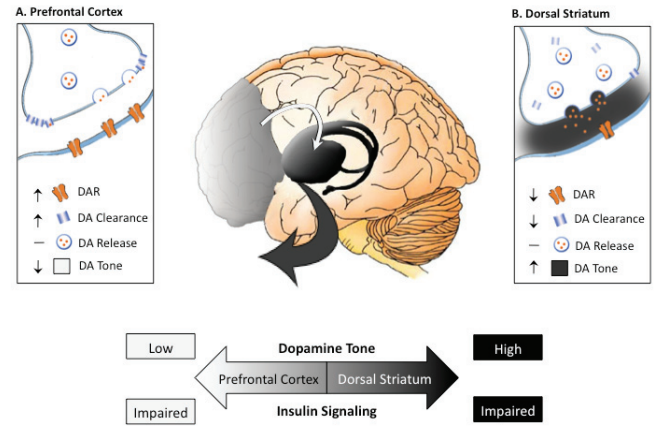

Figure 1. Model for Dopamine Neurotransmission in the context of impaired insulin signaling in A) prefrontal cortex and B) dorsal striatum

downregulation of dopamine receptor function, effectively blunting the impact of dopamine reward signaling and facilitating the emergence of cue-driven food seeking behavior. Further, concomitant insulin-mediated cortical neuroadaptations promote a prefrontal hypodopamineric tone serving to unmask response patterns directed at palatable food acquisition and consumption (see Figure 1). In the next section, we review how available neuroimaging evidence supports this model and identify important next steps for neuroimaging in unraveling obesity pathogenesis and implications for treatment.

Translating Molecules to Systems

Neuroimaging in Obesity

Exploring feeding behavior with molecular (PET) and functional (MR) neuroimaging facilitates the systems-level study of feeding behavior and translation of molecular obesity research to humans. Such methods have in fact convincingly uncovered strong evidence for widespread neural dysregulation in obesity. The multisensory elements of food are reflected as its unified flavor[90] which, combined with individual and environmental factors, contributes to the pleasure derived from food consumption and its hedonic value[91]. In healthy individuals, flavor perception robustly activates the gustatory network, including the thalamus, insula, frontal operculum, inferior frontal gyrus, and orbitofrontal cortex[92-96]. Hunger elicits heightened activity in these areas and further activation in the striatum, midbrain, and prefrontal cortex[95, 96]. This activation correlates with perceived stimulus pleasantness[92, 97] and reward value[95], providing evidence for the representation of hedonic "liking" in these regions.

Yet feeding behavior extends beyond the mere liking of food, and whether food is sought for consumption depends on how much it is "wanted"[4]. This incentive value is influenced by the sight and smell of food; these sensory experiences reflect food availability and the anticipation of food, which can then be contrasted with the effort to achieve them[91]. Indeed, the presentation of a salient food cue elicits reward associated with food anticipation and subsequent food seeking[91, 98, 99]. Visual food stimuli increase activity in areas including the midbrain, amygdala, dorsal striatum, cingulate, insula, and orbitofrontal cortex[94, 96, 98, 100, 101], where activity is amplified by hunger[98, 100, 102, 103]. The fasting state also elicits activation of hedonic brain areas including the ventral striatum[102, 104, 105] which implies that fasting enhances the rewarding properties of food[106], consistent with behavioral studies.

Overweight and obese individuals in the fasting state also demonstrate activation in circuits subserving food reward[107-110]. In contrast to healthy individuals, obese individuals exhibit BMI-dependent potentiation of activation by salient food cues in gustatory areas such as the orbitofrontal cortex and insula, and in brain regions receiving dopaminergic inputs, including dorsal and ventral striatum[107, 108, 110, 111]. Hyperactivity in these areas could represent enhanced expected food reward promoting dopamine release, driving the motivation and behaviors aimed at food consumption[112]. Evidince for greater reward sensitivity in obesity[5, 113] is consistent with this hypothesis. Alternatively, if the obese state is characterized by prefrontal dysregulation of inhihibitory circuits under dopaminergic modulation, the observed hyperactivity results from the unmasking of habitual circuits normally under cortical regulation. One fMRI study examining response inhibition in adolescent girls demonstrated a BMI-dependent loss of activity in the prefrontal areas subserving inhibitory control[114], however the relationship of this attenuation to subcortical activity was not assessed. An important next step will be to determine how prefrontal functional brain activity changes with subcortical activity in light of differential dopamine clearance in these regions.

While fMRI studies indicate neural dysfunction in obesity, they do not assess the mechanism by which it occurs. Molecular imaging evidence directly demonstrating dopamine dysregulation comes from a small number of PET studies finding BMI-dependent decreases in striatal dopamine D2 receptor availability[47, 115]. This reduction depends on the magnitude and duration of overfeeding[42], supporting the hypothesis of an allostatic downregulation of D2 receptors with chronic overeating. However, these PET studies assessed D2 receptor availability using the radioligand [^{11}C]-raclopride which competes with synaptic dopamine for receptor binding, and therefore the decreased binding explained as a reduction in D2 receptor availability could also reflect increases in synaptic dopamine. This interpretation would suggest an elevated basal dopamine tone in obesity. Among adults viewing food cues who received an acute methamphetamine dose (stimulating presynaptic dopamine release), normal-weight individuals demonstrated increases in dopamine[116] while the dopamine levels of obese individuals remained constant[117], providing evidence for blunted dopamine signaling in obesity.

Implications for Treatment

While the precise etiologic nature of dopamine dysregulation in obesity remains unclear, several imaging studies support plasticity in the brain circuits underlying obesity and thus opportunities for treatment. Initial clinical observations of mild weight loss in patients receiving treatment with dopamine agonists[118] have been replicated in animal studies[119], however the cognitive/psychiatric side effects render these drugs problematic. Further, dopamine administration will be ineffective if post-synaptic dopamine signaling is impaired in obesity. Promising observations come from studies demonstrating that bariatric surgery[120] and weight loss[42] increase D2 receptor levels and decrease functional activity in dopamine reward circuitry[121] while increasing activity in the prefrontal cortex[122]. One explanation for these effects on dopamine circuits is the drastic changes in insulin levels following bariatric surgery; however, there have been no longitudinal controlled clinical trials to examine the direct effect of insulin on normalizing dopamine neurotransmission in obesity. Such research will be critical in understanding the pathogenesis of obesity, potential therapeutic targets in insulin signaling pathways, and future opportunities for treatment.

Conclusion

Recent scientific evidence demonstrating that central nervous system dopamine is under the regulatory influence of insulin offers a plausible mechanism for understanding obesity as a dysregulation of neural systems controlling reward, habits, and decision-making. Here we have reviewed the molecular processes underlying insulin's effect on dopamine circuitry and feeding behavior, and how these findings link the opposing theories of a hypodopaminergic reward deficiency versus a hyperresponsiveness to reward in the obese state. We further extend the interpretations of this research by proposing a novel model for obesity as a progressive disruption of subcortical and prefrontal brain

circuitry initiated and perpetuated by insulin resistance and subsequent dysregulation of extracellular dopamine. While future research is necessary, the hypothesis of insulin's ability to reset central dopamine tone and subsequently reshape feeding behavior offers exciting new opportunities for the clinical management of obesity.

References

1. Niswender KD, Baskin DG and Schwartz MW (2004). Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. Trends in endocrinology and metabolism: TEM. 15 (8): 362-369.
2. Flegal KM, Carroll MD, Ogden CL and Curtin LR (2010). Prevalence and trends in obesity among US adults, 1999-2008. JAMA. 303 (3): 235-241.
3. Adam TC and Epel ES (2007). Stress, eating and the reward system. Physiology & behavior. 91 (4): 449-458.
4. Berridge KC, Ho CY, Richard JM and DiFeliceantonio AG (2010). The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain research. 1350: 43-64.
5. Davis C, Strachan S and Berkson M (2004). Sensitivity to reward: implications for overeating and overweight. Appetite. 42 (2): 131-138.
6. Palmiter RD (2007). Is dopamine a physiologically relevant mediator of feeding behavior? Trends in neurosciences. 30 (8): 375-381.
7. Volkow ND, Wang GJ and Baler RD (2011). Reward, dopamine and the control of food intake: implications for obesity. Trends in cognitive sciences. 15 (1): 37-46.
8. Gibson CD, Carnell S, Ochner CN and Geliebter A (2010). Neuroimaging, gut peptides and obesity: novel studies of the neurobiology of appetite. Journal of neuroendocrinology. 22 (8): 833-845.
9. Schwartz MW, Woods SC, Porte Jr. D, Seeley RJ and Baskin DG (2000). Central nervous system control of food intake. Nature. 404: 661-671.
10. Morton GJ, Cummings DE, Baskin DG, Barsh GS and Schwartz MW (2006). Central nervous system control of food intake and body weight. Nature. 443 (7109): 289-295.
11. Moran TH (2006). Gut peptide signaling in the controls of food intake. Obesity. 14 Suppl 5: 250S-253S.
12. Saper CB, Chou TC and Elmquist JK (2002). The need to feed: Homeostatic and hedonic control of eating. Neuron. 36: 199-211.
13. Berridge KC and Robinson TE (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews. 28 (3): 309-369.
14. Koob GF and Volkow ND (2010). Neurocircuitry of addiction. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 35 (1): 217-238.
15. Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M and DiLeone RJ (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron. 51 (6): 801-810.
16. Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E and Flier JS (2006). Leptin regulation of the mesoaccumbens dopamine pathway. Neuron. 51 (6): 811-822.
17. Figlewicz DP, Bennett JL, Naleid AM, Davis C and Grimm JW (2006). Intraventricular insulin and leptin decrease sucrose self-administration in rats. Physiology & behavior. 89 (4): 611-616.
18. Davis JF, Choi DL, Schurdak JD, Fitzgerald MF, Clegg DJ, Lipton JW, Figlewicz DP and Benoit SC (2011). Leptin regulates energy balance and motivation through action at distinct neural circuits. Biological psychiatry. 69 (7): 668-674.
19. Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschop MH, Gao XB and Horvath TL (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. The Journal of clinical investigation. 116 (12): 3229-3239.
20. Carvelli L, Moron JA, Kahlig KM, Ferrer JV, Sen N, Lechleiter JD, Leeb-Lundberg LM, Merrill G, Lafer EM, Ballou LM, Shippenberg TS, Javitch JA, Lin RZ and Galli A (2002). PI 3-kinase regulation of dopamine uptake. J Neurochem. 81 (4): 859-869. Seminal paper defining the molecular mechanism for insulin regulation of the dopamine transporter.
21. Daws LC, Avison MJ, Robertson SD, Niswender K, Galli A and Saunders C (2011). Insulin signalling and addiction. Neuropharmacology. Ahead of Print.
22. Figlewicz DP, Evans SB, Murphy J, Hoen M and Baskin DG (2003). Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. Brain research. 964 (1): 107-115.
23. Garcia BG, Wei Y, Moron JA, Lin RZ, Javitch JA and Galli A (2005). Akt is essential for insulin modulation of amphetamine-induced human dopamine transporter cell-surface redistribution. Molecular pharmacology. 68 (1): 102-109.
24. Johnson PM and Kenny PJ (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nature neuroscience. 13 (5): 635-641.
25. Schultz W (2007). Multiple dopamine functions at different time courses. Annual review of neuroscience. 30: 259-288.
26. Schultz W, Dayan P and Montague PR (1997). A neural substrate of prediction and reward. Science. 275 (5306): 1593-1599.
27. Schultz W (2011). Potential vulnerabilities of neuronal reward, risk, and decision mechanisms to addictive drugs. Neuron. 69 (4): 603-617.
28. Hernandez L and Hoebel BG (1988). Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life sciences. 42 (18): 1705-1712.
29. Salamone JD, Steinpreis RE, McCullough LD, Smith P, Grebel D and Mahan K (1991). Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. Psychopharmacology. 104 (4): 515-521.
30. Bassareo V and Di Chiara G (1997). Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. The Journal of neuroscience : the official journal of the Society for Neuroscience. 17 (2): 851-861.
31. Bassareo V and Di Chiara G (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. The European journal of neuroscience. 11 (12): 4389-4397.
32. Avena NM, Rada P and Hoebel BG (2008). Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. Neuroscience and biobehavioral reviews. 32 (1): 20-39.

33. Avena NM, Rada P, Moise N and Hoebel BG (2006). Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. Neuroscience. 139 (3): 813-820.
34. Rada P, Avena NM and Hoebel BG (2005). Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience. 134 (3): 737-744.
35. Rada P, Bocarsly ME, Barson JR, Hoebel BG and Leibowitz SF (2010). Reduced accumbens dopamine in Sprague-Dawley rats prone to overeating a fat-rich diet. Physiology & behavior. 101 (3): 394-400.
36. Liang NC, Hajnal A and Norgren R (2006). Sham feeding corn oil increases accumbens dopamine in the rat. American journal of physiology Regulatory, integrative and comparative physiology. 291 (5): R1236-1239.
37. Roitman MF, Stuber GD, Phillips PE, Wightman RM and Carelli RM (2004). Dopamine operates as a subsecond modulator of food seeking. The Journal of neuroscience : the official journal of the Society for Neuroscience. 24 (6): 1265-1271.
38. Roitman MF, Wheeler RA and Carelli RM (2005). Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron. 45 (4): 587-597.
39. Roitman MF, Wheeler RA, Wightman RM and Carelli RM (2008). Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nature neuroscience. 11 (12): 1376-1377.
40. Geiger BM, Behr GG, Frank LE, Caldera-Siu AD, Beinfeld MC, Kokkotou EG and Pothos EN (2008). Evidence for defective mesolimbic dopamine exocytosis in obesity-prone rats. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 22 (8): 2740-2746.
41. Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG and Pothos EN (2009). Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience. 159 (4): 1193-1199.
42. Thanos PK, Michaelides M, Piyis YK, Wang GJ and Volkow ND (2008). Food restriction markedly increases dopamine D2 receptor (D2R) in a rat model of obesity as assessed with in-vivo muPET imaging ([11C] raclopride) and in-vitro ([3H] spiperone) autoradiography. Synapse. 62 (1): 50-61.
43. Anderzhanova E, Covasa M and Hajnal A (2007). Altered basal and stimulated accumbens dopamine release in obese OLETF rats as a function of age and diabetic status. American journal of physiology Regulatory, integrative and comparative physiology. 293 (2): R603-611.
44. Bello NT and Hajnal A (2006). Alterations in blood glucose levels under hyperinsulinemia affect accumbens dopamine. Physiology & behavior. 88 (1-2): 138-145.
45. Bello NT, Lucas LR and Hajnal A (2002). Repeated sucrose access influences dopamine D2 receptor density in the striatum. Neuroreport. 13 (12): 1575-1578.
46. Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, Lubar JO, Chen TJ and Comings DE (2000). Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. Journal of psychoactive drugs. 32 Suppl: i-iv, 1-112.
47. Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N and Fowler JS (2001). Brain dopamine and obesity. Lancet. 357 (9253): 354-357. First human PET study of obesity, showing deficits in midbrain dopamine neurotransission in obeseindividuals.
48. Dawe S and Loxton NJ (2004). The role of impulsivity in the development of substance use and eating disorders. Neuroscience and biobehavioral reviews. 28 (3): 343-351.
49. Mathes WF, Nehrenberg DL, Gordon R, Hua K, Garland T, Jr. and Pomp D (2010). Dopaminergic dysregulation in mice selectively bred for excessive exercise or obesity. Behavioural brain research. 210 (2): 155-163.
50. Heusner CL, Hnasko TS, Szczypka MS, Liu Y, During MJ and Palmiter RD (2003). Viral restoration of dopamine to the nucleus accumbens is sufficient to induce a locomotor response to amphetamine. Brain research. 980 (2): 266-274.
51. Darvas M and Palmiter RD (2010). Restricting dopaminergic signaling to either dorsolateral or medial striatum facilitates cognition. The Journal of neuroscience : the official journal of the Society for Neuroscience. 30 (3): 1158-1165.
52. Hnasko TS, Perez FA, Scouras AD, Stoll EA, Gale SD, Luquet S, Phillips PE, Kremer EJ and Palmiter RD (2006). Cre recombinase-mediated restoration of nigrostriatal dopamine in dopamine-deficient mice reverses hypophagia and bradykinesia. Proceedings of the National Academy of Sciences of the United States of America. 103 (23): 8858-8863.
53. Szczypka MS, Kwok K, Brot MD, Marck BT, Matsumoto AM, Donahue BA and Palmiter RD (2001). Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron. 30 (3): 819-828.
54. Faure A, Haberland U, Conde F and El Massioui N (2005). Lesion to the nigrostriatal dopamine system disrupts stimulus-response habit formation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 25 (11): 2771-2780.
55. Graybiel AM (2008). Habits, rituals, and the evaluative brain. Annual review of neuroscience. 31: 359-387.
56. Yin HH, Knowlton BJ and Balleine BW (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. The European journal of neuroscience. 19 (1): 181-189.
57. Berridge KC, Robinson TE and Aldridge JW (2009). Dissecting components of reward: 'liking', 'wanting', and learning. Current opinion in pharmacology. 9 (1): 65-73.
58. Balleine BW and Dickinson A (1998). Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology. 37 (4-5): 407-419.
59. Dickinson A, Nicholas DJ and Adams CD (1983). The Effect of the Instrumental Training Contingency on Susceptibility to Reinforcer Devaluation. Quarterly Journal of Experimental Psychology Section B-Comparative and Physiological Psychology. 35 (Feb): 35-51.
60. Hyman SE, Malenka RC and Nestler EJ (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. Annual review of neuroscience. 29: 565-598.
61. Yin HH (2010). The sensorimotor striatum is necessary for serial order learning. The Journal of neuroscience : the official journal of the Society for Neuroscience. 30 (44): 14719-14723.
62. Yin HH, Knowlton BJ and Balleine BW (2005). Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. The European journal of neuroscience. 22 (2): 505-512.
63. Kalivas PW (2009). The glutamate homeostasis hypothesis

of addiction. Nature reviews Neuroscience. 10 (8): 561-572.
64. Berke JD (2003). Learning and memory mechanisms involved in compulsive drug use and relapse. Methods Mol Med. 79: 75-101.
65. Li CS, Huang C, Constable RT and Sinha R (2006). Imaging response inhibition in a stop-signal task: neural correlates independent of signal monitoring and post-response processing. The Journal of neuroscience : the official journal of the Society for Neuroscience. 26 (1): 186-192. First fMRI study using the stop signal paradigm to assess the neural correlates of response inhibition.
66. Arnsten AF (2009). Stress signalling pathways that impair prefrontal cortex structure and function. Nature reviews Neuroscience. 10 (6): 410-422.
67. Miller EK and Cohen JD (2001). An integrative theory of prefrontal cortex function. Annual review of neuroscience. 24: 167-202.
68. Robbins TW and Arnsten AF (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annual review of neuroscience. 32: 267-287.
69. Goldman-Rakic PS (1998). The cortical dopamine system: role in memory and cognition. Advances in pharmacology. 42: 707-711.
70. Seamans JK and Yang CR (2004). The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol. 74 (1): 1-58.
71. Phillips AG, Ahn S and Floresco SB (2004). Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task. The Journal of neuroscience : the official journal of the Society for Neuroscience. 24 (2): 547-553.
72. Mehta MA and Riedel WJ (2006). Dopaminergic enhancement of cognitive function. Curr Pharm Des. 12 (20): 2487-2500.
73. Chudasama Y and Robbins TW (2004). Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 29 (9): 1628-1636.
74. Kruse MS, Premont J, Krebs MO and Jay TM (2009). Interaction of dopamine D1 with NMDA NR1 receptors in rat prefrontal cortex. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 19 (4): 296-304.
75. Sarantis K, Matsokis N and Angelatou F (2009). Synergistic interactions of dopamine D1 and glutamate NMDA receptors in rat hippocampus and prefrontal cortex: involvement of ERK1/2 signaling. Neuroscience. 163 (4): 1135-1145.
76. Zahrt J, Taylor JR, Mathew RG and Arnsten AF (1997). Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. The Journal of neuroscience : the official journal of the Society for Neuroscience. 17 (21): 8528-8535.
77. Crofts HS, Dalley JW, Collins P, Van Denderen JC, Everitt BJ, Robbins TW and Roberts AC (2001). Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cereb Cortex. 11 (11): 1015-1026.
78. Robbins TW and Roberts AC (2007). Differential regulation of fronto-executive function by the monoamines and acetylcholine. Cereb Cortex. 17 Suppl 1: i151-160.
79. Langley K, Marshall L, van den Bree M, Thomas H, Owen M, O'Donovan M and Thapar A (2004). Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. Am J Psychiatry. 161 (1): 133-138.
80. Eagle DM, Tufft MR, Goodchild HL and Robbins TW (2007). Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. Psychopharmacology. 192 (2): 193-206.
81. Bari A, Mar AC, Theobald DE, Elands SA, Oganya KC, Eagle DM and Robbins TW (2011). Prefrontal and monoaminergic contributions to stop-signal task performance in rats. The Journal of neuroscience : the official journal of the Society for Neuroscience. 31 (25): 9254-9263.
82. Congdon E, Lesch KP and Canli T (2008). Analysis of DRD4 and DAT polymorphisms and behavioral inhibition in healthy adults: implications for impulsivity. Am J Med Genet B Neuropsychiatr Genet. 147B (1): 27-32.
83. Colzato LS, van den Wildenberg WP, van Wouwe NC, Pannebakker MM and Hommel B (2009). Dopamine and inhibitory action control: evidence from spontaneous eye blink rates. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale. 196 (3): 467-474.
84. Moron JA, Brockington A, Wise RA, Rocha BA and Hope BT (2002). Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. The Journal of neuroscience : the official journal of the Society for Neuroscience. 22 (2): 389-395.
85. Wayment HK, Schenk JO and Sorg BA (2001). Characterization of extracellular dopamine clearance in the medial prefrontal cortex: role of monoamine uptake and monoamine oxidase inhibition. The Journal of neuroscience : the official journal of the Society for Neuroscience. 21 (1): 35-44.
86. Robertson SD, Matthies HJ, Owens WA, Sathananthan V, Christianson NS, Kennedy JP, Lindsley CW, Daws LC and Galli A (2010). Insulin reveals Akt signaling as a novel regulator of norepinephrine transporter trafficking and norepinephrine homeostasis. The Journal of neuroscience : the official journal of the Society for Neuroscience. 30 (34): 11305-11316.
87. Schoffelmeer AN, Drukarch B, De Vries TJ, Hogenboom F, Schetters D and Pattij T (2011). Insulin modulates cocaine-sensitive monoamine transporter function and impulsive behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience. 31 (4): 1284-1291.
88. Vijayraghavan S, Wang M, Birnbaum SG, Williams GV and Arnsten AF (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nature neuroscience. 10 (3): 376-384.
89. George O and Koob GF (2010). Individual differences in prefrontal cortex function and the transition from drug use to drug dependence. Neuroscience and biobehavioral reviews. 35 (2): 232-247.
90. Small DM (2008). Flavor and the formation of category-specific processing in olfaction. Chemosensory Perception. 1 (2): 136-146.
91. Small DM (2009). Individual differences in the neurophysiology of reward and the obesity epidemic. International journal of obesity. 33 Suppl 2: S44-48.
92. Bender G, Veldhuizen MG, Meltzer JA, Gitelman DR and Small DM (2009). Neural correlates of evaluative compared with passive tasting. The European journal of neuroscience. 30 (2): 327-338.
93. Felsted JA, Ren X, Chouinard-Decorte F and Small DM (2010). Genetically determined differences in brain response to a pri-

mary food reward. The Journal of neuroscience : the official journal of the Society for Neuroscience. 30 (7): 2428-2432.
94. O'Doherty JP, Deichmann R, Critchley HD and Dolan RJ (2002). Neural responses during anticipation of a primary taste reward. Neuron. 33 (5): 815-826.
95. Small DM, Zatorre RJ, Dagher A, Evans AC and Jones-Gotman M (2001). Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain : a journal of neurology. 124 (Pt 9): 1720-1733.
96. Uher R, Treasure J, Heining M, Brammer MJ and Campbell IC (2006). Cerebral processing of food-related stimuli: effects of fasting and gender. Behavioural brain research. 169 (1): 111-119.
97. Stice E, Yokum S, Blum K and Bohon C (2010). Weight gain is associated with reduced striatal response to palatable food. The Journal of neuroscience : the official journal of the Society for Neuroscience. 30 (39): 13105-13109.
98. Pelchat ML, Johnson A, Chan R, Valdez J and Ragland JD (2004). Images of desire: food-craving activation during fMRI. NeuroImage. 23 (4): 1486-1493.
99. Roefs A, Herman CP, Macleod CM, Smulders FT and Jansen A (2005). At first sight: how do restrained eaters evaluate high-fat palatable foods? Appetite. 44 (1): 103-114.
100. LaBar KS, Gitelman DR, Parrish TB, Kim Y-H, Nobre AC and Mesulam MM (2001). Hunger selectively modulates corticolimbic activation to food stimuli in humans. Behavioral Neuroscience. 115 (2): 493-500.
101. Porubska K, Veit R, Preissl H, Fritsche A and Birbaumer N (2006). Subjective feeling of appetite modulates brain activity: an fMRI study. NeuroImage. 32 (3): 1273-1280.
102. Cornier MA, Salzberg AK, Endly DC, Bessesen DH, Rojas DC and Tregellas JR (2009). The effects of overfeeding on the neuronal response to visual food cues in thin and reduced-obese individuals. PLoS One. 4 (7): e6310.
103. Del Parigi A, Gautier JF, Chen K, Salbe AD, Ravussin E, Reiman E and Tataranni PA (2002). Neuroimaging and obesity: mapping the brain responses to hunger and satiation in humans using positron emission tomography. Annals of the New York Academy of Sciences. 967: 389-397.
104. Beaver JD, Lawrence AD, van Ditzhuijzen J, Davis MH, Woods A and Calder AJ (2006). Individual differences in reward drive predict neural responses to images of food. The Journal of neuroscience : the official journal of the Society for Neuroscience. 26 (19): 5160-5166.
105. Goldstone AP, Prechtl de Hernandez CG, Beaver JD, Muhammed K, Croese C, Bell G, Durighel G, Hughes E, Waldman AD, Frost G and Bell JD (2009). Fasting biases brain reward systems towards high-calorie foods. The European journal of neuroscience. 30 (8): 1625-1635.
106. Stoeckel LE, Cox JE, Cook EW, 3rd and Weller RE (2007). Motivational state modulates the hedonic value of food images differently in men and women. Appetite. 48 (2): 139-144.
107. Fletcher PC, Napolitano A, Skeggs A, Miller SR, Delafont B, Cambridge VC, de Wit S, Nathan PJ, Brooke A, O'Rahilly S, Farooqi IS and Bullmore ET (2010). Distinct modulatory effects of satiety and sibutramine on brain responses to food images in humans: a double dissociation across hypothalamus, amygdala, and ventral striatum. The Journal of neuroscience : the official journal of the Society for Neuroscience. 30 (43): 14346-14355.
108. Rothemund Y, Preuschhof C, Bohner G, Bauknecht HC, Klingebiel R, Flor H and Klapp BF (2007). Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. NeuroImage. 37 (2): 410-421.
109. Stice E, Spoor S, Bohon C and Small DM (2008). Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science. 322 (5900): 449-452.
110. Stoeckel LE, Weller RE, Cook EW, 3rd, Twieg DB, Knowlton RC and Cox JE (2008). Widespread reward-system activation in obese women in response to pictures of high-calorie foods. NeuroImage. 41 (2): 636-647. fMRI study examining the neural correlates of viewing food cues, stratified by nutritional content, in obese and healthy-weight individuals.
111. Yokum S, Ng J and Stice E (2011). Attentional Bias to Food Images Associated With Elevated Weight and Future Weight Gain: An fMRI Study. Obesity.
112. Wang GJ, Volkow ND, Thanos PK and Fowler JS (2009). Imaging of brain dopamine pathways: implications for understanding obesity. J Addict Med. 3 (1): 8-18.
113. Davis C, Levitan RD, Kaplan AS, Carter J, Reid C, Curtis C, Patte K, Hwang R and Kennedy JL (2008). Reward sensitivity and the D2 dopamine receptor gene: A case-control study of binge eating disorder. Progress in neuro-psychopharmacology & biological psychiatry. 32 (3): 620-628.
114. Batterink L, Yokum S and Stice E (2010). Body mass correlates inversely with inhibitory control in response to food among adolescent girls: an fMRI study. NeuroImage. 52 (4): 1696-1703.
115. Volkow ND, Wang GJ, Telang F, Fowler JS, Thanos PK, Logan J, Alexoff D, Ding YS, Wong C, Ma Y and Pradhan K (2008). Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. NeuroImage. 42 (4): 1537-1543.
116. Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, Wong C, Gatley SJ, Gifford AN, Ding YS and Pappas N (2002). "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. Synapse. 44 (3): 175-180.
117. Wang GJ, Geliebter A, Volkow ND, Telang FW, Logan J, Jayne MC, Galanti K, Selig PA, Han H, Zhu W, Wong CT and Fowler JS (2011). Enhanced Striatal Dopamine Release During Food Stimulation in Binge Eating Disorder. Obesity.
118. Bina KG and Cincotta AH (2000). Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing hormone, body weight gain, and hyperglycemia in ob/ob mice. Neuroendocrinology. 71 (1): 68-78.
119. Davis LM, Michaelides M, Cheskin LJ, Moran TH, Aja S, Watkins PA, Pei Z, Contoreggi C, McCullough K, Hope B, Wang GJ, Volkow ND and Thanos PK (2009). Bromocriptine administration reduces hyperphagia and adiposity and differentially affects dopamine D2 receptor and transporter binding in leptin-receptor-deficient Zucker rats and rats with diet-induced obesity. Neuroendocrinology. 89 (2): 152-162.
120. Steele KE, Prokopowicz GP, Schweitzer MA, Magunsuon TH, Lidor AO, Kuwabawa H, Kumar A, Brasic J and Wong DF (2010). Alterations of central dopamine receptors before and after gastric bypass surgery. Obes Surg. 20 (3): 369-374.
121. Ochner CN, Kwok Y, Conceicao E, Pantazatos SP, Puma LM, Carnell S, Teixeira J, Hirsch J and Geliebter A (2011). Selective reduction in neural responses to high calorie foods following gastric bypass surgery. Ann Surg. 253 (3): 502-507.

122. McCaffery JM, Haley AP, Sweet LH, Phelan S, Raynor HA, Del Parigi A, Cohen R and Wing RR (2009). Differential functional magnetic resonance imaging response to food pictures in successful weight-loss maintainers relative to normal-weight and obese controls. The American journal of clinical nutrition. 90 (4): 928-934.

CANDIDATE
REVIEWS

Sensitivity of the Dopamine System to Stress

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Abstract

Pathological conditions such as post traumatic stress disorder (PTSD) and anxiety disorders may result from the inability to properly respond to stress. The extended amygdala is highly involved in the stress response and receives substantial dopaminergic innervation. The dopamine (DA) system is sensitive to stress, but its role in the stress response is not fully understood. Dopamine concentration and metabolism increase within target regions after exposure to stressors. The firing of DA neurons is also altered after stress exposure. The DA system receives norepinephrine (NE) inputs that may, in part, mediate some of these actions. Indeed, NE modulates the firing of DA neurons through the activation of α1 and α2 adrenergic receptors (ARs). This review highlights the evidence of DA's involvement in stress and the potential role NE plays in mediating these actions, with a focus on the dopamine projections to the extended amygdala.

Keywords

Stress
Anxiety
Dopamine
Norepinephrine
Ventral tegmental area
Extended amygdala,
Burst firing

Introduction

Chronic stress or alterations in the appropriate physiological response to stress may lead to pathological conditions such as anxiety, panic disorders, post-traumatic stress disorder (PTSD), or perhaps drug abuse. The extended amygdala, which consists of the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala (CeA), and the nucleus accumbens (NAc) shell, has been shown to play an important role in stress, anxiety, and addiction-related behaviors[1-3]. For example, inhibition of GABA synthesis in the BNST leads to an increase in anxiety-like behavior in rats[4]. Stress-related information is provided to the extended amygdala by a variety of afferents, including catecholamines arising from NE and DA centers. Many stressors increase the firing of NE neurons and increase NE turnover in target regions, such as the BNST[5-7]. Although there is a large literature focused on the role of NE in stress, this review will focus on the actions of stress on the DA system. Dopamine is classically regarded as the reward neurotransmitter, however, the midbrain DA system has been recognized to be sensitive to stress even though this impact is not well understood. Delineating the ways in which stress modulates the DA system will allow a better understanding of the mechanisms mediating the interaction of stress and reward.

Anatomy of dopaminergic innervation of the extended amygdala

It has long been known that the DA neurons that project to the extended amygdala arise from the ventral tegmental area (VTA), substantia nigra pars compacta (SNc), and retrorubral nucleus (RR)[8-13]. The population of DA neurons projecting to each region of the extended amygdala was quantitatively determined using retrograde tracers and tyrosine hydroxylase (TH) immunohistochemistry[14]. The NAc shell receives approximately 80% of its DA projections from the VTA-A10[a] group, with the highest percentage arising from the parabrachial pigmented (PBP) and caudal linear (CLi) nuclei. The CeA and BNST have very similar distributions with approximately 40% of the DA projections coming from the VTA-A10 group (majority from PBP and CLi) and approximately 50% from the A10dc area, which consists of the periaqueductal gray (PAG) and dorsal raphe (DR). The majority of studies that investigate the role of DA neurons in stress and addiction primarily focus on the parabrachial pigmented nucleus of the lateral VTA. Very few studies examine the midline DA neurons of the rostral linear nucleus (RLi), CLi, PAG and DR regions. Given the diverse projection targets of these regions, new insights may be gained from studies focused on the actions of these mid-

a. **Dopaminergic cell groups:** The DA population has been divided into distinct cell groups termed A8-A14. A8 refers to the RR nucleus, A9 is primarily the SNc, and A10 is the VTA. The A10-VTA can be divided into four distinct nuclei: parabrachial pigmented, paranigral, interfascicular, and caudal linear nuclei. The rostral linear nucleus was later added to the A10 group. The DA neurons of the PAG and DR are considered to be a dorsocaudal extent of A10 termed the A10dc. See Hasue and Shammah-Lagnado 2002 for further explanation of the DA cell groups.

line dopamine populations.

Anatomy of noradrenergic innervation of the dopamine system

The role of NE in stress has been widely studied[5-7, 15, 16]. Norepinephrine arises from the locus coeruleus (termed A4 and A6 areas), ventral medulla (A1, A5 and A7 areas), and the dorsomedial medulla (A2 area). The locus coeruleus (LC) has a broad projection field, and NE arising from the LC has been shown to play roles in arousal and cognitive performance[17, 18]. The non-LC NE neurons are located in brainstem, homeostatic centers and have been shown to be involved in a variety of processes. For example, A1 neurons control the release of vasopressin, A2 neurons are involved in regulation of food intake, and A5 neurons regulate the respiratory rhythm generator of the rostral ventrolateral medulla[19-21]. It has long been known that the LC projects to the VTA[22-25]. Recently, it was determined that a large number of non-LC noradrenergic projections innervate DA regions. Using dopamine beta hydroxylase (DBH) immunohistochemistry and anatomical tracing studies, Mejías-Aponte *et al.* found that the midline areas of RLi and CLi receive noradrenergic innervation from A1, A5 and LC[26]. The LC and A5 innervate the medial VTA while the lateral VTA receives innervation from A1, A2, A5 and LC[26]. There is also a noradrenergic input from the LC to the PAG, near the A10dc DA population[27]. These abundant NE innervations make the midbrain DA neurons prime candidates to undergo modulation due to stress. Furthermore, the varying noradrenergic inputs combined with distinct projection targets of diverse DA neuron populations indicate a possible differential sensitivity to stress.

Sensitivity of the dopamine system to stress

Extensive studies have explored the involvement of NE in stress. Furthermore, there is also evidence that DA is important for stress-related behaviors, particularly in the extended amygdala and prefrontal cortex. Acute intermittent tail shock increases extracellular DA concentration in the striatum, NAc, and medial prefrontal cortex (mPFC) with the mPFC showing the largest increase above basal levels[28]. An increase in DA concentration in rats exposed to foot shock stress occurs in the NAc shell[29, 30]. Also, rats who are predisposed to psychostimulant self administration undergo a larger and longer-lasting increase in DA concentration in the NAc, following tail pinch stress, as compared to those who are not predisposed[31]. Furthermore, acute immobilization and restraint stressors increase DA metabolism in the rat mPFC and the NAc shell while exposure to the predator odor, 2,5-dihydro-2,4,5-trimethylthia-zoline (TMT), increases DA metabolism in only the mPFC[32-34]. In summary, different types of acute stressors increase DA concentration and metabolism in numerous brain regions.

There are also actions of DA following stressors in other regions of the extended amygdala. The metabolism of DA increases within the CeA and BNST following foot shock stress in rats[35]. Interestingly, in a study done by Cecchi *et al.,* one session of immobilization stress significantly increases the level of NE, but not DA, in the lateral BNST of rats[7]. However, these rats were singly housed for 5-7 days prior to the immobilization stress and there is evidence that prolonged social isolation in mice leads to an increase in anxiety-like behavior[36]. Also, following chronic stress paradigms such as six days of restraint stress or three weeks of restraint stress combined with unavoidable tail shock, the concentration of DA and its metabolites decreases in the NAc shell[37, 38]. Therefore, the rats in the Cecchi *et al.* study may be undergoing a stress paradigm similar to chronic stress in which an increase in the level of DA in the BNST would not be expected. Further studies are needed to elucidate any changes in DA concentration or metabolism in the BNST and CeA that arise as a result of acute or chronic stress exposure.

Stress may also lead to an increase in *c-fos* expression in animals. In the CeA of the rat, there is an increase in Fos immunoreactivity following acute immobilization stress[39]. This CeA Fos staining is enriched in regions that overlap with TH positive terminals[40]. In rats, exposure to TMT and mild foot shock also leads to an increase in Fos immunoreactivity among DA neurons of the A10-VTA, but not in the A9-SNc region[41]. Furthermore, Deutch and colleagues found that restraint stress increases Fos staining within the VTA of rats[33]. This increase varies by VTA subregions and is highest in the PBP and CLi. The increase in Fos expression is partially blocked with treatment of diazepam prior to administration of the stressor. Interestingly, treatment with the anxiogenic β-carboline, FG 7142, increases Fos expression to a greater extent than restraint stress in the CLi and to a lesser extent in the PBP[33]. This evidence suggests that the CLi may play a larger role in stress and anxiogenic behaviors than other VTA nuclei. Through the use of retrograde tracers, Deutch and colleagues determined that the majority of double-labeled Fos and TH neurons project to the mPFC, with few projecting to the NAc. Additionally, the CLi DA neurons heavily project to the BNST and CeA, but this study did not determine whether the Fos-labeled cells project to these regions of the extended amygdala. As such, more work needs to be done to determine whether or

not the DA neurons that project to the extended amygdala are activated by stress exposure. Such work is important as activation of immediate early genes, such as *c-fos*, represents metabolic activation or increase in neuronal, activity, which may occur due to exposure to stressor. Therefore, based upon the changes in Fos expression and DA concentration in target regions as described above, alterations in firing of DA neurons after stress exposure might be expected.

Exposure to stressors modulates firing in dopamine neurons

The firing of DA neurons is characterized by a low frequency tonic or pacemaker firing that is interspersed with phasic bursting activity. Spontaneous pacemaker firing is independent of afferent input, while bursting activity is stimulated by NMDA receptor activation[42-46]. Bursting activity leads to a larger increase in synaptic DA than regular firing and is thought to occur during the presentation of reward or salient cues[45, 47-49]. There is some evidence that the firing of DA neurons is inhibited by aversive stimuli, such as foot shock, in anesthetized rats[50]. However, there are studies in which firing of putative DA neurons is enhanced with stress. For example, one session of restraint stress enhances firing in VTA DA neurons in awake rats[51]. This increase is only in cells that have a high level of basal bursting activity as compared to those with a regular firing pattern. Also, stress increases the amount of spikes seen within bursts, rather than the amount of bursts themselves. This increase in activity persists for at least twenty-four (24) hours. A second session of restraint stress, on the subsequent day, does not further increase firing activity. Additionally, a single exposure to social defeat stress in rats increases burst firing and DA release in the NAc core[52]. Specifically, this elevated firing occurs as the rat is confronting an aggressor and remains slightly elevated after return to the home cage. An increase in DA firing rate after acute stress correlates with the previously discussed data highlighting increases in DA concentration and metabolism after acute stress.

vRats that are subjected to chronic cold stress undergo a decrease in the number of spontaneously active DA cells compared to control animals[53]. The firing rate and percentage of spikes fired in bursts are not significantly altered as compared to control animals. However, the distribution of bursting across VTA and SN cells differs between the two groups. The decrease in active DA cells with a chronic stressor correlates with data showing decreased levels of DA in target regions after chronic stress[37, 38]. This data also suggests that chronic stress may inactivate one population of DA neurons while increasing burst activity in another. There is evidence that populations of DA neurons possess the ability to switch from single spiking mode to burst firing mode[45]. These populations may represent DA neurons that project to different target regions such as PFC, NAc, BNST or CeA. Different DA populations and their projections may mediate unique responses to stress, perhaps via a diverse sensitivity to stress or ability to mediate a switch in DA neuron firing in response to acute or chronic stress.

Extreme stress may produce diverging results in humans with some people subject to pathological conditions which may lead to depression, anxiety or post-traumatic stress disorder (PTSD), while other individuals appear to escape relatively unharmed. Recent studies in mice attempted to tackle this problem. Cao and colleagues separated mice into susceptible and resilient groups based on their social avoidance behavior after undergoing ten days of social defeat stress[54, 55]. Their studies show that the rate of spontaneous firing and number of bursting events within VTA DA neurons are increased in susceptible but not resilient mice[55]. Chronic social defeat stress increases I_h, a hyperpolarization-activated cation current[55]. I_h has been shown to contribute to the autonomous pacemaker activity of certain neurons and is thought to be activated by the large hyperpolarization following an action potential[56-58]. Therefore, an increase in the size of I_h would facilitate an increase in firing rate or bursting activity. Previous data discussed earlier in the review, details a decrease in spontaneously active DA cells, as well as a decrease in DA concentration and metabolism after chronic stress. One explanation may be that in "susceptible" animals, a greater number of populations of DA neurons make the switch to burst firing after chronic stress. Chronic stress in susceptible animals may be recruiting more components of the DA system than in resilient animals.

Modulation of dopamine firing by norepinephrine

Since stress modifies the firing properties of DA neurons and NE is thought to be a "stress neurotransmitter," it can be postulated that the actions of NE inputs may be partially responsible for the stress effects of DA. The A10 and A10dc DA populations receive substantial NE input, and adrenergic receptor (AR) subtypes are expressed within midbrain dopaminergic areas[26, 59, 60]. Specifically, there is evidence of α_1-AR, α_2-AR and perhaps low levels of β-AR expression in midbrain DA areas[59-61]. There are a few examples of NE producing actions that modulate the firing of DA neurons. A series of studies done by Grenhoff and colleagues show that *in vivo* electrical stimulation of the rat LC and administration of drugs acting on adrenergic receptors can alter DA cell firing within the SNc and VTA[62-65].

Furthermore, it was found that clonidine, an α_2-AR agonist, "regularizes" firing and in some VTA cells, decreases the amount of spikes fired in bursts[63, 64]. This effect was blocked by the α_2-AR antagonists, yohimbine and idazoxan. In fact, the α_2-AR antagonists actually increase burst firing to a level beyond baseline[64, 65]. Application of prazosin, an α_1-AR antagonist, was found to decrease burst firing[65]. In support of this data, Guiard and colleagues also found an α_2-AR mediated decrease in DA firing[66]. Taken together, this data may indicate that NE release onto DA neurons may increase burst firing through postsynaptic α_1-ARs, and this firing can either be increased or decreased through blockade or activation of presynaptic α_2-ARs, respectively.

Paladini and Williams demonstrate that iontophoretic application of NE onto VTA DA neurons in rat brain slices causes an outward current[67]. This outward current can be completely blocked by prazosin and is therefore mediated through the α_1-AR. Furthermore, activation of α_1-ARs causes internal calcium stores to be mobilized. The outward current has a reversal potential near that of potassium and can be blocked by apamin. Collectively, this data indicates that the NE activated outward current is mediated by SK channels which are activated by calcium. Based on these results, NE application and activation of α_1-ARs lead to an inhibition of DA neuron firing while, in the Grenhoff studies, activation of α_1-ARs leads to an increase in burst firing of DA neurons. There are a few possible explanations for this discrepancy. First, in the Grenhoff studies the recordings were done *in vivo* with the drugs applied systemically, but in Paladini and Williams study the recordings were done in *ex vivo* brain slices and drugs were applied iontophoretically directly to the slice. These application variations may cause different NE inputs to be recruited that have unique effects on DA output. Also, the duration of agonist application is very different, with iontophoresis leading to a transient activation of α_1-ARs while the *in vivo* studies will lead to prolonged agonist activation. Indeed, Paladini and Williams found that prolonged application of phenylephrine, an α_1-AR agonist, produces an inward current which would lead to stimulation of DA burst firing as demonstrated in the Grenhoff studies. Iontophoretic application of NE by Guiard and colleagues also leads to a decrease in DA firing[66].

There is also evidence of adrenergic modulation of I_h, the hyperpolarization activated cation current, in putative VTA DA neurons of rat brain slices. Application of clonidine or UK-14304, selective α_2-AR agonists, lead to an inhibition of spontaneous firing and I_h in DA neurons[68]. The inhibition in I_h can be blocked by yohimbine and RS79948, two selective α_2-AR antagonists. RX821002, an antagonist specific for the α_{2A}-AR and α_{2D}-AR subtypes, fails to block the I_h inhibition. Therefore, this modulation of I_h appears to act through the α_{2C}-AR subtype. Further study indicates that I_h inhibition is independent of cAMP levels and instead results from the activation of protein kinase C (PKC). These results are in concordance with the Grenhoff studies, in which activation of α_2-ARs lead to a decrease in firing rate in vivo. Further work needs to be done in this area to determine how NE influences DA firing and the influence of different NE inputs on DA cell firing.

Modulation of excitatory transmission on dopamine neurons by norepinephrine

Stimulation of glutamate afferents facilitates the switch from pacemaker firing to burst firing in midbrain DA neurons[43, 44]. Plasticity at glutamatergic synapses has been found to underlie many learned behaviors[69]. It is possible that changes in glutamatergic transmission occur on DA neurons following acute or chronic stressors. These changes would affect the firing, especially burst firing, of DA neurons. There is evidence that orexin and corticotropin releasing factor (CRF) are powerful modulators of excitatory transmission within the VTA[70]. However, there is very little evidence of NE modulation of glutamatergic transmission on DA neurons in the VTA. Norepinephrine has been shown to modulate excitatory transmission in several other limbic brain regions. For example, it has been shown that activation of α_{2A}-ARs leads to a depression of excitatory transmission and activation of β_1-ARs enhances excitatory transmission within the BNST[71, 72]. In the hippocampus, activation of β-ARs also leads to an enhancement of glutamatergic transmission[73]. Since there are NE inputs in the VTA , as well as evidence of multiple AR subtypes, it is reasonable to hypothesize that NE will modulate glutamatergic transmission onto DA neurons. In fact, unpublished preliminary evidence from our lab indicates that NE leads to an increase in the frequency of spontaneous EPSCs on DA neurons within the RLi. However, there is a great need for studies investigating the role of NE in modulating glutamatergic transmission onto neurons within the VTA subregions and A10dc DA populations.

There is evidence of NE altering mGluR-mediated inhibitory glutamatergic transmission onto VTA DA neurons. Amphetamine may have actions that work to increase DA concentration by altering the firing of midbrain DA neurons, rather than merely altering the function of the dopamine transporter[74]. In this study, amphetamine inhibits mGluR mediated IPSPs with no effect on ionotropic mediated EPSCs. It was determined that this effect can be

blocked by prazosin, an α_1-AR antagonist. Inhibition of the mGluR mediated hyperpolarization may lead to an increase in burst firing of DA neurons and increased DA release. Taken together, this data and previous data indicating an increase in DA burst firing following acute stress lends support to the hypothesis that stress modulates DA firing through the actions of NE.

Conclusions

Acute stress increases DA concentration and metabolism in target regions, while chronic stress may lead to a decrease in DA concentration. Exposure to stressors also leads to an increase in Fos staining and alters the firing of DA neurons. It is possible that these actions of DA in the stress response are mediated by an NE input. The firing of DA neurons can be modulated by NE. Although there seems to be some controversy, it appears that activation of α_1-ARs increases burst firing of DA neurons, but activation of α_2-ARs has an inhibitory effect on DA neuron firing. There is little evidence of NE modulation of glutamatergic transmission onto DA neurons but it is a likely possibility and requires further investigation. Much of the work done regarding stress and DA has been focused on the "classical" VTA and its projections to the mPFC or NAc. However, the BNST and CeA also receive dopaminergic input and play important roles in mediating stress and anxiety behaviors. Dopamine within the BNST has been shown to increase the frequency of spontaneous EPSCs, and in the CeA DA inhibits evoked IPSCs[75, 76]. These actions of DA will alter the output of the extended amygdala and lead to behavioral changes. Therefore it is important to study the effects of stress and NE in the populations of DA neurons that primarily project to the CeA and BNST. Hasue and Shammah-Lagnado found that the extended amygdala receives the majority of its DA innervation from the RLi and CLi regions of the VTA and the A10dc DA neurons arising from the PAG and DR[14]. The A10dc DA neurons have been shown to play roles in the sleep-wake cycle and heroin reward, but there are no studies investigating their role in stress[27, 77]. Moving forward, further investigation into these DA populations that project to the extended amygdala will be required to advance our understanding regarding dopamine's role in the stress response.

References

1. Koob GF (2009). Brain stress systems in the amygdala and addiction. *Brain Res*. **1293**(61-75.
2. Davis M, Walker DL, Miles L and Grillon C (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology*. **35**(1): 105-35.
3. Erb S (2010). Evaluation of the relationship between anxiety during withdrawal and stress-induced reinstatement of cocaine seeking. *Prog Neuropsychopharmacol Biol Psychiatry*. **34**(5): 798-807.
4. Sajdyk T, Johnson P, Fitz S and Shekhar A (2008). Chronic inhibition of GABA synthesis in the bed nucleus of the stria terminalis elicits anxiety-like behavior. *J Psychopharmacol*. **22**(6): 633-41.
5. Abercrombie ED, Keller RW, Jr. and Zigmond MJ (1988). Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: pharmacological and behavioral studies. *Neuroscience*. **27**(3): 897-904.
6. Korf J, Aghajanian GK and Roth RH (1973). Increased turnover of norepinephrine in the rat cerebral cortex during stress: role of the locus coeruleus. *Neuropharmacology*. **12**(10): 933-8.
7. Cecchi M, Khoshbouei H, Javors M and Morilak DA (2002). Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. *Neuroscience*. **112**(1): 13-21.
8. Fallon JH and Moore RY (1978). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J Comp Neurol*. **180**(3): 545-80.
9. Fallon JH, Koziell DA and Moore RY (1978). Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. *J Comp Neurol*. **180**(3): 509-32.
10. Beckstead RM, Domesick VB and Nauta WJ (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res*. **175**(2): 191-217.
11. Ottersen OP (1981). Afferent connections to the amygdaloid complex of the rat with some observations in the cat. III. Afferents from the lower brain stem. *J Comp Neurol*. **202**(3): 335-56.
12. Loughlin SE and Fallon JH (1983). Dopaminergic and nondopaminergic projections to amygdala from substantia nigra and ventral tegmental area. *Brain Res*. **262**(2): 334-8.
13. Swanson LW (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull*. **9**(1-6): 321-53.
14. Hasue RH and Shammah-Lagnado SJ (2002). Origin of the dopaminergic innervation of the central extended amygdala and accumbens shell: a combined retrograde tracing and immunohistochemical study in the rat. *J Comp Neurol*. **454**(1): 15-33.
15. Smagin GN, Swiergiel AH and Dunn AJ (1995). Corticotropin-releasing factor administered into the locus coeruleus, but not the parabrachial nucleus, stimulates norepinephrine release in the prefrontal cortex. *Brain Res Bull*. **36**(1): 71-6.
16. Valentino RJ, Foote SL and Page ME (1993). The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses. *Ann N Y Acad Sci*. **697**(173-88.
17. Clayton EC, Rajkowski J, Cohen JD and Aston-Jones G (2004). Phasic activation of monkey locus ceruleus neurons by simple decisions in a forced-choice task. *J Neurosci*. **24**(44): 9914-20.
18. Aston-Jones G and Cohen JD (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci*. **28**(403-50.
19. Blessing WW and Willoughby JO (1985). Inhibiting the rabbit caudal ventrolateral medulla prevents baroreceptor-initiated secretion of vasopressin. *J Physiol*. **367**(253-65.
20. Rinaman L (2003). Hindbrain noradrenergic lesions attenuate anorexia and alter central cFos expression in rats after gastric vis-

cerosensory stimulation. *J Neurosci*. **23**(31): 10084-92.
21. Hilaire G, Viemari JC, Coulon P, Simonneau M and Bevengut M (2004). Modulation of the respiratory rhythm generator by the pontine noradrenergic A5 and A6 groups in rodents. *Respir Physiol Neurobiol*. **143**(2-3): 187-97.
22. Jones BE and Moore RY (1977). Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain Res*. **127**(1): 25-53.
23. Phillipson OT (1979). Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. *J Comp Neurol*. **187**(1): 117-43.
24. Simon H, Le Moal M, Stinus L and Calas A (1979). Anatomical relationships between the ventral mesencephalic tegmentum--a 10 region and the locus coeruleus as demonstrated by anterograde and retrograde tracing techniques. *J Neural Transm*. **44**(1-2): 77-86.
25. Geisler S and Zahm DS (2005). Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *J Comp Neurol*. **490**(3): 270-94.
26. Mejias-Aponte CA, Drouin C and Aston-Jones G (2009). Adrenergic and noradrenergic innervation of the midbrain ventral tegmental area and retrorubral field: prominent inputs from medullary homeostatic centers. *J Neurosci*. **29**(11): 3613-26.
27. Lu J, Jhou TC and Saper CB (2006). Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *J Neurosci*. **26**(1): 193-202.
28. Abercrombie ED, Keefe KA, DiFrischia DS and Zigmond MJ (1989). Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem*. **52**(5): 1655-8.
29. Kalivas PW and Duffy P (1995). Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. *Brain Res*. **675**(1-2): 325-8.
30. Sutoo D and Akiyama K (2002). Neurochemical changes in mice following physical or psychological stress exposures. *Behav Brain Res*. **134**(1-2): 347-54.
31. Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M and Simon H (1993). Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. *Brain Res*. **602**(1): 169-74.
32. Morrow BA, Lee EJ, Taylor JR, Elsworth JD, Nye HE and Roth RH (1997). (S)-(-)-HA-966, a gamma-hydroxybutyrate-like agent, prevents enhanced mesocorticolimbic dopamine metabolism and behavioral correlates of restraint stress, conditioned fear and cocaine sensitization. *J Pharmacol Exp Ther*. **283**(2): 712-21.
33. Deutch AY, Lee MC, Gillham MH, Cameron DA, Goldstein M and Iadarola MJ (1991). Stress selectively increases fos protein in dopamine neurons innervating the prefrontal cortex. *Cereb Cortex*. 1(4): 273-92.
This paper highlights the stress sensitivity of the rat A10 dopamine neurons and identifies specific subregions of the VTA that have the greatest increase in Fos expression after exposure to stressors.
34. Morrow BA, Roth RH and Elsworth JD (2000). TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. *Brain Res Bull*. **52**(6): 519-23.
35. Coco ML, Kuhn CM, Ely TD and Kilts CD (1992). Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: attenuation by diazepam. *Brain Res*. **590**(1-2): 39-47.
36. Conrad KL, Louderback KM, Gessner CP and Winder DG (2011). Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. *Physiol Behav*. **104**(2): 248-56.
37. Imperato A, Cabib S and Puglisi-Allegra S (1993). Repeated stressful experiences differently affect the time-dependent responses of the mesolimbic dopamine system to the stressor. *Brain Res*. **601**(1-2): 333-6.
38. Mangiavacchi S, Masi F, Scheggi S, Leggio B, De Montis MG and Gambarana C (2001). Long-term behavioral and neurochemical effects of chronic stress exposure in rats. *J Neurochem*. **79**(6): 1113-21.
39. Honkaniemi J, Fuxe K, Rechard L, Koistinaho J, Isola J, Gustafsson JA, Okret S and Pelto-Huikko M (1992). Colocalization of Fos- and Glucocorticoid Receptor-Like Immunoreactivities in the Rat Amygdaloid Complex After Immobilization Stress. *J Neuroendocrinol*. **4**(5): 547-555.
40. Honkaniemi J (1992). Colocalization of peptide- and tyrosine hydroxylase-like immunoreactivities with Fos-immunoreactive neurons in rat central amygdaloid nucleus after immobilization stress. *Brain Res*. **598**(1-2): 107-13.
41. Redmond AJ, Morrow BA, Elsworth JD and Roth RH (2002). Selective activation of the A10, but not A9, dopamine neurons in the rat by the predator odor, 2,5-dihydro-2,4,5-trimethylthiazoline. *Neurosci Lett*. **328**(3): 209-12.
42. Grace AA (1991). Regulation of spontaneous activity and oscillatory spike firing in rat midbrain dopamine neurons recorded in vitro. *Synapse*. **7**(3): 221-34.
43. Overton P and Clark D (1992). Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. *Synapse*. **10**(2): 131-40.
44. Seutin V, Johnson SW and North RA (1993). Apamin increases NMDA-induced burst-firing of rat mesencephalic dopamine neurons. *Brain Res*. **630**(1-2): 341-4.
45. Cooper DC (2002). The significance of action potential bursting in the brain reward circuit. *Neurochem Int*. **41**(5): 333-40.
46. Deister CA, Teagarden MA, Wilson CJ and Paladini CA (2009). An intrinsic neuronal oscillator underlies dopaminergic neuron bursting. *J Neurosci*. **29**(50): 15888-97.
47. Gonon FG (1988). Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. *Neuroscience*. **24**(1): 19-28.
48. Suaud-Chagny MF, Chergui K, Chouvet G and Gonon F (1992). Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. *Neuroscience*. **49**(1): 63-72.
49. Schultz W, Apicella P and Ljungberg T (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J Neurosci*. **13**(3): 900-13.
50. Ungless MA, Magill PJ and Bolam JP (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*. **303**(5666): 2040-2.
51. Anstrom KK and Woodward DJ (2005). Restraint increases dopaminergic burst firing in awake rats. *Neuropsychopharmacology*. 30(10): 1832-40.
This paper shows increases in burst firing in dopamine

neurons of the rat VTA after exposure to restraint stress. Increases in burst firing lead to an increase in dopamine release in target regions. This is one of the first papers to show the effects of stress on dopamine neuron firing in awake animals.

52. Anstrom KK, Miczek KA and Budygin EA (2009). Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. *Neuroscience*. **161**(1): 3-12.

53. Moore H, Rose HJ and Grace AA (2001). Chronic cold stress reduces the spontaneous activity of ventral tegmental dopamine neurons. *Neuropsychopharmacology*. **24**(4): 410-9.

54. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK and Nestler EJ (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. **131**(2): 391-404.

55. Cao JL, Covington HE, 3rd, Friedman AK, Wilkinson MB, Walsh JJ, Cooper DC, Nestler EJ and Han MH (2010). Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. *J Neurosci*. **30**(49): 16453-8.

56. Chan CS, Shigemoto R, Mercer JN and Surmeier DJ (2004). HCN2 and HCN1 channels govern the regularity of autonomous pacemaking and synaptic resetting in globus pallidus neurons. *J Neurosci*. **24**(44): 9921-32.

57. Luthi A and McCormick DA (1998). H-current: properties of a neuronal and network pacemaker. *Neuron*. **21**(1): 9-12.

58. Maccaferri G and McBain CJ (1996). The hyperpolarization-activated current (Ih) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurones. *J Physiol*. 497 (Pt 1)(119-30.

59. Jones LS, Gauger LL and Davis JN (1985). Anatomy of brain alpha 1-adrenergic receptors: in vitro autoradiography with [125I]-heat. *J Comp Neurol*. **231**(2): 190-208.

60. Lee A, Wissekerke AE, Rosin DL and Lynch KR (1998). Localization of alpha2C-adrenergic receptor immunoreactivity in catecholaminergic neurons in the rat central nervous system. *Neuroscience*. **84**(4): 1085-96.

61. Rainbow TC, Parsons B and Wolfe BB (1984). Quantitative autoradiography of beta 1- and beta 2-adrenergic receptors in rat brain. *Proc Natl Acad Sci U S A*. **81**(5): 1585-9.

62. Grenhoff J, Nisell M, Ferre S, Aston-Jones G and Svensson TH (1993). Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *J Neural Transm Gen Sect*. **93**(1): 11-25.

63. Grenhoff J and Svensson TH (1988). Clonidine regularizes substantia nigra dopamine cell firing. *Life Sci*. **42**(20): 2003-9.

64. Grenhoff J and Svensson TH (1989). Clonidine modulates dopamine cell firing in rat ventral tegmental area. *Eur J Pharmacol*. **165**(1): 11-8.

65. Grenhoff J and Svensson TH (1993). Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. *Eur J Pharmacol*. 233(1): 79-84.

This paper is one of the first papers to highlight the modulation of dopamine firing through adrenergic receptors. Blockade of the α_1-AR decreases burst firing, while blockade of the α_2-AR increases burst firing in the VTA of anesthetized rats.

66. Guiard BP, El Mansari M and Blier P (2008). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Mol Pharmacol*. **74**(5): 1463-75.

67. Paladini CA and Williams JT (2004). Noradrenergic inhibition of midbrain dopamine neurons. *J Neurosci*. **24**(19): 4568-75.

68. Inyushin MU, Arencibia-Albite F, Vazquez-Torres R, Velez-Hernandez ME and Jimenez-Rivera CA (2010). Alpha-2 noradrenergic receptor activation inhibits the hyperpolarization-activated cation current (Ih) in neurons of the ventral tegmental area. *Neuroscience*. 167(2): 287-97.

This paper shows that norepinephrine inhibits I_h current through activation of the α_2-AR. I_h may help facilitate the switch from tonic to phasic firing in dopamine neurons.

69. Malenka RC and Bear MF (2004). LTP and LTD: an embarrassment of riches. *Neuron*. **44**(1): 5-21.

70. Bonci A and Borgland S (2009). Role of orexin/hypocretin and CRF in the formation of drug-dependent synaptic plasticity in the mesolimbic system. *Neuropharmacology*. **56 Suppl 1**(107-11.

71. Shields AD, Wang Q and Winder DG (2009). alpha2A-adrenergic receptors heterosynaptically regulate glutamatergic transmission in the bed nucleus of the stria terminalis. *Neuroscience*. **163**(1): 339-51.

72. Nobis WP, Kash TL, Silberman Y and Winder DG (2011). beta-Adrenergic receptors enhance excitatory transmission in the bed nucleus of the stria terminalis through a corticotrophin-releasing factor receptor-dependent and cocaine-regulated mechanism. *Biol Psychiatry*. **69**(11): 1083-90.

73. Gereau RWt and Conn PJ (1994). Presynaptic enhancement of excitatory synaptic transmission by beta-adrenergic receptor activation. *J Neurophysiol*. **72**(3): 1438-42.

74. Paladini CA, Fiorillo CD, Morikawa H and Williams JT (2001). Amphetamine selectively blocks inhibitory glutamate transmission in dopamine neurons. *Nat Neurosci*. **4**(3): 275-81.

75. Kash TL, Nobis WP, Matthews RT and Winder DG (2008). Dopamine enhances fast excitatory synaptic transmission in the extended amygdala by a CRF-R1-dependent process. *J Neurosci*. **28**(51): 13856-65.

76. Naylor JC, Li Q, Kang-Park MH, Wilson WA, Kuhn C and Moore SD (2010). Dopamine attenuates evoked inhibitory synaptic currents in central amygdala neurons. *Eur J Neurosci*. **32**(11): 1836-42.

77. Flores JA, Galan-Rodriguez B, Ramiro-Fuentes S and Fernandez-Espejo E (2006). Role for dopamine neurons of the rostral linear nucleus and periaqueductal gray in the rewarding and sensitizing properties of heroin. *Neuropsychopharmacology*. **31**(7): 1475-88.

Further Information: Lab website http://www.mc.vanderbilt.edu/root/vumc.php?site=winder

Norepinephrine in the Extended Amygdala Regulates Stress-Induced Reinstatement

Stephanie Flavin

Abstract

After treatment for drug addiction, patients remain at high risk for relapse into drug-seeking behavior, especially during stress. A region of the brain that plays a key role in stress-induced relapse into drug-seeking behavior is the extended amygdala. The extended amygdala is anatomically positioned to integrate stress and reward circuitry in the brain. In particular, norepinephrine signaling in the extended amygdala plays an integral role in rodent models of stress-induced reinstatement of drug-seeking. Therefore, understanding how norepinephrine modulates synaptic transmission in the extended amygdala may allow for insight into the mechanisms underlying stress-induced reinstatement of drug-seeking, and may lead to the identification of new pharmacological therapies for treating stress-induced relapse in humans.

Keywords

Stress
Addiction
Norepinephrine,
Extended amygdala
Bed nucleus of the stria terminalis
Central nucleus of the amygdala
Stress-induced reinstatement

Introduction

After undergoing initial treatment for addiction to drugs of abuse, an individual's risk of relapse remains high[1]. Exposure to stressful stimuli greatly increases an individual's risk for relapsing into drug- and alcohol-seeking behavior[2-4]. Relapse into substance abuse upon stress exposure suggests a close relationship between the stress-response circuitry and the reward-seeking circuitry of the brain. The extended amygdala is anatomically situated to participate in both stress and reward circuitry[5]. Further, norepinephrine (NE) in the extended amygdala has been shown to play a critical role in rodent behavioral models of stress-induced relapse into drug-seeking behavior, and to modulate neural activity in the extended amygdala[6-12]. Recent clinical trials have shown certain noradrenergic drugs to be effective in attenuating stress-induced drug cravings in humans[13-15]. Therefore, a better understanding of how NE modulates synaptic transmission in the extended amygdala may provide insight into the underlying mechanisms of stress-induced relapse into drug-seeking behavior, and lead to the identification of new pharmacological therapies. This review will discuss previous findings regarding the role of NE in rodent models of stress-induced reinstatement, as well as findings regarding the role of NE in modulating synaptic transmission in the extended amygdala.

Anatomy of the Extended Amygdala and Its Noradrenergic Innervation

The anatomy of the extended amygdala is critical for its ability to engage both reward and stress circuitry in the brain. The central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) are key components of the extended amygdala[16,17]. The CeA and the BNST are embryologically related[18], and interconnect with one another[19,20], with the CeA exerting inhibitory influence over the BNST[18,21,22]. To participate in stress-response circuitry, the BNST sends an inhibitory projection to the paraventricular nucleus (PVN) of the hypothalamus[20,22-24]. The projection from the BNST to the PVN influences the release of ACTH[25], which in turn leads to the activation of the body's stress response[24,26]. The CeA has some direct connections to the PVN[24], but can also modulate stress activity indirectly through the BNST[24]. The BNST also projects to the nucleus accumbens (NAc)[27] and sends an excitatory projection to the ventral tegmental area (VTA)[28-30]; these projections, along with a projection to the hypothalamus, allow the BNST to modulate reward circuitry [31]. Therefore, the extended amygdala may play a key role in the integration of stress and reward.

The extended amygdala receives an array of synaptic inputs that can modulate its neural activity[6-9]. Modulation of synaptic activity in the extended amygdala can have a profound impact on stress-induced reinstatement[11,12,32]. Two examples of such inputs include excitatory glutamatergic inputs, such as from the basolateral amygdala (BLA)[18], and noradrenergic inputs[6]. The CeA receives its noradren-

ergic input primarily from the A2 cell group of the nucleus tractus solitaris (NTS) through the ventral noradrenergic bundle (VNAB)[33,34], with a small amount of noradrenergic input arising from the locus coeruleus (LC)[35,36]. The BNST receives very dense noradrenergic innervation through the ventral noradrenergic bundle (VNAB) from the A1 and A2 cell groups in the NTS[33,37-39]. The densest noradrenergic input is to the ventral BNST, with the dorsal BNST also receiving noradrenergic input[6]. NE has been shown to be elevated in the extended amygdala during both stress and withdrawal[40-43]; further, NE plays an integral role in stress-induced relapse into drug-seeking behavior[12,44].

The Role of Norepinephrine in the Extended Amygdala in Stress-Induced Reinstatement

NE is released into the extended amygdala during times of stress[40-42,45]. Similarly, neurons in the BNST, and noradrenergic inputs to the BNST, are activated during withdrawal from drugs of abuse[43,46], leading to increased levels of NE[43,46,47]. The release of NE during times of stress and withdrawal affects behavior. Rodent behavioral models implicate NE signaling in the aversive symptoms of withdrawal[12,43,46,48], as well as in behavioral responses to stressors[42]. NE also plays a role in reinstatement of reward-seeking[49], as direct injection of NE into the extended amygdala has been shown to reinstate cocaine-seeking behavior[49]. Similarly, mice lacking dopamine-β-hydroxylase (DBH), an enzyme required for NE synthesis, do not demonstrate morphine-induced conditioned place preference (CPP)[44]. Viral restoration of DBH to the NTS, but not the LC, rescued the morphine-induced CPP behavior[44]. At the integration of stress and reward, NE in the extended amygdala has been shown to be a key mediator of stress-induced reinstatement of drug-seeking[49,50]. For example, lesioning of the VNAB blocks stress-induced reinstatement of morphine-seeking[12]. These studies specifically implicate NE inputs to the extended amygdala in reward-seeking and stress-induced reinstatement. Subsequent work has focused on the role of particular noradrenergic receptors in stress-induced reinstatement.

Adrenergic Receptors Modulate Neuronal Signaling

NE is capable of modulating neurotransmitter release[51] through its actions on adrenergic receptors (ARs). There are nine different ARs[52] divided into three major classes: α_1 receptors, α_2 receptors and β receptors[52]. Each type of receptor has three subtypes: α_1-ARs are composed of α_{1a}, α_{1b}, and α_{1d}; the α_2-ARs are α_{2a}, α_{2b}, and α_{2c}; and the β-ARs are β_1, β_2 and β_3[52]. ARs are G-protein coupled receptors that can modulate synaptic transmission through both pre- and post-synaptic mechanisms. α_1-ARs are linked to G_q signaling, α_2-ARs are linked to $G_{i/o}$ signaling, and β-ARs are linked to G_s signaling[53].

α_2-AR Agonists Block Stress-Induced Reinstatement of Drug-Seeking

Activation of the α_2-AR subtype has repeatedly been shown to block stress-induced reinstatement[11,12,54,55]. Peripheral administration of α_2 agonists blocks stress-induced reinstatement of heroin-seeking[48], and cocaine-seeking[11,54,55]. Specifically in the extended amygdala, α_2-ARs can inhibit stress-induced reinstatement, as administration of an α_2 agonist directly into the BNST blocks footshock-induced reinstatement of morphine-seeking[12]. Of note, α_2-ARs have been implicated in stress-induced reinstatement in humans. Patients being treated for drug addiction who are treated with α_2 agonists have improved relapse outcomes, and show decreased stress-induced drug cravings[13-15]. Therefore, activation of α_2-ARs by NE in the extended amygdala appears to play a crucial role in attenuating stress-induced reinstatement of drug-seeking in both rodents and humans, and could provide an effective therapeutic target.

α_1- and β-AR Antagonists Block Stress-Induced Reinstatement of Drug-Seeking

β-ARs and α_1-ARs also play a role in stress-induced reinstatement. Administration of β_1[10] and β_2 antagonists[10,11] into the CeA or BNST blocks stress-induced reinstatement of cocaine-seeking in rodents[11]. Peripheral administration of an α_1 antagonist, prazosin, can block footshock-induced reinstatement of alcohol-seeking[56]. Therefore, while activating α_2-ARs attenuates stress-induced reinstatement, blocking β- and α_1-ARs appears to be necessary for a similar attenuation of stress-induced reinstatement. However, while α_1-ARs in the BNST have been shown to modulate the stress response, β-ARs have not[42]. For example, while injection of either α_1 antagonists or β_1 and β_2 antagonists in the BNST reduces anxiety after stress[42], only the α_1 antagonist reduces plasma ACTH levels following stress[42]. Therefore, α_1-ARs' modulation of the stress response likely does not contribute to attenuation of stress-induced reinstatement.

Noradrenergic Receptors Modulate Excitatory and Inhibitory Transmission

Evidence suggests that the actions of NE in the extended amygdala influence stress-induced reinstatement of drug-seeking behavior; therefore it is important to understand how NE modulates synaptic transmission to elucidate underlying mechanisms. There has been substantial

evidence to support a heterosynaptic role for ARs in modulating glutamatergic transmission[7-9,57] and inhibitory transmission[30] in the extended amygdala. The effect of NE on synaptic transmission in the BNST appears to depend on duration of NE action[8], previous alterations in noradrenergic signaling[8,58], as well as type of adrenergic receptor activated[6]. Studies have shown α_1-ARs and α_2-ARs to depress excitatory synaptic transmission[6-8,30] as well as to modulate inhibitory transmission[7], while β-ARs are capable of enhancing both excitatory transmission[6,57] and inhibitory transmission[30]. Work has suggested that α_2-ARs are capable of differentially regulating glutamatergic inputs to the extended amygdala[9](unpublished data). The activation of ARs relies on many factors, such as duration of NE action, previous alterations in NE signaling, and activation of other receptors[6,57]. Further, ARs are capable of complex modulations of synaptic transmission in the extended amygdala, such as enhancement or depression of excitatory or inhibitory transmission, and differential regulation of individual excitatory inputs to the BNST. Therefore, ARs can intricately modulate synaptic transmission in the extended amygdala in response to diverse stress and reward stimuli, and these modulations may underlie stress-induced reinstatement.

α_1-ARs Modulate Excitatory Transmission in a Time-Dependent Manner

Noradrenergic modulation of synaptic transmission in the extended amygdala depends on duration of NE action. Extended application of NE to the BNST has been observed to result in an α_1-AR-dependent long term depression (LTD) of glutamatergic transmission in the BNST[8] through a postsynaptic mechanism[8]. However, with a shorter application of NE, only a transient depression or enhancement is seen[6,8]. This LTD is disrupted in mice with chronic alternations in adrenergic signaling, such as α_{2A}-AR- or NET-knockout mice[8], or mice that have undergone chronic stress or chronic ethanol exposure[58]. The absence of α_1-mediated LTD in the context of chronic disruption of noradrenergic signaling suggests that α_1-ARs may be important for long-term regulation of excitatory transmission in the extended amygdala, with prolonged dysregulation of noradrenergic signaling interfering with the α_1-ARs' ability to regulate transmission. Further, evidence suggests α_1-ARs dominate regulation of synaptic transmission after prolonged exposure to NE by ultimately inducing LTD[8], regardless of whether the initial response to NE is a β_2-AR-mediated increase in excitatory transmission, or an α_2-AR-mediated decrease of excitatory transmission[6]. In addition to transient depression in excitatory signaling, acute application of NE to α_1-ARs causes a transient increase of inhibitory transmission through a presynaptic mechanism[30]. Perhaps with prolonged stimulation by NE, α_1-ARs switch from a short-term presynaptic mechanism that enhances inhibitory transmission, to a long-term postsynaptic mechanism that depresses excitatory transmission[8,58]. α_1-ARs would then have the ability to depress activity in the BNST both short-term, through enhancement of $GABA_A$ inhibitory postsynaptic currents (IPSCs), and well as long term, through LTD. The ability of α_1-ARs to induce LTD in the extended amygdala suggests a possible mechanism for α_1-ARs in modulating the stress-response after exposure to a prolonged stressor. α_1-ARs in the extended amygdala have been shown to be capable of modulating the stress response, with injection of α_1 antagonists into the BNST decreasing levels of plasma ACTH[42]. By modulating excitatory or inhibitory transmission in the BNST, α_1-ARs may modulate the stress response by affecting the strength of the BNST's inhibitory projection to the PVN.

β-ARs Enhance Excitatory and Inhibitory Transmission in the BNST

Prior alterations in noradrenergic signaling can influence which ARs are recruited by NE. For example, with brief application of NE, α_1-ARs have been shown to enhance IPSC frequency in the BNST[30]; during acute withdrawal from morphine, NE-treated slices also demonstrate increased IPSC frequency through β-ARs[30]. Therefore, although the overall outcome of enhanced inhibitory transmission is the same whether through α_1- or β-ARs, the physiological circumstances under which NE is released in the extended amygdala seem to influence whether or not β-ARs are recruited. Brief application of NE to a slice might mimic a brief stressor that predominantly acts through α_1-ARs. In contrast, withdrawal may lead to long-term changes in NE signaling that effect the basal activity of β-ARs, and thus their likelihood of recruitment by subsequent NE signaling. Further evidence suggests that the recruitment of β-ARs by NE depends on their initial state of activity before NE application[6]. If enhanced excitatory transmission does not occur with initial NE application, subsequent treatment with β-AR agonists will not lead to β-AR-mediated enhancement of excitatory transmission[6]. However, if excitatory transmission does enhance with initial NE application, subsequent β-AR agonists will cause a similar enhancement of excitatory transmission[6]. Withdrawal may therefore influence the initial state of β-ARs, increasing their likelihood of recruitment by NE signaling. In other studies, β-ARs have been shown to enhance excitatory synaptic transmission through

processes that rely on the activity of other receptors, such as α_2-ARs[6] and CRFR1 receptors[57]. Therefore, the initial state of the β-ARs may also rely on signaling through other receptors. As a result, β-ARs may be poised to integrate stress and reward information received from inputs that signal though different neurotransmitters, for example integrating NE neurotransmission with CRF neurotransmission. The ability of β-ARs to enhance synaptic transmission in the extended amygdala may rely on both prior noradrenergic signaling, and on activation of other receptors.

α_2-ARs Mediate Short-term Depression of Excitatory and Inhibitory Transmission

Like α_1-ARs, α_2-ARs depress synaptic transmission in the BNST through heterosynaptic mechanisms[7,9]. Distribution of α_{2A}-ARs in the BNST suggests a prominent role for α_{2A}-ARs in modulating glutamatergic transmission. Immunohistochemical studies reveal that α_{2A}-ARs in the BNST are more broadly distributed than noradrenergic terminals, and instead closely resemble distribution of glutamatergic terminals[7]. Functionally, activation of α_2-ARs in the BNST leads to a decrease in excitatory transmission[6,7]. In a later study, application of a specific α_{2A}-AR agonist to BNST slices led to a decrease in both excitatory and inhibitory synaptic transmission[7]. Unlike α_1-ARs, the depression of synaptic transmission by α_2-ARs occurs through a presynaptic mechanism[7]. Also in contrast to α_1-ARs, α_2-ARs may play a greater role in short term depression of synaptic transmission[6] (unpublished data). Studies have not yet shown α_2-ARs to be capable of modulating plasticity of the BNST through LTD.

α_2-ARs Differentially Modulate Individual Inputs to the Extended Amygdala

α_2-ARs may differentially regulate synaptic transmission from individual inputs to the extended amygdala[9,59](unpublished data). As in the BNST, NE signaling in the CeA has been shown to heterosynaptically modulate glutamatergic transmission through α_2-ARs[9]. Further, NE has differential effects on the modulation of the glutamatergic inputs to the CeA from the parabrachial nucleus and the BLA[9]. Application of NE depresses glutamatergic transmission from the parabrachial nucleus to the CeA, but had no effect on transmission between the BLA and the CeA, with this effect depending on α_2-AR activation[9]. The differential modulation of glutamatergic transmission by NE is a particularly interesting finding, as it suggests that NE action through the α_2-AR, for a similar duration of time, could lead to afferent-specific effects on excitatory transmission. Differential excitatory modulation has important implications for understanding the circuitry underlying the relationship between stress and reward-seeking, as specific glutamatergic inputs could have a stronger influence on synaptic transmission in the extended amygdala, contingent on activation of α_2-ARs. Further evidence of differential regulation of glutamatergic inputs to the extended amygdala through α_2-ARs has been shown by increased c-fos expression after treatment with an α_{2A} agonist[59]. C-fos expression following treatment with an α_{2A}-AR agonist may indicate an excitatory role for α_{2A}-ARs in modulating glutamatergic transmission, which contrasts with previous work showing α_{2A}-ARs depress excitatory transmission[7]. Unpublished data using optogenetic approaches has also provided evidence for α_{2A}-AR-mediated enhancement of excitatory transmission. Behaviorally, guanfacine, an α_{2A} agonist, was recently shown to be less effective than prazosin at blocking yohimbine-induced reinstatement of alcohol-seeking[56]. The decreased effectiveness of guanfacine could result from guanfacine enhancing excitatory transmission from certain inputs while depressing others, therefore having less of an overall effect on synaptic transmission in the extended amygdala. However, work still needs to be done to determine if glutamatergic inputs to the extended amygdala are indeed differentially regulated by α_2-ARs, and if so, how α_2-ARs modulate each of these inputs. Optogenetic approaches may provide a powerful tool to resolve the effect of α_2-AR activation on specific inputs to the extended amygdala.

Conclusion

Evidence implicates ARs in the extended amygdala as being important in stress-induced reinstatement of drug-seeking. In the BNST, activation of α_1-ARs depresses excitatory synaptic transmission, enhances inhibitory synaptic transmission, and modulates the stress response following prolonged exposure to stressors. Depressing excitatory transmission or enhancing inhibitory transmission in the BNST could lead to decreased strength of the inhibitory projection from the BNST to the PVN, and therefore decreased inhibition of the PVN and an enhanced stress response in the body. Therefore, α_1-AR antagonists in the extended amygdala may attenuate these effects on excitatory and inhibitory transmission, thus attenuating the stress response of the body, which is consistent with previous findings of decreased plasma ACTH upon injection of α_1-AR antagonists into the BNST[42]. This attenuation of the stress response likely does not contribute to α_1-AR antagonists' ability to block stress-induced reinstatement in the BNST, as β-AR antagonists injected into the BNST also block re-

instatement, but do not decrease plasma ACTH[42]. Perhaps instead, block of stress-induced reinstatement is mediated through changes in strength of the BNST projection to the VTA. α_2- and β-ARs seem to influence negative symptoms of withdrawal, as α_2-AR agonists and β-AR antagonists can block withdrawal-mediated conditioned place aversion[43], perhaps implicating reward, as opposed to stress, circuitry in attenuating stress-induced reinstatement. Further, β- and α_2-ARs may be critical in integrating information from different inputs to the extended amygdala. β-ARs may integrate signals from different neurotransmitters, as β-AR-mediated increases in excitatory transmission rely on signaling through other receptors, such as α_2-AR[6] and CRFR1[57]. Activation of these other receptors may help to determine the initial state of β-AR responsiveness to NE, thus determining subsequent response to β-AR agonists[6]. Finally, α_2-ARs play a role in transient depression of excitatory transmission, and may differentially modulate excitatory inputs to the extended amygdala. Differential modulation would allow for certain inputs to dominate regulation of synaptic transmission in the extended amygdala, depending on the neural context of information reaching the BNST. Integration of inputs to the extended amygdala, and modulation of neural activity within the region, may allow noradrenergic receptors to regulate stress-induced reinstatement to drug-seeking.

References

1. Weiss F and Koob GF (2001). Drug addiction: functional neurotoxicity of the brain reward systems. Neurotoxicity research. 3 (1): 145-156.

2. Brown S, Vik PW, Patterson TL, Grant I and Shuckit MA (1995). Stress, Vulnerability and Adult Alcohol Relapse. Journal of Studies on Alcohol. 56: 538-545.

3. Sinha RDC and O'Malley S (1999). Stress-induced craving and stress resoponse in cocaine dependent individuals. Psychopharmacology. 142: 343-351.

4. Sinha R, Shaham Y and Heilig M (2011). Translational and reverse translational research on the role of stress in drug craving and relapse. Psychopharmacology.

5. Egli RE and Winder DG (2003). Dorsal and ventral distribution of excitable and synaptic properties of neurons of the bed nucleus of the stria terminalis. Journal of neurophysiology. 90 (1): 405-414.

6. Egli RE, Kash TL, Choo K, Savchenko V, Matthews RT, Blakely RD and Winder DG (2005). Norepinephrine modulates glutamatergic transmission in the bed nucleus of the stria terminalis. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 30 (4): 657-668.

This paper demonstrates that norepinephrine applied to the BNST is capable of either enhancing or depressing excitatory transmission. The enhancement of excitatory transmission was shown to occur through β-ARs, with depression of excitatory transmission being mediated by α_2-ARs.

7. Shields AD, Wang Q and Winder DG (2009). alpha2A-adrenergic receptors heterosynaptically regulate glutamatergic transmission in the bed nucleus of the stria terminalis. Neuroscience. 163 (1): 339-351.

This paper demonstrates the heterosynaptic role of α_{2A}-ARs in depressing glutamatergic synaptic transmission. It also provides evidence that α_{2A}-ARs may also depress inhibitory transmission in the BNST. Finally, the paper provides immunohistochemical evidence for the heterosynaptic role of α_2-ARs in regulating glutamatergic transmission in the BNST.

8. McElligott ZA and Winder DG (2008). Alpha1-adrenergic receptor-induced heterosynaptic long-term depression in the bed nucleus of the stria terminalis is disrupted in mouse models of affective disorders. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 33 (10): 2313-2323.

9. Delaney AJ, Crane JW and Sah P (2007). Noradrenaline modulates transmission at a central synapse by a presynaptic mechanism. Neuron. 56 (5): 880-892.

This paper demonstrates interesting evidence for the differential modulation of individuals inputs to the extended amygdala by α_2-ARs. α_2-AR activation is shown to depress the excitatory input from the parabrachial nucleus to the CeA, but α_2-AR activation has no effect on the excitatory input from the BLA to the CeA. My studies will look at the differential modulation of excitatory inputs to the BNST by α_2-ARs.

10. Leri F, Flores J, Rodaros D and Stewart J (2002). Blockade of stress-induced but not cocaine-induced reinstatement by infusion of noradrenergic antagonists into the bed nucleus of the stria terminalis or the central nucleus of the amygdala. The Journal of neuroscience : the official journal of the Society for Neuroscience. 22 (13): 5713-5718.

11. Mantsch JR, Weyer A, Vranjkovic O, Beyer CE, Baker DA and Caretta H (2010). Involvement of noradrenergic neurotransmission in the stress- but not cocaine-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: role for beta-2 adrenergic receptors. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 35 (11): 2165-2178.

12. Wang X, Cen X and Lu L (2001). Noradrenaline in the bed nucleus of the stria terminalis is critical for stress-induced reactivation of morphine-conditioned place preference in rats. Eur J Pharmacol. 432 (2-3): 153-161.

13. Sinha R, Kimmerling A, Doebrick C and Kosten TR (2007). Effects of lofexidine on stress-induced and cue-induced opioid craving and opioid abstinence rates: preliminary findings. Psychopharmacology. 190 (4): 569-574.

14. Jobes ML, Ghitza UE, Epstein DH, Phillips KA, Heishman SJ and Preston KL (2011). Clonidine blocks stress-induced craving in cocaine users. Psychopharmacology.

15. Sallee FR and Eaton K (2010). Guanfacine extended-release for attention-deficit/hyperactivity disorder (ADHD). Expert opinion on pharmacotherapy. 11 (15): 2549-2556.

16. Alheid GF and Heimer L (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience. 27 (1): 1-39.

17. Alheid GF (2003). Extended amygdala and basal forebrain. Annals of the New York Academy of Sciences. 985: 185-205.

18. Walker D (2003). Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. European Journal

of Pharmacology. 463 (1-3): 199-216.
19. Dong HW and Swanson LW (2006). Projections from bed nuclei of the stria terminalis, anteromedial area: cerebral hemisphere integration of neuroendocrine, autonomic, and behavioral aspects of energy balance. The Journal of comparative neurology. 494 (1): 142-178.
20. Shammah-Lagnado SJ, Beltramino CA, McDonald AJ, Miselis RR, Yang M, de Olmos J, Heimer L and Alheid GF (2000). Supracapsular bed nucleus of the stria terminalis contains central and medial extended amygdala elements: evidence from anterograde and retrograde tracing experiments in the rat. The Journal of comparative neurology. 422 (4): 533-555.
21. Dong HW, Petrovich GD and Swanson LW (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain research. Brain research reviews. 38 (1-2): 192-246.
22. Choi DC, Furay AR, Evanson NK, Ostrander MM, Ulrich-Lai YM and Herman JP (2007). Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic-pituitary-adrenal axis activity: implications for the integration of limbic inputs. The Journal of neuroscience : the official journal of the Society for Neuroscience. 27 (8): 2025-2034.
23. Cullinan WE, Herman JP and Watson SJ (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. The Journal of comparative neurology. 332 (1): 1-20.
24. Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC and Cullinan WE (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers in neuroendocrinology. 24 (3): 151-180.
25. Herman JP, Cullinan WE and Watson SJ (1994). Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. Journal of neuroendocrinology. 6 (4): 433-442.
26. Harris GW (1948). Neural control of the pituitary gland. Physiological reviews. 28 (2): 139-179.
27. Dong HW, Petrovich GD, Watts AG and Swanson LW (2001). Basic organization of projections from the oval and fusiform nuclei of the bed nuclei of the stria terminalis in adult rat brain. The Journal of comparative neurology. 436 (4): 430-455.
28. Georges F and Aston-Jones G (2001). Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. The Journal of neuroscience : the official journal of the Society for Neuroscience. 21 (16): RC160.
29. Georges F and Aston-Jones G (2002). Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 22 (12): 5173-5187.
30. Dumont EC and Williams JT (2004). Noradrenaline triggers GABAA inhibition of bed nucleus of the stria terminalis neurons projecting to the ventral tegmental area. The Journal of neuroscience: the official journal of the Society for Neuroscience. 24 (38): 8198-8204.
31. White FJ (1996). Synaptic regulation of mesocorticolimbic dopamine neurons. Annual review of neuroscience. 19: 405-436.
32. Briand LA, Vassoler FM, Pierce RC, Valentino RJ and Blendy JA (2010). Ventral tegmental afferents in stress-induced reinstatement: the role of cAMP response element-binding protein. The Journal of neuroscience: the official journal of the Society for Neuroscience. 30 (48): 16149-16159.
33. Forray MI and Gysling K (2004). Role of noradrenergic projections to the bed nucleus of the stria terminalis in the regulation of the hypothalamic-pituitary-adrenal axis. Brain research. Brain research reviews. 47 (1-3): 145-160.
34. Zardetto-Smith AM and Gray TS (1990). Organization of peptidergic and catecholaminergic efferents from the nucleus of the solitary tract to the rat amygdala. Brain research bulletin. 25 (6): 875-887.
35. Fallon JH and Moore RY (1978). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. The Journal of comparative neurology. 180 (3): 545-580.
36. Moore RY and Bloom FE (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annual review of neuroscience. 2: 113-168.
37. Ricardo JA and Koh ET (1978). Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. Brain research. 153 (1): 1-26.
38. Woulfe JM, Hrycyshyn AW and Flumerfelt BA (1988). Collateral axonal projections from the A1 noradrenergic cell group to the paraventricular nucleus and bed nucleus of the stria terminalis in the rat. Experimental neurology. 102 (1): 121-124.
39. Banihashemi L and Rinaman L (2006). Noradrenergic inputs to the bed nucleus of the stria terminalis and paraventricular nucleus of the hypothalamus underlie hypothalamic-pituitary-adrenal axis but not hypophagic or conditioned avoidance responses to systemic yohimbine. The Journal of neuroscience: the official journal of the Society for Neuroscience. 26 (44): 11442-11453.
40. Pacak K, McCarty R, Palkovits M, Kopin IJ and Goldstein DS (1995). Effects of immobilization on in vivo release of norepinephrine in the bed nucleus of the stria terminalis in conscious rats. Brain research. 688 (1-2): 242-246.
41. Cecchi M, Khoshbouei H and Morilak DA (2002). Modulatory effects of norepinephrine, acting on alpha1 receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. Neuropharmacology. 43 (7): 1139-1147.
42. Cecchi M, Khoshbouei H, Javors M and Morilak DA (2002). Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. Neuroscience. 112 (1): 13-21.
43. Delfs JM, Zhu Y, Druhan JP and Aston-Jones G (2000). Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. Nature. 403 (6768): 430-434.
44. Olson VG, Heusner CL, Bland RJ, During MJ, Weinshenker D and Palmiter RD (2006). Role of noradrenergic signaling by the nucleus tractus solitarius in mediating opiate reward. Science. 311 (5763): 1017-1020.
45. Ma S and Morilak DA (2005). Norepinephrine release in medial amygdala facilitates activation of the hypothalamic-pituitary-adrenal axis in response to acute immobilisation stress. Journal of neuroendocrinology. 17 (1): 22-28.
46. Aston-Jones G, Delfs JM, Druhan J and Zhu Y (1999). The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Annals of the New York Academy of Sciences. 877: 486-498.
47. Fuentealba JA, Forray MI and Gysling K (2000). Chronic

morphine treatment and withdrawal increase extracellular levels of norepinephrine in the rat bed nucleus of the stria terminalis. Journal of neurochemistry. 75 (2): 741-748.
48. Shaham Y, Highfield D, Delfs J, Leung S and Stewart J (2000). Clonidine blocks stress-induced reinstatement of heroin seeking in rats: an effect independent of locus coeruleus noradrenergic neurons. The European journal of neuroscience. 12 (1): 292-302.
49. Brown ZJ, Nobrega JN and Erb S (2011). Central injections of noradrenaline induce reinstatement of cocaine seeking and increase c-fos mRNA expression in the extended amygdala. Behavioural brain research. 217 (2): 472-476.
50. Shaham Y, Shalev U, Lu L, De Wit H and Stewart J (2003). The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology. 168 (1-2): 3-20.
51. Carter AJ (1997). Hippocampal Noradrenaline Release in Awak, Freely Moving Rats Is Regulated by Alpha-2 Adrenoceptors but Not be Adenosinse Receptors. The Journal of Pharmacology and Experimental Therapeutics. 281 (2): 648-654.
52. Bylund DB ED, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR, Trendelenburg U (1994). International Union of Pharmacology Nomenclature of Adrenoreceptors. Pharmacology Reviews. 46 (2): 121-136.
53. Hein L (2006). Adrenoceptors and signal transduction in neurons. Cell and tissue research. 326 (2): 541-551.
54. Highfield D, Yap J, Grimm JW, Shalev U and Shaham Y (2001). Repeated lofexidine treatment attenuates stress-induced, but not drug cues-induced reinstatement of a heroin-cocaine mixture (speedball) seeking in rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 25 (3): 320-331.
55. Erb S, Hitchcott PK, Rajabi H, Mueller D, Shaham Y and Stewart J (2000). Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 23 (2): 138-150.
56. Le AD, Funk D, Juzytsch W, Coen K, Navarre BM, Cifani C and Shaham Y (2011). Effect of prazosin and guanfacine on stress-induced reinstatement of alcohol and food seeking in rats. Psychopharmacology.
57. Nobis WP, Kash TL, Silberman Y and Winder DG (2011). beta-Adrenergic receptors enhance excitatory transmission in the bed nucleus of the stria terminalis through a corticotrophin-releasing factor receptor-dependent and cocaine-regulated mechanism. Biological psychiatry. 69 (11): 1083-1090.
58. McElligott ZA, Klug JR, Nobis WP, Patel S, Grueter BA, Kash TL and Winder DG (2010). Distinct forms of Gq-receptor-dependent plasticity of excitatory transmission in the BNST are differentially affected by stress. Proceedings of the National Academy of Sciences of the United States of America. 107 (5): 2271-2276.
59. Savchenko VL and Boughter JD, Jr. (2011). Regulation of Neuronal Activation by Alpha2A Adrenergic Receptor Agonist. Neurotoxicity research. 20 (3): 226-239.
This paper provides further evidence for a potential excitatory role for α_{2A}-ARs' modulation of glutamatergic transmission, as measured by c-fos staining. This paper is interesting because it contradicts with previous findings that show α_{2A}-ARs as depressing excitatory transmission.

Further Information
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CANDIDATE
REVIEWS

Reversal of Dopamine Flux in Health and Disease

Peter Hamilton

Abstract

The dopamine transporter (DAT) is responsible for regulating the concentration of dopamine (DA) in the synaptic space via a substrate re-uptake mechanism. This re-uptake mechanism can occur both as a facilitated exchange process, commonly described as the alternating access model (which is dependent on the concentration of Na^+, Cl^-, and DA), as well as a channel-like diffusion process where DA transport does not appear to be linked to Na^+ or Cl^- concentrations. The DAT is also capable of reverse transport of DA (DAT-RT) or moving DA from the cell cytosol into the extracellular space. This DAT-RT has been observed to occur under specific conditions: 1) upon the introduction of amphetamines (AMPH), 2) upon the activation of specific second messenger, DAT-influencing proteins, and 3) upon conditions of DAT sequence mutations, or coding variants of the DAT. Similar to DA re-uptake, this DAT-RT can occur both as a facilitated exchange process and a channel-like diffusion process. It is possible that the process of DA efflux, via a DAT-RT mechanism, plays a major, yet poorly understood, role in the DA homeostasis in the central nervous system. By further understanding the process of DAT-RT, it may be possible to shed new light on DA homeostasis and the diseases associated with alterations in this DA homeostasis.

Keywords

Addiction
Amphetamine
Dopamine
Transport
Mutation

Background

The neurotransmitter, dopamine (DA), plays an important role in the central nervous system by exerting influence over functions like voluntary movement, motivation, and reward[1]. DA's function as a neurotransmitter is regulated, in part, by the dopamine transporter (DAT) [2]. The DAT is a plasmalemmal phosphoprotein capable of DA re-uptake from the synaptic space, thereby limiting the post-synaptic exposure to DA which, in turn, regulates the intensity and duration of the dopaminergic response[2-4]. The DAT is also a vector for DA release, or DAT mediated reverse transport (DAT-RT), wherein the DAT is capable of slowly releasing large quantities of DA into the synaptic space via a vesicle-independent mechanism[2, 4, 5]. The DAT function, particularly DAT-RT function, can be altered by genetic mutations, activation of second messenger systems, and under the influence of exogenous pharmacological agents, such as psychostimulants like cocaine and amphetamines (AMPH)[6-8]. Therefore, alterations in DAT function by any of the aforementioned mechanisms will result in altered DA homeostasis.

Diseases implicated in altered DA homeostasis include attention deficit hyperactivity disorder (ADHD), affective disorders, schizophrenia, and drug abuse[6]. The National Institute of Mental Heath estimates that ADHD is exhibited by 3-5% of the American population. Adderal, a racemic mixture of S(+) and R(-) AMPH enantiomers, is the most prescribed treatment for juvenile ADHD in the United States. Furthermore, the number of American children exposed to an AMPH congener rose from 0.6 per 100 in 1987 to 2.4 per 100 in 1996[9]. The total annual economic cost of drug abuse in the United States rose from US$102 billion in 1992 to US$143 billion in 1998 and is currently estimated to be well above US$200 billion[10, 11]. Affective disorders and schizophrenia were attributed with contributing over US$300 billion to the global burden of disease in 2008 alone[11]. Given these data and innumerable other ways in which DA oriented diseases have affected individual and global health, it is important that the regulation of DA homeostasis be further understood.

This review will focus on the phenomenon of reverse transport of DA through the DAT and its role in DA homeostasis. This process of delivering large amounts of DA over extended periods may play a major, yet underappreciated, regulatory role in DA homeostasis. In this review, I will outline the mechanism for DA re-uptake via the DAT, followed by an overview of DAT-RT, detailing three documented factors that induce DAT-RT: (1) exposure to psy-

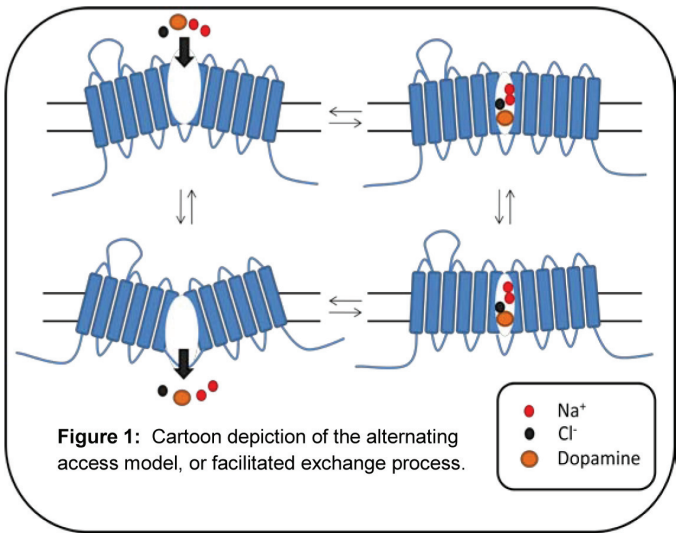

Figure 1: Cartoon depiction of the alternating access model, or facilitated exchange process.

chostimulants, particularly AMPH, (2) activation of second messenger signaling pathways, and (3) coding variants of the DAT.

Mechanism of Re-uptake

The DAT is a member of the solute carrier 6 (SLC6) gene family of Na^+/Cl^- symporters, which includes other monoamine transporters such as the serotonin transporter and the norepinephrine transporter. A characteristic of the transporters in this gene family is the use of the ion concentration gradient as the driving force for transporter-mediated re-uptake of their respective substrate[12]. In the case of the DAT, the uptake of DA is coupled with the translocation of two Na^+ ions and one Cl^- ion, resulting in a net movement of two positive charges per DA molecule (DA is positively charged at physiological pH)[13]. This results in the generation of an inward current in conditions of DA re-uptake. This model of moving two positive charges per transporter cycle stems from the classical alternating-access model of transporter function, which essentially assumes that the DAT function is analogous to a revolving door[14,15]. The model assumes that DAT is capable of an "outward-facing" conformation in which DA, Na^+, and Cl^- are required to bind to the transporter in a fixed ratio and induce a conformational change. This conformational change results in an "inward-facing" transporter and the dissociation of the cargo, thereby, completing the transport of DA from the synaptic space into the cytosol[14] (Figure 1).

Since the creation of the alternating-access model, researchers have been able to describe the types of conductance that occur in conjunction with substrate re-uptake through the DAT. They discovered a coupled "transport-associated" current, which obeys the properties of the alternating-access model and, notably, has a constitutive leakage conductance due to a previously unknown channel-like activity of the DAT where the transporter experiences a rapid, inward flux of ions and/or DA molecules without the requirement of Na^+ or Cl^-[16, 17]. The observation of the channel-like, constitutive leak conductance demonstrates that the DA re-uptake through the DAT behaves in a way that cannot be fully explained with classical alternating-access model of transporter function. This insight into the DAT function raises interesting questions about the molecular regulatory events that are responsible for this channel-like activity of the DAT, as well as questions about the directionality of the channel.

Reverse Transport of Dopamine via the Dopamine Transporter

There is an inherent asymmetry of ion concentration, substrate concentration, membrane potential, and DAT protein structure when using the neuronal cell membrane as the axis of symmetry. This asymmetry of obligate components for DA re-uptake would seem to suggest that the DAT is only capable of DA re-uptake. However there are very clear and documented instances of the reversal of the DAT function where the net flow of DA, mediated by the DAT, travels from the cell cytosol to the extracellular space[2, 4, 5, 7, 8, 17]. Therefore, these asymmetric conditions point to an asymmetry in the DAT function, with some molecular elements important for re-uptake and entirely different ones important for DAT-RT, depending on local and transient conditions. I will explore three conditions in which DAT-RT is known to occur: (1) exposure to psychostimulants, focusing on AMPH, (2) activation of second messenger signaling pathways, and (3) DAT coding variants.

Reverse Transport - Amphetamines

Pharmacological substances have long been used to study the process of DAT-RT, not only to understand the molecular events that surround the abuse of these substances, but also to study the molecular mechanisms that are required for reverse transport. Of these pharmacological substances, AMPH is the gold standard.

AMPH is a psychostimulant that exerts its physiological effect by primarily influencing the DAT[4, 18]. AMPH's molecular structure is very similar to DA and, as a result, is a substrate for the DAT. AMPH competitively inhibits DA re-uptake and ultimately promotes DAT-RT of

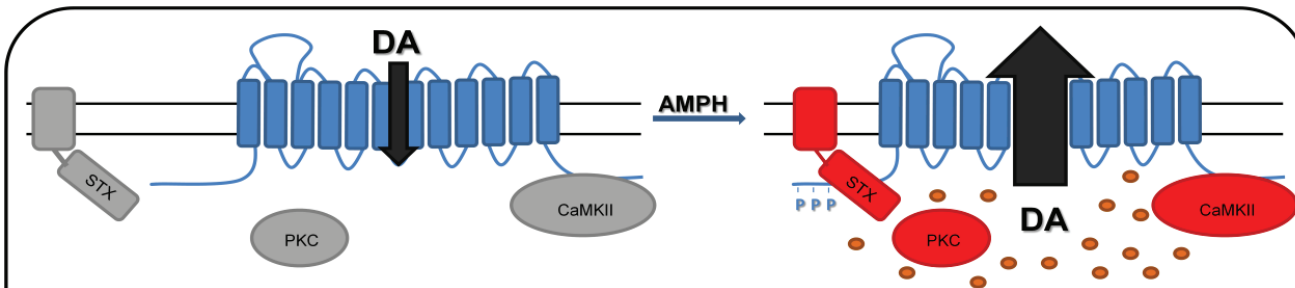

Figure 2: Cartoon depiction of molecular events surrounding the administration of AMPH. In basal conditions, the DAT favors re-uptake of DA. Administration of AMPH activates second messenger proteins, resulting in phosphorylation of N-terminus, promoting DAT-RT of DA molecules released from vesicles.

DA[18, 19]. Following the introduction of AMPH in the extracellular space, AMPH competes with other DAT substrates and interacts with the "outward-facing" conformation of the DAT. Thereafter, AMPH is transported into the intracellular space and interacts with the vesicular monoamine transporter. Being a weak base, AMPH alters the pH gradient between the cytoplasm and vesicles, resulting in the release of DA into the intracellular space. The newly released DA accesses the "inward-facing" conformation of the DAT and, along with the increased Na^+ concentration, results in DAT-RT of DA via a facilitated exchange process[20-22]. Other studies have revealed that this DAT-RT of DA can also occur in rapid bursts of DA efflux through the channel-like mode of the DAT which is independent of the facilitated exchange mechanism[17]. This process allowed researchers to conclude that the previously observed channel-like mechanism of DA re-uptake[16] is bi-directional and occurs in instances of DA re-uptake and DA efflux.

The scope of AMPH's influence on the DAT is not limited to direct interactions with the DAT protein itself; AMPH also appears to influence the local and transient environment surrounding the DAT which ultimately influences the properties of this transporter. Administration of extracellular AMPH increases the frequency of channel-like DA release via the DAT by approximately 8-fold[23]. Furthermore, exposure to AMPH shifts the DAT from a "reluctant" to a "willing" state-- an asymmetric state that favors AMPH-induced DA efflux via the DAT without disturbing normal DA re-uptake[24]. These modulations in DAT efflux are thought to be executed by the activities of DAT-interacting proteins (proteins that have become catalytically active in response to the administration of AMPH). The AMPH-dependent and –independent second messengers and associated proteins will be more thoroughly discussed in the next section.

Reverse Transport - Signaling Pathways

The model of AMPH-induced DA efflux cannot be accurately conveyed without considering its regulation by second messenger systems. As mentioned above, the administration of AMPH is thought to influence DAT-RT by altering the properties of DAT-associated proteins. For example, the first 22 amino acids of the intracellular-facing, N-terminal region of the DAT contain crucial serine residues that, when phosphorylated, alter the properties of DAT-RT. When truncated or replaced with non-phosphorylatable alanine residues, the AMPH-mediated DA efflux was reduced by 80%[24]. These N-terminal residues are phosphorylated by protein kinase C (PKC)[25, 26]. AMPH can increase the activity of PKC in vivo, and inhibition of PKC prevents AMPH-induced DAT-RT[25, 27]. Also, in the rat striatum, it was observed that there is a physical interaction between the DAT and $PKC\beta_{II}$[26].

Another major kinase implicated in the regulation of DAT-RT is CaMKII. CaMKII is also capable of phosphorylating the DAT N-terminal serine residues, and researchers have demonstrated that inactivation or inhibition of CaMKIIα reduces AMPH-induced DAT-RT[28]. A physical interaction between CaMKII and the DAT C-terminus is observed, and disruption of this interaction results in a diminished AMPH-induced DAT-RT[28]. A potential mediator of CaMKII's influence on the DAT-RT is the protein syntaxin 1 (STX). STX is a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) which is required for the docking and release of synaptic vesicles[29]. However, there is a growing body of evidence to suggest that STX associates with and regulates a variety of ion channels and neurotransmitter transporters, including the DAT[30, 31].

These DAT-STX interactions are demonstrated to increase in response to AMPH and are important for the regulation of AMPH-induced DAT-RT[30, 31]. The physical association between DAT and STX is also shown to influence the channel-like, AMPH-induced DA efflux via the DAT, and in C. Elegans a STX homologue suppresses the channel-like properties of the DAT-1[32, 33]. Moreover, researchers have established that the activity of the C-terminal associated CaMKII is responsible for mediating the DAT-STX interaction, and this interaction can be inhibited through the use of CaMKII inhibitors. Figure 2 depicts a representation of some of the molecular events that surround DAT-RT.

Reverse Transport – DAT Coding Variants

In the previous examples of DAT-RT, the efflux of DA via the DAT was stimulated by exogenous compounds (AMPH) and/or modulated by key second messenger proteins. However, researchers have recently described a DAT coding variant that is observed to produce an anomalous DAT-RT under physiologically normal conditions and without the administration of AMPH.

The A559V mutant of the DAT was identified in two brothers diagnosed with ADHD[6]. By cloning the DAT coding variant and expressing it in stable cell lines, the researchers were able to observe that the A559V mutant exhibited an anomalous DAT-RT that occurred under basal conditions[34]. The A559V DAT variant was also more sensitive to intracellular Na^+ concentrations and cell depolarization than wild-type DAT, as seen by increased magnitude of DAT-RT under these conditions[34]. These findings demonstrate the following: 1) a single amino-acid mutation in the DAT can produce significant functional differences in terms of DAT-RT properties and 2) the amino acid sequence of the DAT is important in regulating the dynamics of the DAT-RT.

Conclusions

As demonstrated by the studies presented in this review, the DAT is capable of both re-uptake of DA from the extracellular space and the release of DA via a non-vesicular, DAT-RT mechanism. The mechanisms of the DA re-uptake and DAT-RT are asymmetric; conditions that govern the properties of one mode of transport may not govern the other mode of transport. Despite this, both DA re-uptake and DAT-RT are capable of transporting DA via a facilitated exchange process and a channel-like process.

The significance of the DAT-RT mechanism is particularly remarkable considering that the amount of DA delivered via this flux can be larger than a vesicle-mediated, quantal release of DA delivered during activity dependent exocytosis[35]. In physiological terms, this indicates that the phenomenon of DAT-RT is capable of providing enough neurotransmitter to contribute to the micro-environment surrounding the neuron. This fact coupled with the observation that the DAT is localized not only in the synapse, but also extra-synaptically, on neuronal cell bodies, axons, as well as dendrites, and it becomes clear that dopaminergic neurons possess the capacity to significantly alter DA homeostasis through a potential DAT-RT mechanism[36, 37]. It is also clear that the DAT constantly maintains the capacity for DAT-RT as demonstrated by administration of AMPH, activation of key proteins, or expression of DAT coding variants that activate the DAT's endogenous potential for DAT-RT. Given these three scenarios for triggering DAT-RT, it is reasonable to begin asking the following questions: Does the DAT-RT occur in the CNS, under physiological conditions, without exposure to psychostimulants or genetic mutations? Given that the DAT-RT machinery exists and is exploited by the mechanisms outlined in this review, are there endogenous mechanisms for activating the second messenger proteins that result in DAT-RT? Is this potential DA release enough to act as a viable mechanism of neurotransmission? If any of these questions were even partly true, it would not be difficult to imagine the influence that DAT-RT would have on DA homeostasis in both health and disease.

References

1. Bjorklund A and Dunnett SB (2007). Fifty years of dopamine research. Trends Neurosci. 30 (5): 185-187.
2. Giros B, Jaber M, Jones SR, Wightman RM and Caron MG (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature. 379 (6566): 606-612.
3. Amara SG and Kuhar MJ (1993). Neurotransmitter transporters: recent progress. Annu Rev Neurosci. 16: 73-93.
4. Jones SR, Gainetdinov RR, Wightman RM and Caron MG (1998). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci. 18 (6): 1979-1986. This paper demonstrated the DA releasing actions of AMPH in striatal tissue slices of both wild-type and DAT knock-out mice. Using fast scan cyclic voltammatry, the authors could demonstrate the kinetics of vesicular DA release, DA diffusion, and AMPH-induced DA efflux.
5. Sulzer D, Maidment NT and Rayport S (1993). Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. J Neurochem. 60 (2): 527-535.
6. Mazei-Robison MS, Couch RS, Shelton RC, Stein MA and Blakely RD (2005). Sequence variation in the human dopamine transporter gene in children with attention deficit hyperactivity disorder.

Neuropharmacology. 49 (6): 724-736.
7. Bowton E, Saunders C, Erreger K, Sakrikar D, Matthies HJ, Sen N, Jessen T, Colbran RJ, Caron MG, Javitch JA, Blakely RD and Galli A (2010). Dysregulation of dopamine transporters via dopamine D2 autoreceptors triggers anomalous dopamine efflux associated with attention-deficit hyperactivity disorder. J Neurosci. 30 (17): 6048-6057.
8. Eshleman AJ, Henningsen RA, Neve KA and Janowsky A (1994). Release of dopamine via the human transporter. Mol Pharmacol. 45 (2): 312-316.
9. Olfson M, Marcus SC, Weissman MM and Jensen PS (2002). National trends in the use of psychotropic medications by children. J Am Acad Child Adolesc Psychiatry. 41 (5): 514-521.
10. Cartwright WS (2008). Economic costs of drug abuse: Financial, cost of illness, and services. Journal of Substance Abuse Treatment. 34 (2): 224-233.
11. Eaton WW, Martins SS, Nestadt G, Bienvenu OJ, Clarke D and Alexandre P (2008). The burden of mental disorders. Epidemiol Rev. 30: 1-14.
12. Gu H, Wall SC and Rudnick G (1994). Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. J Biol Chem. 269 (10): 7124-7130.
13. Krueger BK (1990). Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus. J Neurochem. 55 (1): 260-267.
14. Bogdanski DF and Brodie BB (1969). The effects of inorganic ions on the storage and uptake of H3-norepinephrine by rat heart slices. J Pharmacol Exp Ther. 165 (2): 181-189.
15. Jardetzky O (1966). Simple allosteric model for membrane pumps. Nature. 211 (5052): 969-970.
16. Sonders MS, Zhu SJ, Zahniser NR, Kavanaugh MP and Amara SG (1997). Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. J Neurosci. 17 (3): 960-974.
This paper demonstrated that the re-uptake of DA via the DAT occurs both through a facilitated exchange mechanism, which follows the dynamics of the alternating access model, and through a channel-like re-uptake mechanism ,which is independent of the alternating access model.
17. Kahlig KM, Binda F, Khoshbouei H, Blakely RD, McMahon DG, Javitch JA and Galli A (2005). Amphetamine induces dopamine efflux through a dopamine transporter channel. Proc Natl Acad Sci U S A. 102 (9): 3495-3500.
This paper demonstrated that the previously discovered channel-like property of the DAT is bi-directional and also occurs for the AMPH-induced efflux of DA. This channel-like property of the DAT is responsible for transporting large quantities of DA.
18. Sitte HH, Huck S, Reither H, Boehm S, Singer EA and Pifl C (1998). Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells transfected with the human dopamine transporter. J Neurochem. 71 (3): 1289-1297.
19. Wall SC, Gu H and Rudnick G (1995). Biogenic amine flux mediated by cloned transporters stably expressed in cultured cell lines: amphetamine specificity for inhibition and efflux. Mol Pharmacol. 47 (3): 544-550.
20. Sulzer D and Rayport S (1990). Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. Neuron. 5 (6): 797-808.
21. Pifl C, Drobny H, Reither H, Hornykiewicz O and Singer EA (1995). Mechanism of the dopamine-releasing actions of amphetamine and cocaine: plasmalemmal dopamine transporter versus vesicular monoamine transporter. Mol Pharmacol. 47 (2): 368-373.
22. Wilhelm CJ, Johnson RA, Lysko PG, Eshleman AJ and Janowsky A (2004). Effects of methamphetamine and lobeline on vesicular monoamine and dopamine transporter-mediated dopamine release in a cotransfected model system. J Pharmacol Exp Ther. 310 (3): 1142-1151.
23. Pothos EN, Davila V and Sulzer D (1998). Presynaptic recording of quanta from midbrain dopamine neurons and modulation of the quantal size. J Neurosci. 18 (11): 4106-4118.
24. Khoshbouei H, Sen N, Guptaroy B, Johnson L, Lund D, Gnegy ME, Galli A and Javitch JA (2004). N-terminal phosphorylation of the dopamine transporter is required for amphetamine-induced efflux. PLoS Biol. 2 (3): E78.
25. Giambalvo CT (1992). Protein kinase C and dopamine transport--1. Effects of amphetamine in vivo. Neuropharmacology. 31 (12): 1201-1210.
26. Johnson LA, Guptaroy B, Lund D, Shamban S and Gnegy ME (2005). Regulation of amphetamine-stimulated dopamine efflux by protein kinase C beta. J Biol Chem. 280 (12): 10914-10919.
27. Kantor L and Gnegy ME (1998). Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices. J Pharmacol Exp Ther. 284 (2): 592-598.
28. Fog JU, Khoshbouei H, Holy M, Owens WA, Vaegter CB, Sen N, Nikandrova Y, Bowton E, McMahon DG, Colbran RJ, Daws LC, Sitte HH, Javitch JA, Galli A and Gether U (2006). Calmodulin kinase II interacts with the dopamine transporter C terminus to regulate amphetamine-induced reverse transport. Neuron. 51 (4): 417-429.
29. McMahon HT and Sudhof TC (1995). Synaptic core complex of synaptobrevin, syntaxin, and SNAP25 forms high affinity alpha-SNAP binding site. J Biol Chem. 270 (5): 2213-2217.
30. Binda F, Dipace C, Bowton E, Robertson SD, Lute BJ, Fog JU, Zhang M, Sen N, Colbran RJ, Gnegy ME, Gether U, Javitch JA, Erreger K and Galli A (2008). Syntaxin 1A interaction with the dopamine transporter promotes amphetamine-induced dopamine efflux. Mol Pharmacol. 74 (4): 1101-1108.
This paper highlights the interactions between syntaxin 1A and the N-terminus of the DAT. The authors demonstrate that this physical interaction is facilitated by the first 33 amino acids on the N-terminus of the DAT, and overexpression of syntaxin 1A results in significantly greater AMPH-induced DA efflux. It is an example of the role of a DAT-regulating protein in DAT-RT.
31. Dipace C, Sung U, Binda F, Blakely RD and Galli A (2007). Amphetamine induces a calcium/calmodulin-dependent protein kinase II-dependent reduction in norepinephrine transporter surface expression linked to changes in syntaxin 1A/transporter complexes. Mol Pharmacol. 71 (1): 230-239.
32. Sung U, Apparsundaram S, Galli A, Kahlig KM, Savchenko V, Schroeter S, Quick MW and Blakely RD (2003). A regulated interaction of syntaxin 1A with the antidepressant-sensitive norepinephrine transporter establishes catecholamine clearance capacity. J Neurosci. 23 (5): 1697-1709.
33. Carvelli L, Blakely RD and DeFelice LJ (2008). Dopamine transporter/syntaxin 1A interactions regulate transporter channel activity and dopaminergic synaptic transmission. Proc Natl Acad Sci U S A. 105 (37): 14192-14197.

34. Mazei-Robison MS, Bowton E, Holy M, Schmudermaier M, Freissmuth M, Sitte HH, Galli A and Blakely RD (2008). Anomalous dopamine release associated with a human dopamine transporter coding variant. J Neurosci. 28 (28): 7040-7046.
35. Grace AA (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience. 41 (1): 1-24.
36. Nirenberg MJ, Vaughan RA, Uhl GR, Kuhar MJ and Pickel VM (1996). The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. J Neurosci. 16 (2): 436-447.
37. Mengual E and Pickel VM (2004). Regional and subcellular compartmentation of the dopamine transporter and tyrosine hydroxylase in the rat ventral pallidum. J Comp Neurol. 468 (3): 395-409.

FURTHER INFORMATION

Aurelio Galli's Lab: https://medschool.mc.vanderbilt.edu/facultydata/php_files/part_dept/show_faculty/show_part-molecular.php?id3=4070

CANDIDATE
REVIEWS

Linking Together the Mammalian Circadian Clock

Jeffrey Jones

Abstract

Mammalian circadian rhythms are thought to be driven by the suprachiasmatic nucleus (SCN), the master biological pacemaker. Each SCN neuron contains a set of "clock genes" that are transcribed, translated, and degraded every ~24 hours. Outputs from the molecular clock influence intrinsic ionic currents to generate cell-autonomous rhythms in action potential frequency. The firing pattern output of the SCN neural network ultimately synchronizes daily rhythms in behavior and physiology. This review presents our current understanding of how clock genes influence electrical activity, and how electrical activity influences circadian behavior.

Keywords

Circadian
Suprachiasmatic
Electrophysiology
Clock genes
Firing rate
Behavior

Introduction

The fact that we can wake and sleep on a regular schedule without the help of an alarm clock hints at the existence of some internal time-keeping mechanism that influences our daily behavior. Indeed, nearly every organism on the planet, from cyanobacteria and plants to mice and humans, has evolved a way to anticipate the daily light-dark cycle that results from the Earth's approximately 24-hour, or circadian, rotation about its axis. In mammals, the timekeeper is found in the SCN, a small, bilateral collection of ~20,000 neurons located above the optic chiasm in the hypothalamus[1]. The SCN is unique in that it not only generates self-sustaining oscillations, but also directly receives photic input from the retina. This input allows it to entrain to the external light cycle by synchronizing downstream cellular oscillators, ultimately leading to overt circadian behavioral and physiological rhythms[2]. Locomotor rhythms are lost when the SCN is lesioned, but can be restored by grafting SCN tissue into the arrhythmic animal[3]. The period of the restored rhythmicity is determined by the period of the donor SCN rather than that of the lesioned host, thus firmly establishing the SCN as the master circadian pacemaker[4-6].

While circadian outputs involve the emergent properties of the entire interconnected SCN neural network, individual SCN neurons are autonomous pacemakers with a period of approximately 24 hours. Each neuron exhibits both endogenous molecular rhythms and endogenous electrophysiological rhythms of high daytime and low nighttime spontaneous firing rate[1]. A key question in chronobiology is how these parts interact to make a coherent circadian pacemaker: how genes are linked to electrical rhythms, and how electrical rhythms are linked to behavior. A helpful analogy for this interconnection of circadian clock components is to think of the molecular oscillations as the "gears" of a clock, which move the clock's "hands" – its daily rhythms in electrical activity[7]. And just as looking at the hands on a clock can let a student know that he is late for class, the hands of the SCN clock seem to be able to dictate behavioral rhythms. Understanding the interconnectivity of the SCN is essential because the dysregulation of the core clock components plays a role in a variety of neuropsychiatric disorders including depression, sleep disorders, and autism[1,8,9]. Therefore this review will introduce the genetic, electrical, and behavioral components of the circadian clock and will examine the evidence linking together the gears and the hands, and the hands and behavior.

Gears

Molecular basis for rhythmicity. In each SCN neuron there exists an auto-regulatory transcription / translation negative feedback loop of core clock genes consisting of the *Period* genes *Per1* and *Per2*, the *Cryptochrome* genes *Cry1* and *Cry2*, *Clock* and *Bmal1*[1,10-12] (Figure 1). The positive arm of the core feedback loop begins when CLOCK and BMAL1 are translated and heterodimerize outside the nucleus. The complex then enters the nucleus and binds to the E-box sequence (CACGTG) of the *Per* and *Cry* promoter regions, activates their transcription, and begins the negative arm of the feedback loop. Various combinations of PER and CRY proteins then heterodimerize outside the nucleus where they are first phosphorylated by casein kinase 1 ε/δ (CK1ε/δ) and then enter the nucleus to inhibit the CLOCK/BMAL1 heterodimer, effectively inhibiting their own transcription.

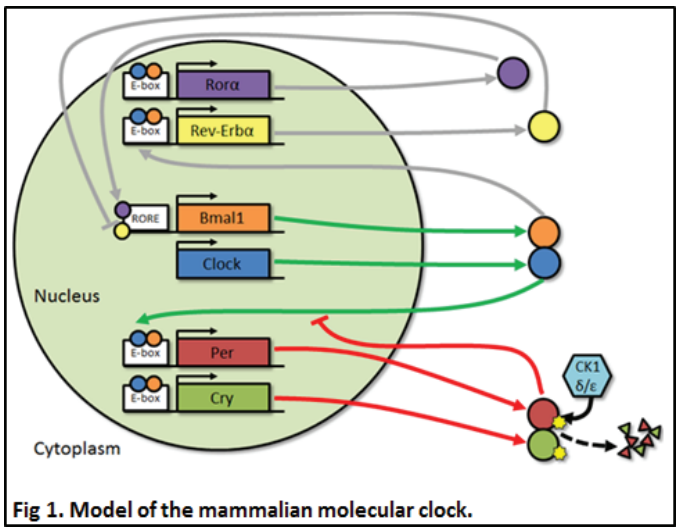

Fig 1. Model of the mammalian molecular clock.

Progressive phosphorylation of PER and CRY by CK1ε/δ leads to their degradation, which releases their inhibition of CLOCK/BMAL1 and restarts the feedback loop. A second negative feedback loop consisting of REV-ERBα and RORα contributes to clock precision and robustness by regulating the transcription of *Bmal1*[1,10,11].

Linking the gears to the hands. A series of experiments involving animals with mutations in their molecular clockwork provided the first evidence linking the gears of the clock to its hands. Multielectrode array recordings of spontaneous neural activity from hamsters with the dominant-negative *tau* (R178C) mutation in CK1ε showed a drastic reduction in the normal 24-hour period of electrical activity with homozygous mutants exhibiting shorter periods than heterozygotes[13]. *tau* was later found to promote the degradation of PER protein, causing an acceleration of the molecular feedback loop that paralleled the reduction in the period of electrical activity[14]. Using the same recording technique, neurons from mice heterozygous for an exon-19 deletion in the core clock gene *Clock* were found to have a lengthened period of electrical activity. Neurons from homozygous mutants, depending on the study, exhibited either an even longer period or complete arrhythmicity[15,16]. Likewise, when again recorded with a multielectrode array, mice with a double knockout of the essential core clock genes *Cry1* and *Cry2* exhibited a complete lack of circadian oscillation in firing rate[17].

These results with mutant animals clearly indicate a necessary interaction between the molecular clock and electrical activity but raise the question of how clock genes interact in a wild-type mouse. To address this, brain slices from mice expressing a degradable form of green fluorescent protein (GFP) under the control of the *Per1* promoter were used as a real-time indicator of single-cell molecular rhythms in combination with patch-clamp electrophysiological recording[18,19]. It was initially found that after a nighttime light pulse, the degree of *Per1* induction as reported by the fluorescent reporter was positively correlated with the frequency of spontaneous firing in individual neurons[18]. Similar experiments showed that such a correlation between GFP fluorescence and firing rate could be obtained at midday[19]. These results seem to indicate that SCN neurons increase their firing rate in tandem with an increase in *Per1* promoter activity. A computational model of SCN neuron pacemaker activity also suggests that there is a positive correlation between *Per1* mRNA levels and firing rate[20]. Evidence from a more recent study seems to contradict these previous results by suggesting that SCN neurons expressing maximal levels of *Per1* during midday are in fact electrically silent. These *Per1*-positive neurons are so depolarized that they exhibit depolarization block and cannot fire action potentials. The cells exhibiting the previously reported high daily firing rate appear to be *Per1*-negative[21]. Future experiments are necessary to clarify the relationship between *Per1* and firing rate.

Hands

Ionic basis of electrical rhythms. SCN neurons are unique in that not only do they fire spontaneously, but they also rhythmically alter their firing rate with a high firing rate during the day and a low firing rate at night (Figure 2). In most neurons, firing rate is set by synaptic activity, which is required for a cell's resting membrane potential to reach spike threshold and elicit an action potential. SCN neurons, however, are intrinsically driven towards spike threshold by a depolarizing persistent, slowly-inactivating Na^+ current[22-24], which opens voltage-gated Na^+ and L-type Ca^{2+} channels and initiates the characteristic "spike" of an action potential[23-25]. The membrane subsequently hyperpolarizes due to the opening of fast-delayed rectifier (FDR) and A-type (IA) K^+ channels[26,27]. After-hyperpolarization and repolarization back to resting membrane potential are thought to be due to the closing of voltage-gated Na^+ and Ca^{2+} channels and the opening of Ca^{2+}-dependent K^+ channels, particularly the large conductance BK channel[28-30]. The cell is then ready to fire again – at a rate that is circadianly modulated.

The first evidence for the ionic mechanism behind the circadian modulation of firing rate came from record-

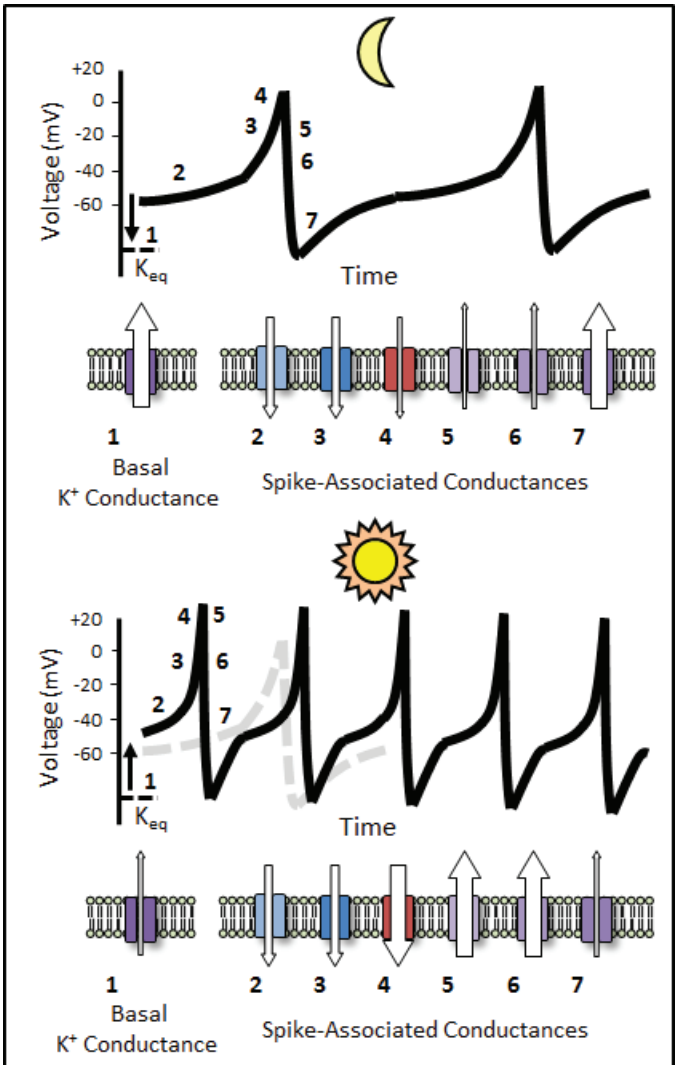

Fig 2. Ionic basis of SCN electrical rhythms. *(1)* Leak K^+ channel; *(2)* Persistent Na^+ channel; *(3)* Voltage-gated Na^+ channel; *(4)* L-type Ca^{2+} channel; *(5)* FDR K^+ channel; *(6)* A-type K^+ channel; *(7)* BK channel. Size of arrows represents relative size of current passing through channels.

ings taken from the basal retinal neurons of the sea slug *Bulla gouldiana*. Circadian rhythms in compound action potentials were shown to be driven by rhythms in resting membrane potential[31]. These daily membrane potential rhythms also exist in the neurons of the mammalian SCN, which, due to a daytime decrease in "leak" K^+ channel conductance, are depolarized and have a high input resistance (low conductance) during the day and are hyperpolarized and have a low input resistance at night[32,33]. A nighttime increase in K^+ conductance drives the neuron's resting membrane potential closer to the hyperpolarized equilibrium potential for K^+ and makes it harder for the cell to elicit a spike. Conversely, a daytime decrease in K^+ conductance brings the cell closer to spike threshold[34]. In addition to the rhythms in K^+ conductance, SCN neurons also show diurnal L-type Ca^{2+} channel-dependent current oscillations that may facilitate the high firing rate during the day[25]. Finally, circadian modulation of ionic conductance contributes to circadian rhythms in firing rate: the depolarizing L-type Ca2+ and the hyperpolarizing FDR and IA currents are higher during the day, which contribute to higher daytime spike rate[25-27,35]. The repolarizing BK current is higher during the night, which most likely contributes to a low nighttime spike rate by lengthening the refractory period required to initiate a new spike[28-30,35].

Linking the hands to behavior. Over two decades ago, it was shown that blocking electrical activity by transiently infusing the Na^+ channel blocker tetrodotoxin (TTX) into the SCN of freely behaving rats abolishes locomotor activity rhythms. However, once the drug was washed out, the animals resumed locomotor activity with an unaltered phase. These results strongly suggest that electrical activity is required for overt expression of circadian rhythms, but not for accurate molecular timekeeping[7]. More recent work either agrees with this[29,36] or instead implicates SCN electrical activity as being essential for robust molecular oscillations, as, for example, TTX or membrane hyperpolarization appears to dramatically but reversibly dampen clock gene rhythms[1,37,38]. Either way, it is now clear that electrical activity serves as the output of the intracellular clock. The calcium-activated BK channel, which normally suppresses spontaneous firing rate in the SCN at night, has been found to be necessary for circadian rhythms in locomotor activity. Mutant mice lacking the BK channel exhibit severely disrupted circadian behavioral rhythms and altered neuronal activity. Extracellular recordings taken during the daytime and nighttime from wild-type and knockout mice showed that BK-null mice had much higher firing rates at night while still having normal expression of clock genes such as *Bmal1*[29].

From this it seems probable that BK channels comprise at least part of the SCN output leading to behavior. Indeed, long-term multielectrode array recordings from BK-null mice demonstrate that the behavioral deficit appears to be due to a disruption of circadian rhythms in spontaneous firing rate[30]. Likewise, mice with a genetic knockout that attenuates the current carried by FDR K^+ channels, which greatly reduces the daytime spontaneous firing rate recorded from SCN neurons, were either behaviorally arrhythmic or exhibited severely attenuated rhythms. However, they did not show altered PER2 expression[36]. *In vivo* multiple-unit

recordings in freely-moving mice further clarified the link between electrical activity and behavior by determining that the onset and offset of behavioral activity corresponded with the half-maximal spontaneous firing rate[39].

Together, these studies indicate that firing rate is highly correlated with behavior. Intriguingly, however, a recent study found that the behavioral activity phase of nocturnal mice that were forced to work for food was shifted into the day. The behavior of these mice immediately reversed to the nocturnal phase when given *ad libitum* access to food, suggesting that the internal circadian pacemaker was maintained at its original phase[40]. A possible, though controversial, interpretation of this result is that the SCN is inherently rhythmic solely to inform the brain about the current light/dark cycle, and that an unexplored, malleable downstream brain region dictates circadian behavior from this input. Whether the SCN truly drives circadian behavior is yet to be determined.

Behavior

Rhythmic outputs of the SCN network. Individual SCN neurons are cell-autonomous circadian oscillators in both firing rate and clock gene expression[41], yet such an oscillation (in clock gene expression) has been observed in a variety of tissues outside the SCN[42]. What makes the SCN unique, however, is that it is a neuronal network of many coupled single-cell oscillators that together are able to produce robust behavioral and physiological rhythms. The SCN network can be organized into two broad regions based on their neuropeptide content: the vasoactive intestinal peptide (VIP)-expressing ventrolateral core, and the arginine vasopressin-expressing dorsomedial shell, which is densely innervated by the core[2]. VIP, the core's main neurotransmitter, is thought to mediate coupling in the SCN; VIP's effects may be modulated by the inhibitory neurotransmitter GABA, which is expressed in most, if not all, SCN neurons[1,43,44]. When coupled by VIP, the periods of the individual SCN neural oscillators are essentially identical; however, their phases are widely distributed[1,34]. Recent evidence suggests that an animal's behavioral phase and coherence are determined by the timing and degree of synchrony of individual rhythms, respectively, encoded by the average temporal population vector of the SCN network. The more synchronized the SCN, the more coherent the phases of its constituent neurons. Consequently, the average phase of the individual oscillators, represented as a time vector, determines the circadian time of activity onset[45]. Mutant animals lacking SCN synchrony, such as VIP knockouts, are behaviorally arrhythmic[45,46], and when behavioral arrhythmicity is induced in animals exposed to constant light, the synchrony within their SCN is disrupted[47].

The coordinated firing of the SCN network is sufficient to induce behavioral rhythmicity, but whether this output is synaptic or humoral in nature is unclear. Major axonal projections from the SCN synapse in other hypothalamic areas, including the subparaventricular zone and dorsomedial hypothalamus[48]. Some SCN neurons also secrete signaling molecules such as transforming growth factor alpha[49], prokineticin 2[50], and cardiotrophin-like cytokine[51]. Implanting encapsulated SCN tissue into SCN-lesioned animals, thus preventing neural outgrowth but allowing diffusion of secreted factors, is able to restore locomotor rhythms but not rhythms in melatonin or corticosterone secretion[52,53]. This suggests that both synaptic innervation and humoral secretion are necessary for complete circadian rhythmicity. Through downstream signaling, the SCN rhythmically regulates such diverse functions as corticosterone and melatonin release, orexin secretion, and cortical arousal[34,48]. Ultimately, these SCN outputs likely lead to overt circadian regulation of behavior.

Conclusions

The dynamic interaction between the molecular transcription/translation feedback loop, oscillations in electrical activity, and behavioral and physiological rhythms results in a functioning mammalian circadian clock, which provides an extremely useful model system to further understand the basic science question of how genes, through membrane events, can control behavior. The analogy for this interconnectivity presented in this review – the gears moving the hands, which in turn control behavior – is simplified. There is ample evidence that, unlike a physical clock, electrical activity can feed back onto the molecular clock[37,38], and that behavior can influence electrical activity[1,18]. However, to fully understand the mechanics of the biological clock, and to understand how its dysfunction can produce neuropsychiatric illness, it is necessary to understand the outputs of the circadian system: to link the gears to the hands, and to link the hands to behavior.

References

1. Welsh DK, Takahashi JS and Kay SA (2010). Suprachiasmatic nucleus: cell autonomy and network properties. *Annu. Rev. Physiol*. **72** 551-77.

2. Abrahamson EE and Moore RY (2001). Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res*. **916** (1-2): 172-91.

3. Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M and Bittman EL (1987). Circadian rhythmicity restored by neural transplant. Immunocytological characterization of the graft and its integration with the host brain. *J. Neurosci*. **7** (6): 1626-1638.
4. Ralph MR, Foster RG, Davis FC and Menaker M (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science* **247** (4945): 975-8
5. Sujino M, Masumoto K, Yamaguchi S, van der Horst GTJ, Okamura H and Inouye ST (2003). Suprachiasmatic nucleus grafts restore circadian behavioral rhythms of genetically arrhythmic mice. *Curr. Biol*. **13** (8): 664-668.
6. Guo H, Brewer JM, Lehman MN and Bittman EL (2006). Suprachiasmatic regulation of circadian rhythms of gene expression in hamster peripheral organs: effects of transplanting the pacemaker. *J. Neurosci*. **26** (24): 6406-12.
7. Schwartz WJ, Gross RA and Morton MT (1987). The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker. *Proc. Natl. Acad. Sci*. **84** (6): 1694-8.
8. Terracciano A, Tanaka T, Sutin AR, Sanna S, Deiana B, Lai S, Uda M, Schlessinger D, Abecasis GR, Ferrucci L and Costa PT (2010). Genome-wide association scan of trait depression. *Biol. Psychiatry* **68** (9): 811-7.
9. Glickman G (2010). Circadian rhythms and sleep in children with autism. *Neurosci. Biobehav. Rev*. **34** (5): 755-68.
10. Mackey SR (2007). Biological rhythms workshop IA: molecular basis of rhythms generation. *Cold Spring Harb. Symp. Quant. Biol*. **72** 7-19.
11. Glossop NRJ (2011). Circadian timekeeping in Drosophila melanogaster and Mus musculus. *Essays Biochem*. **49** (1): 19-35.
12. Debruyne JP, Noton E, Lambert CM, Maywood ES, Weaver DR and Reppert SM (2006). A clock shock: mouse CLOCK is not required for circadian oscillator function. *Neuron* **50** (3): 465-77.
13. Liu C, Weaver DR, Strogatz SH and Reppert SM (1997). Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. *Cell* **91** (6): 855-60.
14. Meng QJ, Logunova L, Maywood ES, Gallego M, Lebiecki J, Brown TM, Sladek M, Semikhodskii AS, Glossop NRJ, Piggins HD, Chesham JE, Bechtold DA, Yoo SH, Takahashi JS, Virshup DM, Boot-Handford RP, Hastings MH and Loudon ASI (2008). Setting clock speed in mammals: the CK1 epsilon tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. *Neuron* **58** (1): 78-88.
15. Herzog ED, Takahashi JS and Block GD (1998). Clock controls circadian period in isolated suprachiasmatic nucleus neurons. *Nature Neurosci*. **1** (8): 708-13.
16. Nakamura W, Honma S, Shirakawa T and Honma K (2002). Clock mutation lengthens the circadian period without damping rhythms in individual SCN neurons. *Nature Neurosci*. **5** (5): 399-400.
This paper provides some of the first evidence showing that the molecular clockwork is critical for electrical activity rhythms.
17. Albus H, Bonnefont X, Chaves I, Yasui A, Doczy J, van der Horst GTJ and Meijer JH (2002). Cryptochrome-deficient mice lack circadian electrical activity in the suprachiasmatic nuclei. *Curr. Biol*. **12** (13): 1130-3.
18. Kuhlman SJ, Silver R, Le Sauter J, Bult-Ito A and McMahon DG (2003). Phase resetting light pulses induce Per1 and persistent spike activity in a subpopulation of biological clock neurons. *J. Neurosci*. **23** (4): 1441-50.
Fluorescence imaging and electrophysiology are used to directly demonstrate for the first time that *Per1* promoter activity and spontaneous firing rate are positively correlated.
19. Quintero JE, Kuhlman SJ and McMahon DG (2003). The biological clock nucleus: a multiphasic oscillator network regulated by light. *J. Neurosci*. **23** (22): 8070-6.
20. Vasalou C and Henson MA (2010). A multiscale model to investigate circadian rhythmicity of pacemaker neurons in the suprachiasmatic nucleus. *PLoS Comp. Biol*. **6** (6): e1000706.
21. Belle MDC, Diekman CO, Forger DB and Piggins HD (2009). Daily electrical silencing in the mammalian circadian clock. *Science* **326** (5950): 281-4.
This work suggests that *Per1*-positive neurons are so depolarized during midday that they can no longer generate action potentials, thus providing another perspective on the correlation between *Per1* and firing rate.
22. Pennartz CMA, Bierlaagh MA and Geurtsen AMS (1997). Cellular mechanisms underlying spontaneous firing in rat suprachiasmatic nucleus : involvement of a slowly inactivating component of sodium current. *J. Neurophysiol*. **78** (4): 1811-1825.
23. Jackson AC, Yao GL and Bean BP (2004). Mechanism of spontaneous firing in dorsomedial suprachiasmatic nucleus neurons. *J. Neurosci*. **24** (37): 7985-98.
24. Kononenko NI, Shao LR and Dudek FE (2004). Riluzole-sensitive slowly inactivating sodium current in rat suprachiasmatic nucleus neurons. *J. Neurophysiol*. **91** (2): 710-8.
25. Pennartz CMA, de Jeu MTG, Bos NPA, Schaap J and Geurtsen AMS (2002). Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. *Nature* **416** (6878): 286-90.
26. Itri JN, Michel S, Vansteensel MJ, Meijer JH and Colwell CS (2005). Fast delayed rectifier potassium current is required for circadian neural activity. *Nature Neurosci*. **8** (5): 650-6.
27. Itri JN, Vosko AM, Schroeder A, Dragich JM, Michel S and Colwell CS (2010). Circadian regulation of a-type potassium currents in the suprachiasmatic nucleus. J. *Neurophysiol*. **103** (2): 632-40.
28. Pitts GR, Ohta H and McMahon DG (2006). Daily rhythmicity of large-conductance Ca2+ -activated K+ currents in suprachiasmatic nucleus neurons. *Brain Res*. **1071** (1): 54-62.
29. Meredith AL, Wiler SW, Miller BH, Takahashi JS, Fodor AA, Ruby NF and Aldrich RW (2006). BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nature Neurosci*. **9** (8): 1041-1049.
An important study that demonstrates the link between singular ionic currents with circadian behavioral activity using BK channel knockout mice.
30. Kent J and Meredith AL (2008). BK channels regulate spontaneous action potential rhythmicity in the suprachiasmatic nucleus. *PloS ONE* **3** (12): e3884.
31. McMahon DG, Wallace SF and Block GD (1984). Cellular analysis of the *Bulla* ocular circadian pacemaker system. *J. Comp. Physiol. A* 155 (3): 387-395.
32. de Jeu MTG, Hermes M and Pennartz CMA (1998). Circadian modulation of membrane properties in slices of rat suprachiasmatic nucleus. *Neuroreport* **9** (16): 3725-9.
33. Kuhlman SJ and McMahon DG (2004). Rhythmic regulation of membrane potential and potassium current persists in SCN neurons in the absence of environmental input. *Eur. J. Neurosci*. **20** (4): 1113-7.
34. Kuhlman SJ (2007). Biological rhythms workshop IB: neu-

rophysiology of SCN pacemaker function. *Cold Spring Harb. Symp. Quant. Biol.* **72** 21-33.
35. Ko GYP, Shi L and Ko ML (2009). Circadian regulation of ion channels and their functions. *J. Neurochem.* **110** (4): 1150-69.
36. Kudo T, Loh DH, Kuljis D, Constance C and Colwell CS (2011). Fast delayed rectifier potassium current: critical for input and output of the circadian system. *J. Neurosci.* **31** (8): 2746-55.
37. Yamaguchi S, Isejima H, Matsuo T, Okura R, Yagita K, Kobayashi M and Okamura H (2003). Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* **302** (5649): 1408-12.
38. Lundkvist GB, Kwak Y, Davis EK, Tei H and Block GD (2005). A calcium flux is required for circadian rhythm generation in mammalian pacemaker neurons. *J. Neurosci.* **25** (33): 7682-6.
39. Houben T, Deboer T, van Oosterhout F and Meijer JH (2009). Correlation with behavioral activity and rest implies circadian regulation by SCN neuronal activity levels. *J. Biol. Rhythms* **24** (6): 477-87.
40. Hut RA, Pilorz V, Boerema AS, Strijkstra AM and Daan S (2011). Working for food shifts nocturnal mouse activity into the day. *PloS ONE* **6** (3): e17527.
41. Webb AB, Angelo N, Huettner JE and Herzog ED (2009). Intrinsic, nondeterministic circadian rhythm generation in identified mammalian neurons. *Proc. Natl. Acad. Sci.* **106** (38): 16493-8.
42. Welsh DK, Yoo SH, Liu AC, Takahashi JS and Kay SA (2004). Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Curr. Biol.* **14** (24): 2289-2295.
43. Aton SJ, Huettner JE, Straume M and Herzog ED (2006). GABA and Gi/o differentially control circadian rhythms and synchrony in clock neurons. *Proc. Natl. Acad. Sci.* **103** (50): 19188-93.
44. Harmar AJ, Marston HM, Shen S, Spratt C, West KM, Sheward WJ, Morrison CF, Dorin JR, Piggins HD, Reubi JC, Kelly JS, Maywood ES and Hastings MH (2002). The VPAC2 receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell* **109** (4): 497-508.
45. Ciarleglio CM, Gamble KL, Axley JC, Strauss BR, Cohen JY, Colwell CS and McMahon DG (2009). Population encoding by circadian clock neurons organizes circadian behavior. *J. Neurosci.* **29** (6): 1670-6.
46. Aton SJ, Colwell CS, Harmar AJ, Waschek J and Herzog ED (2005). Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nature Neurosci.* **8** (4): 476-83.
47. Ohta H, Yamazaki S and McMahon DG (2005). Constant light desynchronizes mammalian clock neurons. *Nature Neurosci.* **8** (3): 267-9.
48. Saper CB, Lu J, Chou TC and Gooley J (2005). The hypothalamic integrator for circadian rhythms. *Trends Neurosci.* **28** (3): 152-7.
49. Kramer A, Yang FC, Snodgrass P, Li X, Scammell TE, Davis FC and Weitz CJ (2001). Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science* **294** (5551): 2511-5.
50. Cheng MY, Bullock CM, Li C, Lee AG, Bermack JC, Belluzzi J, Weaver DR, Leslie FM and Zhou QY (2002). Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* **417** (6887): 405-10.
51. Kraves S and Weitz CJ (2006). A role for cardiotrophin-like cytokine in the circadian control of mammalian locomotor activity. *Nature Neurosci.* **9** (2): 212-9.
52. Silver R, LeSauter J, Tresco PA and Lehman MN (1996). A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature* **382** (6594): 810–813.
53. Meyer-Bernstein EL, Jetton AE, Matsumoto SI, Markuns JF, Lehman MN and Bittman EL (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* **140** (1): 207-18.

The Use of Mouse Models to Study Genetic Modifiers of Neurological Disease

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Abstract

Neurological disorders are a significant public health concern affecting as many as one billion people worldwide, and this number is expected to grow in the coming years. Many neurological disorders exhibit some degree of heritability, and susceptibility genes have been identified for several of the heritable disorders. A common feature of heritable neurological disorders is phenotype heterogeneitya observed in families carrying identical mutations at disease genes or loci. This is an indication that genetic modifiers may be influencing the disease phenotype. Genetic modifiers are variation(s) in loci or genes that, when inherited along with a primary disease-causing mutation, alter some aspect of the disease phenotype. Genetic modifiers are prevalent among a wide variety of neurological diseases. Genetic modifiers increase the phenotypic complexity of neurological diseases, making diagnoses and treatment more difficult. Identifying genetic modifiers can enhance our understanding of neurological diseases and reveal new therapeutic targets. Mouse models of neurological disease are an excellent resource for the identification and characterization of genetic modifiers, as they can help circumvent many of the problems that are encountered when studying genetic modifiers in humans. This review highlights some of the ways in which mouse models can be used in conjunction with human studies to enhance our understanding of neurological diseases.

Keywords

Neurological Disease
Mouse Models
Modifier genes
Genetics
Complex Traits

Introduction

Neurological disorders are a significant public health concern. As many as one billion people worldwide are affected by neurological disorders, and this number is expected to increase in the coming years[1]. Many neurological disorders are heritable to some degree, and in recent years susceptibility genes have been identified for several neurological disorders[a]. In most cases, variation in the primary susceptibility gene has not been sufficient to explain the full range of phenotypes observed in the affected population. A common feature of heritable neurological disorders is phenotype heterogeneity observed among and between families carrying identical mutations at disease genes or loci. This indicates that additional factors contribute to the phenotype heterogeneity exhibited by many neurological disorders. Among the factors that can contribute to phenotype heterogeneity are environmental influence, stochastic events, and genetic modifiers, which are the focus of this review[2]. Understanding how genetic modifiers influence neurological disease phenotypes can enhance our knowledge of disease processes by uncovering disease-related pathways. Components of these pathways represent potential targets for novel therapies, which could improve the lives of patients with neurological disorders. This review highlights some of the ways in which genetic modifiers influence neurological disorder phenotypes and includes a discussion on the use of mouse models for the identification and characterization of genetic modifiers.

Genetic modifiers are genes or loci that alter the phenotypic expression of other, non-allelic[b] genes or loci (target genes). In the context of neurological disorders, variation in genetic modifiers generally does not have a noticeable phenotypic effect unless it is inherited in the presence of a pathogenic variant of a non-allelic susceptibility gene or locus (target genes) (see Fig. 1). For example, alleles that modify Huntington's disease (HD) do not produce their own discrete phenotypes in the absence of the pathogenic CAG repeats in the Huntingtin gene. However, when they are inherited in the presence of CAG repeats, they can affect the age of onset of HD[3]. Genetic modifiers are prevalent among a wide variety of inherited neurological disorders.

a. The proportion of phenotype heterogeneity that can be explained by genetic variation.

b. Located at different genetic loci.

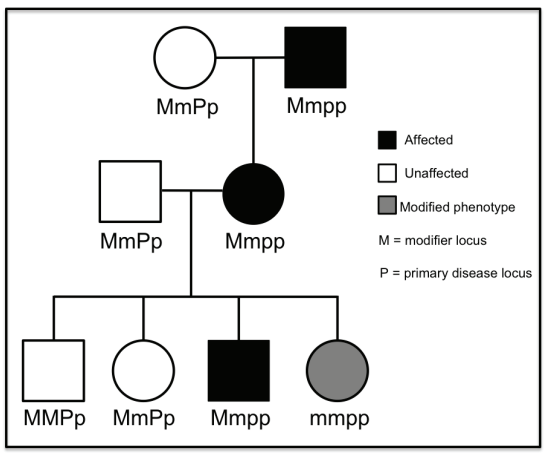

Figure 1. Phenotype heterogeneity within a family. In this representative family pedigree both the primary disease mutation and the modifier exhibit recessive modes of inheritance. Alleles at the modifier locus (designated M or m) segregate independently from alleles at the primary disease locus (designated P or p). In this particular case the phenotype is not modified unless two recessive alleles are inherited at both loci.

Disorders for which genetic modifiers have been implicated include: epilepsy, amyotrophic lateral sclerosis (ALS), Alzheimer disease, HD, tuberous sclerosis, and Hirschsprung disease, among others[4-9]. When present, genetic modifiers increase the complexity of the disease phenotype. This can complicate both the diagnoses and treatment of patients. Therefore, it is important to understand the ways in which genetic modifiers influence disease phenotypes.

Genetic modifiers can interact with target genes at any level of biological function to alter disease phenotypes in a wide variety of ways. Scnm1, one of the earliest modifiers of a neurological phenotype to be identified, acts at the level of transcription. Scnm1 modifies the neurologic movement disorder phenotype of $Scn8a^{medJ/medJ}$ mice, which results from a splice site mutation in Scn8a that leads to improper splicing and a reduction in functional Scn8a sodium channels[10]. Scnm1 is an RNA splicing factor that normally facilitates the proper splicing of Scn8a transcript. Buchner et al. identified a mutation in Scnm1 that exacerbates the $Scn8a^{medJ/medJ}$ phenotype by further reducing the amount of correctly spliced Scn8a transcript[11, 12]. Kcnv2, a genetic modifier of a seizure phenotype in the $Scn2a^{Q54}$ mouse model of epilepsy, works at the system level. $Scn2a^{Q54}$ mice have an epilepsy phenotype due to a gain-of-function mutation in the Scn2a sodium channel that results in excess sodium current [13]. Kcnv2 is a potassium channel subunit that reduces Kv2.1-mediated delayed rectifier potassium current. The exacerbation of the epilepsy phenotype is likely a result of a decrease in this delayed-rectifier potassium current [14]. This is an example in which the modifier gene (Kcnv2) does not directly interact with the target gene (Scn2a); instead it perturbs the system in which the target gene operates. There seems to be no limitations on the manner in which genetic modifiers can interact with their targets, or on the phenotypic effects that can result from these interactions. Specific examples of these interactions are too numerous to mention, but other phenotypic properties that can be altered include penetrance[c], disease progression, age of onset, and severity of the disease[15].

Genetic modifiers can offer insight into disease processes to help us better understand neurological diseases. The identification of the gene encoding microtubule-associated protein 1a (Mtap1a) as a modifier of hearing loss in tubby mice is a good example. Tubby mice have hearing loss as a result of a mutation in the tub gene. Before the identification of Mtap1a, the function of the tub gene was unknown. Ikeda et al. identified sequence polymorphisms in Mtap1a that were required for the hearing loss phenotype of tubby mice. These sequence polymorphisms reduced the binding efficiency of Mtap1a to Psd95, a gene encoding a synaptic scaffolding protein that helps coordinate synaptic function. These observations provided some of the earliest evidence of tub gene involvement in synaptic function[16]. Another example is the aforementioned discovery of Scnm1 as a modifier of the $Scn8a^{medJ/medJ}$ phenotype, which demonstrated that genes involved in mRNA splicing can modulate the phenotypic effects of splice-site mutations. This is of particular importance as splice site mutations are believed to compose approximately 10% of human disease mutations [17]. Identifying genetic modifiers can help us discover novel, disease-related pathways. These pathways not only help us to better understand pathogenic processes, but they may also contain therapeutic targets that could help us to better treat patients with neurological diseases. Therefore, studying genetic modifiers can be an important inroad to the successful treatment of neurological disorders. However, studying genetic modifiers in humans is challenging for a variety of reasons. Using mouse models of neurological disease can help researchers to circumvent some of these challenges. This review highlights some of the ways in which mouse models can facilitate the study of genetic modifiers of neurological disease.

Identifying Genetic Modification

The first step in the study of genetic modifiers is to establish that genetic modification is occurring. In hu-

c. The fraction of individuals with a particular genotype that express the associated phenotype.

mans, genetic modification is manifested as phenotype heterogeneity among or between families or populations that carry the same genotype at a primary disease gene or locus (see Fig.1). It can be difficult to distinguish between genetic modifiers and environmental sources of phenotype heterogeneity in humans. To do so, one must show that a portion of the phenotype heterogeneity is heritable. In HD, for example, there is considerable variability in the age of onset among patients with equivalent CAG repeat expansions in the HTT gene. It has been estimated that factors other than the length of the expansion account for approximately 30-50% of the total variability in age of onset[3, 18-20]. Using a large, well-characterized cohort of Venezuelan HD patients, Wexler et al. were able to show that a portion of this variability was heritable[3]. Oftentimes, large, well-characterized human cohorts are not available. In such cases, mouse models of the disease of interest can be employed. Genetic modification in mice is manifested as strain-dependent phenotype variability. Because mice can be reared and evaluated in similar environments, this strain-dependent variation is sufficient to establish that genetic modification is occurring[2]. Mouse models of neurological diseases frequently exhibit strain-dependent phenotypes. For example, the Hdh^{Q111} knock-in mouse model of HD exhibits several HD-related phenotypes that vary depending on genetic background, including: intergenerational repeat instability, somatic repeat instability, nuclear accumulation of full-length mutant huntingtin, and intranuclear N-terminal huntingtin inclusions[21]. Using mouse models to establish genetic modification can save researchers valuable time and money.

Genetic Mapping

Once it has been established that genetic modification of a disease phenotype is occurring within a population, genetic mapping is used to identify the genomic locations of the modifying genes/loci. Genetic mapping requires DNA samples from large, well-characterized populations of affected individuals, which are frequently unavailable in human populations. As an alternative approach, genetic mapping can be done in mouse models. This approach allows for the use of strategic breeding to take advantage of strain-dependent phenotypes to identify modifier loci. Once modifier loci/genes have been identified in mice, then researchers can screen a smaller number of patients for variants in the homologous loci/genes, thereby circumventing the need for large populations of human patients. This combination of genetic mapping and candidate gene screening was used to identify Kcnv2 as a modifier of epilepsy in mice and for the subsequent identification of two novel KCNV2 variants in pediatric epilepsy patients[14]. This approach has been used to successfully identify a number of other modifier loci/genes in mice and humans as well.

Forward Genetic Screen

For any potential modifier loci, the genetic mapping approach requires that there be genetic variation between individuals at that locus. Without this variation, there will be no discernible differences in phenotype with which to map the locus[22]. A forward genetic screen employs the use of a mutagen to induce polymorphisms throughout the genome, including potential modifier loci. This approach is commonly used in lower model organisms for pathway analysis, but it can also be used in mice to identify genetic modifiers. Using this approach in mice increases the number of potential modifiers that can be identified. Instead of relying on natural genetic variation between inbred mouse strains, N-ethyl-N-nitrosourea (ENU) is used to induce mutations throughout the genome. Mice carrying ENU-induced mutations can be crossed with any mouse model of interest to produce progeny that carry the primary disease-causing mutation along with ENU-induced mutations in potential modifier loci. These progeny are screened for phenotype modification, and standard genetic mapping is employed to identify modifier loci. This approach was first used by Matera et al. to identify a modifier of hypopigmentation in a Sox10 haploinsufficient[d] mouse model of Waardenburg syndrome[23].

Candidate Gene Approach

Genetic mapping and forward genetic screens are both unbiased approaches to identifying genetic modifiers. These approaches maximize the number of modifiers that can be identified. However, they can be time-consuming, even in mice. A less time-consuming alternative is the candidate gene approach. This approach involves screening candidate genes for genetic variation that is inherited along with the altered phenotype. Reducing the number of genes interrogated can increase statistical power, resulting in a reduced number of mice or patients required for the study. This can save both time and money, and is the approach most often used in human studies. Several modifiers of tuberous sclerosis complex phenotypes have been identified in humans by screening genes that interact with the tuberin-hamartin complex formed by TSC1 and TSC2, the target genes in which the primary tuberous sclerosis mutations occur[9]. Additionally, a number of different studies have identi-

d. A condition in which one allele is not sufficient for normal function.

fied putative modifiers in pathways believed to be involved in HD, including: glutamatergic transmission, protein degradation, gene transcription, stress response/apoptosis, lipoprotein metabolism, axonal trafficking, and energy metabolism[24-31]. Similarly, several modifiers of ALS have been identified in ALS-related pathways[32]. This approach can also be effective in mouse models. Cantrell et al. made use of this approach to identify Ednrb, a modifier of aganglionosis[e], in the $Sox10^{Dom}$ mouse model of Hirschsprung disease[8]. Instead of searching the whole genome for possible modifiers, they restricted their search to genes involved in the endothelin signaling pathway based on the knowledge that mutations in this pathway had been previously shown to cause Hirschsprung disease in humans[33]. For this approach to be effective in mice or humans, such previous knowledge is required to inform the search. This means that the search is generally restricted to genes found in pathways already known to be involved in the disease. Thus, modifiers identified using this method may not be as informative as modifiers identified using one of the unbiased approaches.

Validating Genetic Modifiers

Although genetic mapping and candidate gene screening establishes an association between a modifier gene and phenotype variation, this does not imply a causal relationship. In order to validate a putative modifier, the genetic variation at a modifier locus/gene must be shown to be sufficient to alter the phenotype of interest. This is commonly demonstrated by expressing the putative modifier as a transgene in the relevant mouse model. This approach was used to validate Kcnv2 as a quantitative modifier of the $Scn2a^{Q54}$ seizure phenotype. Several Kcnv2 transgenic mouse lines expressing different levels of the Kcnv2 transgene transcript were developed and bred to $Scn2a^{Q54}$ transgenic mice to produce double transgenic mice expressing $Scn2a^{Q54}$ and various levels of the Kcnv2 transcript. A comparison of the seizure phenotypes of each mouse line demonstrated that increased Kcnv2 expression is sufficient to exacerbate the $Scn2a^{Q54}$ epilepsy phenotype[4]. Mouse models are also useful for validating genetic modifiers that were identified in humans. A study by Giess et al. used this approach to validate CNTF as a modifier of ALS, which was first identified by screening candidate modifier genes in a family with ALS resulting from a SOD-1 mutation. To determine whether a CNTF deficiency could modify ALS onset, they crossbred hSOD-1G93A mice with $CNTF^{-/-}$ mice and compared disease onset to that of hSOD-1G93A mice expressing wild-type CNTF. They found that the CNTF-deficient mice had an earlier disease onset, validating CNTF as a modifier of the SOD-1 ALS phenotype[5]. This study demonstrates the benefit of combining both mouse and human approaches to study genetic modifiers.

e. The absence of parasympathetic ganglion cells in the myenteric plexus of the digestive system.

Considerations for Using Mouse Models

When using mouse models to study modifiers of human diseases, there are several considerations that one must take into account. First, not all modifiers identified in mouse models will be relevant to human diseases. This is because genetic and cellular pathways are not always conserved between mice and humans. Therefore, it is necessary to use caution when drawing conclusions from mice about human diseases. Second, there may be modifiers present in humans that cannot be identified in mice. This could occur because the homologous gene is not present in mice; because the pathways are not conserved; or because the imbred mouse strains are not polymorphic at the relevant locus. Third, mouse models of human disease may not recapitulate every aspect of the human phenotype. Even when the underlying mutation is the same, mouse model phenotypes can differ from human phenotypes. When observing mouse phenotypes, it is important to pick one that is conserved in humans. Even with these limitations in mind, mouse models remain an indispensable tool for studying genetic modifiers of human disease.

The Future of the Search for Genetic Modifiers

Large-scale, high-throughput sequencing techniques are developing at a rapid pace and becoming cheaper by the day. These techniques can greatly improve the speed and efficiency with which genetic modifiers can be identified. There are a number of ion channel variants that have been associated with epilepsy, and it is known that these variants can interact to influence epilepsy phenotypes[34]. Traditionally, these interactions have been tested one at a time. Recently, Klassen et al. performed exome sequencing on 237 ion channel genes and created ion channel variant profiles of individuals with sporadic idiopathic epilepsy and unaffected individuals, revealing a surprising degree of genetic complexity. Such an approach would not have been feasible just a few years ago. In the future, powerful techniques like these may help us to unravel some of the complexity underlying neurological diseases. Yet, with these techniques come new challenges. Though the discovery of genetic modifiers will come much faster than it has in the past, each putative modifier must be validated. As the complexity of the data increases, so must the means with which to evaluate it.

And with all of this new data come hypotheses that must be tested. Though high-throughput sequencing methods stand to decrease our reliance on mouse models for the discovery of modifiers, we will need mouse models more than ever to validate newly discovered modifiers and to test the hypotheses that we derive from them.

Conclusion

Genetic modifiers are a major contributor to the phenotypic heterogeneity observed in a wide variety of neurological diseases. Identifying modifier genes and elucidating the mechanisms by which they influence their targets is an important step in understanding neurological diseases. Mouse models are an indispensable resource in which to identify and characterize genetic modifiers. Knowledge gleaned through the skillful use of mouse models can be used to inform human studies, saving time and resources. When used thoughtfully and in combination with human studies, mouse models can help elucidate disease-related pathways, giving insight into pathogenic mechanisms. Knowledge of genetic modifiers and the pathways in which they operate can yield new therapeutic targets for the treatment of neurological disorders.

References

1. World Health Organization. (2006). Neurological disorders : public health challenges. Geneva: World Health Organization.

2. Nadeau JH (2003). Modifier genes and protective alleles in humans and mice. Curr Opin Genet Dev. 13 (3): 290-295.

3. Wexler NS, Lorimer J, Porter J, Gomez F, Moskowitz C, Shackell E, Marder K, Penchaszadeh G, Roberts SA, Gayán J, Brocklebank D, Cherny SS, Cardon LR, Gray J, Dlouhy SR, Wiktorski S, Hodes ME, Conneally PM, Penney JB, Gusella J, Cha J-H, Irizarry M, Rosas D, Hersch S, Hollingsworth Z, MacDonald M, Young AB, Andresen JM, Housman DE, De Young MM, Bonilla E, Stillings T, Negrette A, Snodgrass SR, Martinez-Jaurrieta MD, Ramos-Arroyo MA, Bickham J, Ramos JS, Marshall F, Shoulson I, Rey GJ, Feigin A, Arnheim N, Acevedo-Cruz A, Acosta L, Alvir J, Fischbeck K, Thompson LM, Young A, Dure L, O'Brien CJ, Paulsen J, Brickman A, Krch D, Peery S, Hogarth P, Higgins DS, Landwehrmeyer B and Project US-VCR (2004). Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci USA. 101 (10): 3498-3503.

4. Jorge BS, Campbell CM, Miller AR, Rutter ED, Gurnett CA, Vanoye CG, George AL and Kearney JA (2011). Voltage-gated potassium channel KCNV2 (Kv8. 2) contributes to epilepsy susceptibility. Proceedings of the National Academy of Sciences. 108 (13): 5443.

5. Giess R, Holtmann B, Braga M, Grimm T, Müller-Myhsok B, Toyka KV and Sendtner M (2002). Early onset of severe familial amyotrophic lateral sclerosis with a SOD-1 mutation: potential impact of CNTF as a candidate modifier gene. Am J Hum Genet. 70 (5): 1277-1286.

This paper demonstrates how genetic modifiers identified in humans can be functionally tested using mouse models.

6. Carrasquillo MM, Zou F, Pankratz VS, Wilcox SL, Ma L, Walker LP, Younkin SG, Younkin CS, Younkin LH, Bisceglio GD, Ertekin-Taner N, Crook JE, Dickson DW, Petersen RC, Graff-Radford NR and Younkin SG (2009). Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. Nat Genet. 41 (2): 192-198.

7. Rubinsztein DC, Leggo J, Chiano M, Dodge A, Norbury G, Rosser E and Craufurd D (1997). Genotypes at the GluR6 kainate receptor locus are associated with variation in the age of onset of Huntington disease. Proc Natl Acad Sci USA. 94 (8): 3872-3876.

8. Cantrell VA, Owens SE, Chandler RL, Airey DC, Bradley KM, Smith JR and Southard-Smith EM (2004). Interactions between Sox10 and EdnrB modulate penetrance and severity of aganglionosis in the Sox10Dom mouse model of Hirschsprung disease. Human Molecular Genetics. 13 (19): 2289-2301.

This paper exemplifies how a candidate gene approach can be used to identify genetic modifiers.

9. Au KS, Ward CH and Northrup H (2008). Tuberous sclerosis complex: disease modifiers and treatments. Curr Opin Pediatr. 20 (6): 628-633.

10. Kohrman DC, Harris JB and Meisler MH (1996). Mutation detection in the med and medJ alleles of the sodium channel Scn8a. Unusual splicing due to a minor class AT-AC intron. J Biol Chem. 271 (29): 17576-17581.

11. Buchner DA, Trudeau M and Meisler MH (2003). SCNM1, a putative RNA splicing factor that modifies disease severity in mice. Science. 301 (5635): 967-969.

This seminal paper describes the identification and characterization of Scnm1, one of the first modifiers of a neurological phenotype to be identified.

12. Howell VM, Jones JM, Bergren SK, Li L, Billi AC, Avenarius MR and Meisler MH (2007). Evidence for a direct role of the disease modifier SCNM1 in splicing. Hum Mol Genet. 16 (20): 2506-2516.

13. Kearney JA, Plummer NW, Smith MR, Kapur J, Cummins TR, Waxman SG, Goldin AL and Meisler MH (2001). A gain-of-function mutation in the sodium channel gene Scn2a results in seizures and behavioral abnormalities. Neuroscience. 102 (2): 307-317.

14. Jorge BS, Campbell CM, Miller AR, Rutter ED, Gurnett CA, Vanoye CG, George AL and Kearney JA (2011). Voltage-gated potassium channel KCNV2 (Kv8.2) contributes to epilepsy susceptibility. Proceedings of the National Academy of Sciences. 108 (13): 5443-5448.

This paper highlights the utility of transgenic mouse models for validating putative modifier genes.

15. Kearney JA (2011). Genetic modifiers of neurological disease. Curr Opin Genet Dev. 21 (3): 349-353.

16. Ikeda A, Zheng QY, Zuberi AR, Johnson KR, Naggert JK and Nishina PM (2002). Microtubule-associated protein 1A is a modifier of tubby hearing (moth1). Nature genetics. 30 (4): 401-405.

17. Buchner DA, Trudeau M and Meisler MH (2003). SCNM1, a Putative RNA Splicing Factor That Modifies Disease Severity in Mice. Science. 301 (5635): 967-969.

18. Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, MacDonald ME, Gusella JF, Harper PS and Shaw DJ (1993). Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. Nature genetics. 4 (4): 393-397.

19. Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti

F, Frontali M, Folstein S, Ross C, Franz M and Abbott M (1993). Trinucleotide repeat length instability and age of onset in Huntington's disease. Nature genetics. 4 (4): 387-392.
20. Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, Starr E, Squitieri F, Lin B and Kalchman MA (1993). The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nature genetics. 4 (4): 398-403.
21. Lloret A, Dragileva E, Teed A, Espinola J, Fossale E, Gillis T, Lopez E, Myers RH, MacDonald ME and Wheeler VC (2006). Genetic background modifies nuclear mutant huntingtin accumulation and HD CAG repeat instability in Huntington's disease knock-in mice. Hum Mol Genet. 15 (12): 2015-2024.
22. Houlston RS and Tomlinson IP (1998). Modifier genes in humans: strategies for identification. Eur J Hum Genet. 6 (1): 80-88.
23. Matera I, Watkins-Chow DE, Loftus SK, Hou L, Incao A, Silver DL, Rivas C, Elliott EC, Baxter LL and Pavan WJ (2008). A sensitized mutagenesis screen identifies Gli3 as a modifier of Sox10 neurocristopathy. Hum Mol Genet. 17 (14): 2118-2131.
24. MacDonald ME, Vonsattel JP, Shrinidhi J, Couropmitree NN, Cupples LA, Bird ED, Gusella JF and Myers RH (1999). Evidence for the GluR6 gene associated with younger onset age of Huntington's disease. Neurology. 53 (6): 1330-1332.
25. Metzger S, Bauer P, Tomiuk J, Laccone F, Didonato S, Gellera C, Soliveri P, Lange HW, Weirich-Schwaiger H, Wenning GK, Melegh B, Havasi V, Baliko L, Wieczorek S, Arning L, Zaremba J, Sulek A, Hoffman-Zacharska D, Basak AN, Ersoy N, Zidovska J, Kebrdlova V, Pandolfo M, Ribai P, Kadasi L, Kvasnicova M, Weber BH, Kreuz F, Dose M, Stuhrmann M and Riess O (2006). The S18Y polymorphism in the UCHL1 gene is a genetic modifier in Huntington's disease. Neurogenetics. 7 (1): 27-30.
26. Holbert S, Denghien I, Kiechle T, Rosenblatt A, Wellington C, Hayden MR, Margolis RL, Ross CA, Dausset J, Ferrante RJ and Neri C (2001). The Gln-Ala repeat transcriptional activator CA150 interacts with huntingtin: neuropathologic and genetic evidence for a role in Huntington's disease pathogenesis. Proc Natl Acad Sci U S A. 98 (4): 1811-1816.
27. Arning L, Monte D, Hansen W, Wieczorek S, Jagiello P, Akkad DA, Andrich J, Kraus PH, Saft C and Epplen JT (2008). ASK1 and MAP2K6 as modifiers of age at onset in Huntington's disease. J Mol Med (Berl). 86 (4): 485-490.
28. Kehoe P, Krawczak M, Harper PS, Owen MJ and Jones AL (1999). Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length. J Med Genet. 36 (2): 108-111.
29. Metzger S, Rong J, Nguyen HP, Cape A, Tomiuk J, Soehn AS, Propping P, Freudenberg-Hua Y, Freudenberg J, Tong L, Li SH, Li XJ and Riess O (2008). Huntingtin-associated protein-1 is a modifier of the age-at-onset of Huntington's disease. Hum Mol Genet. 17 (8): 1137-1146.
30. Brune N, Andrich J, Gencik M, Saft C, Muller T, Valentin S, Przuntek H and Epplen JT (2004). Methyltetrahydrofolate reductase polymorphism influences onset of Huntington's disease. J Neural Transm Suppl. (68): 105-110.
31. Weydt P, Soyal SM, Gellera C, Didonato S, Weidinger C, Oberkofler H, Landwehrmeyer GB and Patsch W (2009). The gene coding for PGC-1alpha modifies age at onset in Huntington's Disease. Mol Neurodegener. 4: 3.
32. Riboldi G, Nizzardo M, Simone C, Falcone M, Bresolin N, Comi GP and Corti S (2011). ALS genetic modifiers that increase survival of SOD1 mice and are suitable for therapeutic development. Progress in neurobiology. 95 (2): 133-148.
33. Badner JA, Sieber WK, Garver KL and Chakravarti A (1990). A genetic study of Hirschsprung disease. Am J Hum Genet. 46 (3): 568-580.
34. Kearney JA, Yang Y, Beyer B, Bergren SK, Claes L, Dejonghe P and Frankel WN (2006). Severe epilepsy resulting from genetic interaction between Scn2a and Kcnq2. Hum Mol Genet. 15 (6): 1043-1048.

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CANDIDATE
REVIEWS

Cortical Multisensory Processes: Neural Encoding Strategies

Juliane Krueger

Abstract

In our everyday environment, we are constantly bombarded with cues from different sensory modalities. Essential mechanisms within our brain have evolved that integrate signals from multiple sensory sources shaping perception and behavior. Much headway has been made characterizing subcortical multisensory processes, particularly in the cat superior colliculus (SC), which has also lead to the establishment of three working principles for multisensory integration. Although a good first step in understanding multisensory integration, studying subcortical processes has its limitations. In order to understand perception and behavior, we have to understand cortical processes. Most studies thus far have detailed the mechanics of cortical multisensory interactions in the primate brain, but direct links between these mechanics and perception and behavior have not been made. In the cat, there exists a unique multisensory structure – the anterior ectosylvian sulcus (AES) that is well-suited to study cortical multisensory processes. The AES is comprised of three unisensory zones and a region at their respective overlapping domains that has a high incidence of multisensory neurons. Studying how AES neurons integrate multiple modalities and establishing links to perceptual and behavioral processes will not only shed light on multisensory encoding strategies and their contributions to perception and behavior but may also further our understanding of how cortical processes lead to perception and behavior in general.

Keywords

Cortex
Multisensory
Cat
Primate
Electrophysiology

Introduction

In our daily environment we constantly experience sensory signals arising from various events. Sometimes, these cues take place alone but more often multiple signals occur in combination. In order to convey an accurate percept of our world, the brain must be equipped to manage and synthesize sensory information from a variety of sensory sources (Figure 1A). Research over the past four decades has focused on identifying structures within the brain that actively integrate multisensory signals and subsequent studies have also investigated the neural properties within these specific multisensory brain regions. Current data shows that there is something special about these multisensory neurons as their firing rates during multisensory trials most often significantly differ compared to the component unisensory responses alone[1, 2]. On a behavioral level, this is frequently indicated by speeded response times[3], higher response accuracies[2, 4], and increased detection rates[2, 5] during multisensory tasks. These neuronal as well as behavioral gains underscore that multisensory processes greatly contribute to the processes that shape perception and behavior. Nevertheless, these exact contributions have yet to be determined.

The superior colliculus in the cat and the principles of multisensory integration

Most studies to date have been carried out in the cat superior colliculus (SC) – a multisensory subcortical structure. The SC contains visual, auditory, and somatosensory neurons with around 50% of intermediate and deep layer neurons shown to be multisensory[6]. Research in the SC lead to the establishment of three widely-recognized working principles that clearly delineate a set of rules to which multisensory neurons adhere. These principles have subsequently been shown to be valid in various cortical regions within the cat and primate brain. The principle of spatial coincidence states that pairing spatially coincident unisensory stimuli will more likely elicit response enhancements (Figure 1B) as measured by firing rate changes than stimuli that are separated in space[7]. The principle of temporal coincidence applies the same idea to time. Two temporally coincident stimuli are more likely to lead to an enhanced neuronal response during multisensory trials than two temporally separated stimuli[8]. Temporal coincidence generally encompasses a range of stimulus onset asynchronies (SOAs; often also referred to as the temporal binding window[9]) brought about by the fact that different sensory signals propagate at differ-

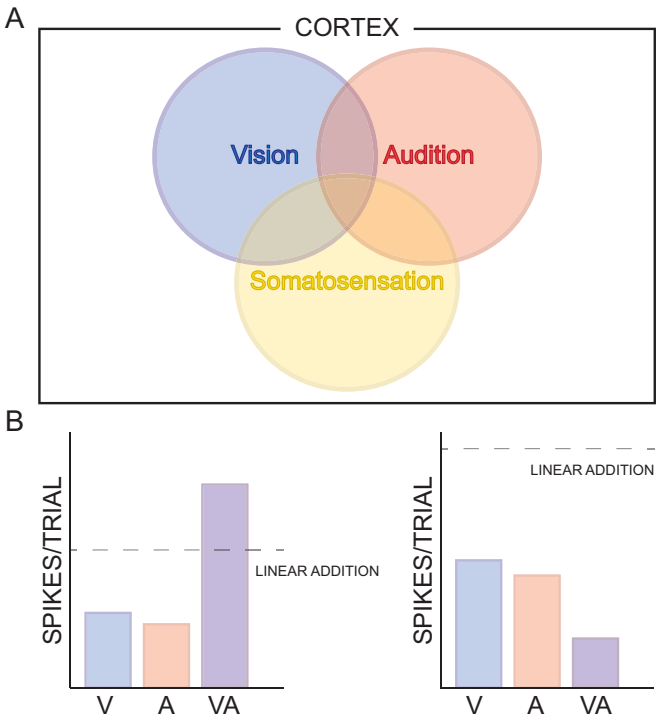

Figure 1. A. Schematic representation of multisensory interactions in cortex brought about by sensory overlap. **B.** Neuronal response profiles (visual - V, auditory - A, audiovisual - VA) demonstrating response enhancement (left) and depression (right) under multisensory (VA) conditions.

ent speeds. The principle of inverse effectiveness relates to stimulus efficacy. Combining two weakly effective unisensory stimuli will more often lead to response enhancements. Having spatially-offset stimuli, temporally-offset stimuli or strongly effective unisensory stimuli can either lead to a lack of integration or a response depression (Figure 1B). Thus far, these principles were studied in isolation but recent research indicates strong interactions within the principles[10, 11] leading to theories about one overarching principle - the principle of inverse effectiveness. Data analysis looking at these interactions implies that space and time just merely affect stimulus efficacy, meaning certain spatial locations within a receptive field (RF) or certain SOAs render the stimuli more or less effective, and consequently greatly influence neuronal responses and multisensory integration as defined by the principle of inverse effectiveness.

Cortical multisensory processes

Although, subcortical processing is undoubtedly important and has clearly established a set of principles that characterizes multisensory neurons, it cannot explain how multisensory processes shape and influence perception and behavior. Multisensory cortical processes have been mostly studied in the primate brain and so far, research has primarily focused on identifying regions within the primate brain that respond to multiple sensory signals and some headway has been made identifying whether or not these areas actively integrate multisensory cues. Studies involving the superior temporal sulcus (STS[12, 13]), the ventral intraparietal area (VIP[14]), and the ventrolateral prefrontal cortex (VLPFC[15]) demonstrated that they are involved in face-voice integration (STS, VLPFC) , speech perception (STS), and space representations (VIP), and that they display multisensory interactions but the exact contributions of multisensory processes to behavior and perception have not been established.

The superior temporal sulcus in the primate. STS is located within the temporal lobe in the primate brain. Studies identifying the roles of STS have identified a strong involvement in face[16, 17] and voice[18] processing, perception of biological motion[19, 20], and visual object recognition[21]. Moreover, very early on, STS was recognized as a region of sensory overlap with interactions between visual and auditory cues at the single neuron level[22-24]. Ghazanfar et al in 2005[13] demonstrated by pairing species-specific dynamic faces and vocalizations that auditory belt integrates multisensory signals as measured by local field potentials (LFPs) while multisensory responses were strongly face-voice specific. They also observed that response enhancements occurred significantly more often than response suppression. One interesting caveat is that STS neuron activity did not change with SOA since there was no correlation between SOA and magnitude of multisensory response. Prior to this study, Schroeder and Foxe in 2002[12] illustrated that visual, auditory, and somatosensory inputs to the STS are most likely feedforward projections as revealed by current source density (CSD) analysis. As of late, STS has also been shown to work in conjunction with auditory cortex to appropriately manage multisensory looming signals and bimodal speech[25, 26]. Further studies indicate that STS may be a locus for bimodal representations of observed actions[27] and studies in humans using fMRI also indicate a potential involvement in multisensory object recognition and object categorization[28].

The ventral intraparietal area in the primate. VIP, is buried within the fundus of the intraparietal sulcus of the posterior parietal cortex[29], a processing core for spatial coordinate transformations[30]. VIP is thought to play a role during visual motion processing with neurons being strongly driven by direction of movement[29] and may contribute to movements

associated with defense or avoidance behaviors[31]. Anatomical tracer studies have shown VIP's strong connectivity patterns with visual, somatosensory, and motor areas[32] and thus recent research has focused on identifying multisensory interactions within VIP. Initial studies demonstrated vestibular-visual[33] and somatosensory-visual[34] interactions whereas neurons were responsive to bimodal stimuli that had RFs in close spatial registry. Schlack et al in 2005[14] showed for the first time that VIP neurons are responsive to auditory in addition to visual stimulation. Although auditory and visual RFs were generally well aligned, most bimodal neurons encoded space in their native reference frames (auditory – head-centered and visual – eye-centered) and yet significant multisensory interactions could be observed. Whether or not these neurons actively integrate these sensory modalities remains unclear. Altogether, these findings demonstrate that VIP may play an integral role in multisensory coordinate transformations as seen during peripersonal space and movement processing, in particular during tasks requiring shifts within modality specific reference frames. The lateral and the medial intraparietal areas (LIP and MIP) are also found within the posterior parietal cortex and both have been implicated in coordinate transformation[35]. Multisensory integration has not been overtly studied but LIP has been shown to be responsive to auditory cues[36], particularly in context of a saccade task[37, 38].

The ventrolateral prefrontal cortex in the primate. VLPFC, has extensive connections from sensory cortices and strong projections to the motor cortex and areas involved in cognitive processes[39-41]. VLPFC has been associated with memory retrieval[42], processes involving behavior inhibition[43], and visual object recognition[44, 45]. Research also shows that VLPFC neurons are responsive to visuo-spatial cues[46] and to conspecific vocalizations[47-49]. Sugihara et al in 2006[15] were the first to demonstrate that VLPFC neurons actively integrate audiovisual stimuli with strong preferences for interactions of face and vocalization stimuli. Moreover, neurons abode by the principles of multisensory integration exhibiting enhancement as well as suppression as seen in spike firing changes depending upon stimulus efficacy.

The anterior ectosylvian sulcus in the cat. To date, the AES is the most studied multisensory cortical region in the cat. AES is located within the parietotemporal lobe and is comprised of three distinct unisensory zones: the auditory field AES[50-52] (FAES), the anterior ectosylvian visual area[53-55] (AEV), and the fourth somatosensory area[56, 57] (SIV), as well as multisensory domains at the respective overlapping unisensory representations[58]. Auditory neurons within FAES exhibit short latencies, broad tuning curves, and are mostly monaural[50]. FAES has been associated with sound localization as shown by considerable behavioral detection deficits caused by cooling FAES[59], particularly the deeper layers[60]. Visual neurons within AEV are characterized by a robust preference for small and quickly moving stimuli – frequently being strongly directionally sensitive, usually have large RFs often spanning the entire contralateral hemifield and respond most vigorously to binocular stimulation. Moreover, no obvious retinotopic organization could be detected[53]. Although AEV has substantial connections to the frontal eye field[61] (FEF), an area highly important for saccade production, microstimulation studies have shown that eye movements can be evoked with intracortical stimulation of AEV[61] even after removal of FEF. Furthermore, within this eye-movement area in the ventral bank of AES, a large percentage of neurons respond to multisensory stimuli[62] suggesting that AES may have a potential role in multisensory coordinate transformation (or sensory transformation in general), a process often used during orientation behavior. Additionally, AEV neurons seem to be selective for pattern over component motion, which has been hypothesized to signal the salience of local motion information[63]. Area SIV contains a somatotopic map that represents the head rostrally and the hind legs caudally. SIV neurons can be stimulated by hair displacement, low threshold cutaneous stimulation, or distortion of subcutaneous tissue[56]. Further studies have shown that deactivating AES impedes successful integration of multisensory stimuli within the SC[64] and alters approach and orientation behaviors thought to be mediated by the SC so that accuracy gains with spatially coincident stimuli as well as response inhibitions associated with spatially disparate stimuli are significantly reduced[65]. A first attempt to better characterize multisensory AES neurons has utilized spatial receptive field (SRF) analysis – an approach that looks at the spatial influences on multisensory integration across the RF of a neuron. SRF analysis has demonstrated that RFs are heterogeneous in nature and that often multisensory SRFs differ markedly from the prediction plots (linear addition of the unisensory SRFs) frequently including one very defined hot spot surrounded by regions of subadditivity[10]. SRF analysis, as a first step to see how spatial location can influence stimulus effectiveness, suggests that there are strong interactions between stimulus location and efficacy in that location with low neuronal firing rates during unisensory conditions show strong response profiles during multisensory trials. A first effort to characterize temporal coding strategies within the AES revealed higher peak firing

rates, shorter response latencies, and longer discharge durations during multisensory stimulation[11]. Altogether, due to its unique anatomical layout including a high incidence of multisensory neurons, the AES is an ideal candidate to study multisensory processes in the cortex. Furthermore, establishing the role of AES in perception and behavior may allow for direct links between multisensory processes and perception and behavior.

Spatiotemporal receptive field (STRF) analysis. STRF analysis is a method to better characterize the interactions of the three principles of multisensory integration and to aid in determining the role of the AES. It examines how space and time within the RF of a multisensory neuron affect stimulus efficacies and the neuron's response to these multisensory stimuli. This becomes increasingly important when examining biologically relevant stimuli as they often have complex spatial and temporal features. STRFs are constructed using neuronal firing data at the tested stimuli locations and SOAs (in spikes/trial) across the RF of a neuron. Figure 2 illustrates a hypothetical audiovisual STRF at four locations with 6 different SOAs. Within the classical RF presentation (azimuth versus elevation), neuronal discharge profiles at the tested locations depict the different SOAs (x-axis) and the resulting response spike rates in spikes/trial (y-axis). Multisensory responses at each SOA are subsequently compared to the maximum unisensory response (referred to as multisensory index) and to the linear addition of both unisensory response profiles (referred to as multisensory contrast). Both measures will give a detailed look at response enhancements (superadditivity) as well as response suppressions (subadditivity) across space and time within the RF of the tested neuron. STRF analysis does not just give insight into encoding strategies but may also indicate AES function. For example, having heterogeneous STRFs, similarly to SRFs[10], could be a means to code for moving stimuli (i.e. firing rate differences within and outside of a hot spot) in relation to head/eye orientation and thus may give further evidence for a role in motion perception and sensory transformation.

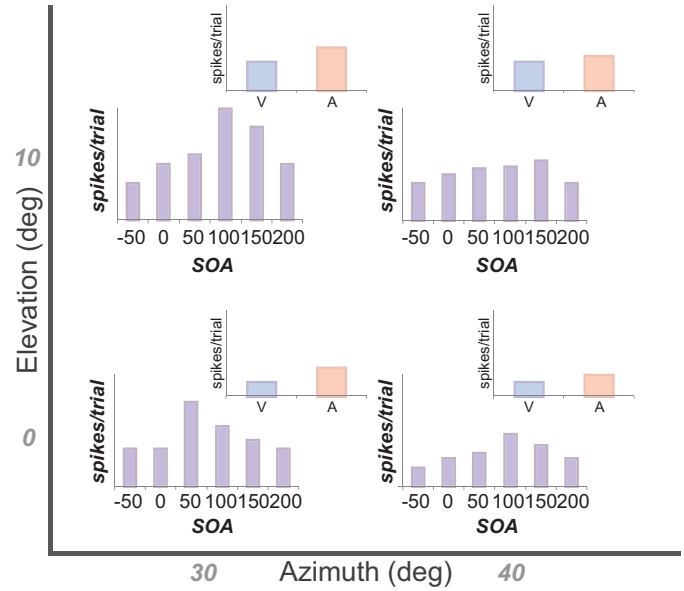

Figure 2. Hypothetical multisensory spatiotemporal receptive field at four locations. Bar graphs represent the neuronal firing rates at the different locations for the unisensory visual (V, blue) and auditory (A, red) trials as well as the audiovisual trials (VA, purple) over all tested SOAs. Note the different firing rates depending upon SOA, giving a temporal window over which multisensory integration occurs. Furthermore, the temporal window varies with spatial location.

Concluding remarks

In our everyday environment, the brain is constantly tasked to integrate signals from several sensory modalities. Understanding cortical multisensory processing is essential in understanding perception and behavior. The cat AES is a well suited model structure to identify multisensory encoding strategies and their effects on perception and behavior. STRF analysis is a great tool to investigate multisensory neuronal responses as determined by time, space and stimulus effectiveness and will aid in establishing AES function. Furthermore, comparing findings across species may allow for generalizations about multisensory processes in the healthy brain, which may contribute to research targeting disorders of the central nervous system.

References

1. Meredith, M.A. and B.E. Stein, Interactions among converging sensory inputs in the superior colliculus. Science, 1983. 221(4608): p. 389-91.
2. Stein, B.E., W.S. Huneycutt, and M.A. Meredith, Neurons and behavior: the same rules of multisensory integration apply. Brain Res, 1988. 448(2): p. 355-8.
3. Engelken, E.J. and K.W. Stevens, Saccadic eye movements in response to visual, auditory, and bisensory stimuli. Aviat Space Environ Med, 1989. 60(8): p. 762-8.
4. Hairston, W.D., et al., Multisensory enhancement of localization under conditions of induced myopia. Experimental Brain Research, 2003. 152(3): p. 404-408.
5. Lovelace, C.T., B.E. Stein, and M.T. Wallace, An irrelevant light enhances auditory detection in humans: a psychophysical analysis of multisensory integration in stimulus detection. Brain Res Cogn Brain Res, 2003. 17(2): p. 447-53.
6. Meredith, M.A. and B.E. Stein, Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. J Neurophysiol, 1986. 56(3): p. 640-62.

7. Meredith, M.A. and B.E. Stein, Spatial factors determine the activity of multisensory neurons in cat superior colliculus. Brain Res, 1986. 365(2): p. 350-4.
8. Meredith, M.A., J.W. Nemitz, and B.E. Stein, Determinants of multisensory integration in superior colliculus neurons. I. Temporal factors. J Neurosci, 1987. 7(10): p. 3215-29.
9. Hairston, W.D., et al., Altered temporal profile of visual-auditory multisensory interactions in dyslexia. Experimental Brain Research, 2005. 166(3-4): p. 474-480.
10. Carriere, B.N., D.W. Royal, and M.T. Wallace, Spatial Heterogeneity of Cortical Receptive Fields and Its Impact on Multisensory Interactions. J Neurophysiol, 2008.
11. Royal, D.W., B.N. Carriere, and M.T. Wallace, Spatiotemporal architecture of cortical receptive fields and its impact on multisensory interactions. Exp Brain Res, 2009. 198(2-3): p. 127-36.
12. Schroeder, C.E. and J.J. Foxe, The timing and laminar profile of converging inputs to multisensory areas of the macaque neocortex. Brain Res Cogn Brain Res, 2002. 14(1): p. 187-98.
13. Ghazanfar, A.A., et al., Multisensory integration of dynamic faces and voices in rhesus monkey auditory cortex. J Neurosci, 2005. 25(20): p. 5004-12.
This study gives a first look at multisensory integration of face and species-specific vocalizations within STS indicating that multisensory processes within STS adhere to the principles of multisensory inetgration.
14. Schlack, A., et al., Multisensory space representations in the macaque ventral intraparietal area. J Neurosci, 2005. 25(18): p. 4616-25.
This study shows for the first time that VIP neurons are responsive to auditory and visual stimuli while emphasizing that multisensory interactions occur albeit the differences in reference frame coding of auditory and visual cues.
15. Sugihara, T., et al., Integration of auditory and visual communication information in the primate ventrolateral prefrontal cortex. J Neurosci, 2006. 26(43): p. 11138-47.
This study demonstrates for the first time that VLPFC neurons actively integrate face and vocalization cues exhibiting response enhancements and suppression depending on stimulus effectiveness.
16. Mikami, A., K. Nakamura, and K. Kubota, Neuronal responses to photographs in the superior temporal sulcus of the rhesus monkey. Behav Brain Res, 1994. 60(1): p. 1-13.
17. Baylis, G.C., E.T. Rolls, and C.M. Leonard, Selectivity between faces in the responses of a population of neurons in the cortex in the superior temporal sulcus of the monkey. Brain Res, 1985. 342(1): p. 91-102.
18. Petkov, C.I., et al., A voice region in the monkey brain. Nat Neurosci, 2008. 11(3): p. 367-74.
19. Saito, H., et al., Integration of direction signals of image motion in the superior temporal sulcus of the macaque monkey. J Neurosci, 1986. 6(1): p. 145-57.
20. Oram, M.W., D.I. Perrett, and J.K. Hietanen, Directional tuning of motion-sensitive cells in the anterior superior temporal polysensory area of the macaque. Exp Brain Res, 1993. 97(2): p. 274-94.
21. Oram, M.W. and D.I. Perrett, Integration of form and motion in the anterior superior temporal polysensory area (STPa) of the macaque monkey. J Neurophysiol, 1996. 76(1): p. 109-129.
22. Benevento, L.A., et al., Auditory--visual interaction in single cells in the cortex of the superior temporal sulcus and the orbital frontal cortex of the macaque monkey. Exp Neurol, 1977. 57(3): p. 849-72.
23. Hikosaka, K., et al., Polysensory properties of neurons in the anterior bank of the caudal superior temporal sulcus of the macaque monkey. J Neurophysiol, 1988. 60(5): p. 1615-37.
24. Watanabe, J. and E. Iwai, Neuronal activity in visual, auditory and polysensory areas in the monkey temporal cortex during visual fixation task. Brain Res Bull, 1991. 26(4): p. 583-92.
25. Ghazanfar, A.A., C. Chandrasekaran, and N.K. Logothetis, Interactions between the superior temporal sulcus and auditory cortex mediate dynamic face/voice integration in rhesus monkeys. J Neurosci, 2008. 28(17): p. 4457-69.
26. Maier, J.X., C. Chandrasekaran, and A.A. Ghazanfar, Integration of bimodal looming signals through neuronal coherence in the temporal lobe. Curr Biol, 2008. 18(13): p. 963-8.
27. Barraclough, N.E., et al., Integration of visual and auditory information by superior temporal sulcus neurons responsive to the sight of actions. J Cogn Neurosci, 2005. 17(3): p. 377-91.
28. Beauchamp, M.S., et al., Integration of auditory and visual information about objects in superior temporal sulcus. Neuron, 2004. 41(5): p. 809-23.
29. Colby, C.L., J.R. Duhamel, and M.E. Goldberg, Ventral intraparietal area of the macaque: anatomic location and visual response properties. J Neurophysiol, 1993. 69(3): p. 902-14.
30. Colby, C.L. and M.E. Goldberg, Space and attention in parietal cortex. Annu Rev Neurosci, 1999. 22: p. 319-49.
31. Cooke, D.F., et al., Complex movements evoked by microstimulation of the ventral intraparietal area. Proc Natl Acad Sci U S A, 2003. 100(10): p. 6163-8.
32. Lewis, J.W. and D.C. Van Essen, Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey. J Comp Neurol, 2000. 428(1): p. 112-37.
33. Bremmer, F., et al., Visual-vestibular interactive responses in the macaque ventral intraparietal area (VIP). Eur J Neurosci, 2002. 16(8): p. 1569-86.
34. Duhamel, J.R., C.L. Colby, and M.E. Goldberg, Ventral intraparietal area of the macaque: congruent visual and somatic response properties. J Neurophysiol, 1998. 79(1): p. 126-36.
35. Mullette-Gillman, O.A., Y.E. Cohen, and J.M. Groh, Eye-centered, head-centered, and complex coding of visual and auditory targets in the intraparietal sulcus. J Neurophysiol, 2005. 94(4): p. 2331-52.
36. Gifford, G.W., 3rd and Y.E. Cohen, Spatial and non-spatial auditory processing in the lateral intraparietal area. Exp Brain Res, 2005. 162(4): p. 509-12.
37. Grunewald, A., J.F. Linden, and R.A. Andersen, Responses to auditory stimuli in macaque lateral intraparietal area. I. Effects of training. J Neurophysiol, 1999. 82(1): p. 330-42.
38. Linden, J.F., A. Grunewald, and R.A. Andersen, Responses to auditory stimuli in macaque lateral intraparietal area. II. Behavioral modulation. J Neurophysiol, 1999. 82(1): p. 343-58.
39. Petrides, M. and D.N. Pandya, Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. Eur J Neurosci, 2002. 16(2): p. 291-310.
40. Barbas, H. and D.N. Pandya, Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. J Comp Neurol, 1989. 286(3): p. 353-75.
41. Chavis, D.A. and D.N. Pandya, Further observations on cor-

ticofrontal connections in the rhesus monkey. Brain Res, 1976. 117(3): p. 369-86.
42. Nakahara, K., et al., Functional MRI of macaque monkeys performing a cognitive set-shifting task. Science, 2002. 295(5559): p. 1532-6.
43. Sakagami, M., et al., A code for behavioral inhibition on the basis of color, but not motion, in ventrolateral prefrontal cortex of macaque monkey. J Neurosci, 2001. 21(13): p. 4801-8.
44. Miller, E.K., The prefrontal cortex: complex neural properties for complex behavior. Neuron, 1999. 22(1): p. 15-7.
45. Wilson, F.A., S.P. Scalaidhe, and P.S. Goldman-Rakic, Dissociation of object and spatial processing domains in primate prefrontal cortex. Science, 1993. 260(5116): p. 1955-8.
46. Funahashi, S., C.J. Bruce, and P.S. Goldman-Rakic, Visuospatial coding in primate prefrontal neurons revealed by oculomotor paradigms. J Neurophysiol, 1990. 63(4): p. 814-31.
47. Romanski, L.M. and P.S. Goldman-Rakic, An auditory domain in primate prefrontal cortex. Nat Neurosci, 2002. 5(1): p. 15-6.
48. Gifford, G.W., 3rd, et al., The neurophysiology of functionally meaningful categories: macaque ventrolateral prefrontal cortex plays a critical role in spontaneous categorization of species-specific vocalizations. J Cogn Neurosci, 2005. 17(9): p. 1471-82.
49. Romanski, L.M., B.B. Averbeck, and M. Diltz, Neural representation of vocalizations in the primate ventrolateral prefrontal cortex. J Neurophysiol, 2005. 93(2): p. 734-47.
50. Clarey, J.C. and D.R. Irvine, Auditory response properties of neurons in the anterior ectosylvian sulcus of the cat. Brain Res, 1986. 386(1-2): p. 12-9.
51. Clarey, J.C. and D.R. Irvine, The anterior ectosylvian sulcal auditory field in the cat: I. An electrophysiological study of its relationship to surrounding auditory cortical fields. J Comp Neurol, 1990. 301(2): p. 289-303.
52. Clarey, J.C. and D.R. Irvine, The anterior ectosylvian sulcal auditory field in the cat: II. A horseradish peroxidase study of its thalamic and cortical connections. J Comp Neurol, 1990. 301(2): p. 304-24.
53. Mucke, L., et al., Physiologic and anatomic investigation of a visual cortical area situated in the ventral bank of the anterior ectosylvian sulcus of the cat. Exp Brain Res, 1982. 46(1): p. 1-11.
54. Norita, M., et al., Connections of the anterior ectosylvian visual area (AEV). Exp Brain Res, 1986. 62(2): p. 225-40.
55. Olson, C.R. and A.M. Graybiel, Ectosylvian visual area of the cat: location, retinotopic organization, and connections. J Comp Neurol, 1987. 261(2): p. 277-94.
56. Clemo, H.R. and B.E. Stein, Organization of a fourth somatosensory area of cortex in cat. J Neurophysiol, 1983. 50(4): p. 910-25.
57. Clemo, H.R. and B.E. Stein, Somatosensory cortex: a 'new' somatotopic representation. Brain Res, 1982. 235(1): p. 162-8.
58. Wallace, M.T., M.A. Meredith, and B.E. Stein, Integration of multiple sensory modalities in cat cortex. Exp Brain Res, 1992. 91(3): p. 484-8.
59. Malhotra, S., A.J. Hall, and S.G. Lomber, Cortical control of sound localization in the cat: unilateral cooling deactivation of 19 cerebral areas. J Neurophysiol, 2004. 92(3): p. 1625-43.
60. Lomber, S.G., S. Malhotra, and A.J. Hall, Functional specialization in non-primary auditory cortex of the cat: areal and laminar contributions to sound localization. Hear Res, 2007. 229(1-2): p. 31-45.
61. Tamai, Y., E. Miyashita, and M. Nakai, Eye movements following cortical stimulation in the ventral bank of the anterior ectosylvian sulcus of the cat. Neurosci Res, 1989. 7(2): p. 159-63.
62. Kimura, A. and Y. Tamai, Sensory response of cortical neurons in the anterior ectosylvian sulcus, including the area evoking eye movement. Brain Res, 1992. 575(2): p. 181-6.
63. Scannell, J.W., et al., Visual motion processing in the anterior ectosylvian sulcus of the cat. J Neurophysiol, 1996. 76(2): p. 895-907.
64. Alvarado, J.C., et al., Cortex mediates multisensory but not unisensory integration in superior colliculus. J Neurosci, 2007. 27(47): p. 12775-86.
This study illustrates the role of AES as a mediator for cross-modal but not within-modal integration in the SC.
65. Wilkinson, L.K., M.A. Meredith, and B.E. Stein, The role of anterior ectosylvian cortex in cross-modality orientation and approach behavior. Exp Brain Res, 1996. 112(1): p. 1-10.

CANDIDATE
REVIEWS

Glucocorticoid Receptor-Mediated Effects within the Extended Amygdala

Katherine Louderback

Abstract

Stress has been associated with a number of adverse effects, including anxiety disorders and addiction. Glucocorticoids are released during stress and bind to glucocorticoid receptors (GRs) present in every cell of the body. Limbic areas, such as the hippocampus, express high levels of GR, and the receptors have been extensively examined within these regions for their ability to alter synaptic plasticity. GRs within one limbic region, the extended amygdala - consisting of the shell of the nucleus accumbens (NAc-Sh), the central nucleus of the amygdala (CeA), and the bed nucleus of the stria terminalis (BNST), have received relatively less attention. Given the prominent role of the extended amygdala in the integration of stress and reward circuitry, and the demonstrated capability of GRs to alter synaptic plasticity, it is somewhat surprising that GRs within the region have not been studied to a greater extent. This review will examine current literature of GR-mediated effects within the extended amygdala. In short, GR activation seems to increase excitability in the extended amygdala. GRs facilitate dopamine release in response to drugs of abuse and stress within the NAc-Sh, facilitate fear conditioning and anxiety within the CeA, and decrease anxiety and maintain excitability within the BNST. Activation of GRs within the extended amygdala plays a pivotal role in response to stress and reward, and disregulation of GRs within the region could lead to maladaptive responses that typify anxiety disorders and addiction.

Keywords

Stress
Anxiety
Addiction
Plasticity
Glucocorticoid Receptor
Extended Amygdala
Nucleus Accumbens
Central Nucleus of the Amygdala
Bed Nucleus of the Stria Terminalis

Introduction

Stress is prevalent in everyday life, and can be defined as "a condition or feeling experienced when a person perceives that demands exceed the personal and social resources the individual is able to mobilize"[1]. While surmounting stress experienced is crucial to survival, maladaptive responses to stress or prolonged stress can prove detrimental. Stress has been associated with anxiety disorders such as generalized anxiety disorder, post-traumatic stress disorder (PTSD), and panic disorder, as well as addiction. Indeed, stress is a commonly cited reason for relapse to drug use in addicts, and diagnosis of an anxiety disorder is significantly associated with drug use[2]. A deeper understanding of the effects of stress on anxiety and reward circuitry will prove invaluable for treating and preventing anxiety and addictive behaviors such as stress-induced reinstatement of drug seeking.

The HPA Axis and Glucocorticoid Release

Upon exposure to a stressful stimulus, the hypothalamic-pituitary-adrenal (HPA) axis is activated. Corticotropin releasing factor (CRF) is first released from the parvocellular neurons of the hypothalamus to the pituitary through the portal system, triggering the release of adrenocorticotropin (ACTH)[3]. ACTH then acts upon the adrenal cortex, leading to the release of glucocorticoids (cortisol in humans and corticosterone in rodents; CORT) into the blood stream. CORT binds to two receptor types, the mineralocorticoid receptor (MR) and the GR, which has about 10-fold lower affinity for CORT than MR[4]. While MRs are almost entirely occupied under basal conditions, the lower affinity GR is only activated when high circulating concentrations of CORT are present – such as during the circadian peak of CORT release and during stress – and acts as negative feedback to inhibit the HPA axis[4]. Interestingly, it has also been demonstrated that administration of drugs of abuse and drug withdrawal leads to an increase in plasma CORT levels in rodents[5-6] and humans[7-9] and subsequent activation of GRs. Thus, GRs may play a role in drug addiction and withdrawal, in addition to its role in the stress response.

Complete knockout of GR is lethal in mice, indicating that this receptor is necessary for survival[10]. Site-specific genetic or pharmacological alterations in GR function have proved more useful in assessing the receptors' roles. GRs are expressed ubiquitously within the brain[4,11], and show highest expression within a number of limbic regions, including the hippocampus, CeA, BNST.

Effects of Glucocorticoid Receptor Activation

The effects of GR activation are extensive, and involve two distinct mechanisms: the genomic pathway and the non-genomic pathway[12]. In the genomic pathway, GRs within the cytosol bind CORT that diffuses freely through the plasma membrane. Unbound GR is maintained in a protein heterocomplex in the cytoplasm[13-14]. The binding of ligand leads to the increased phosphorylation of GR[15]. This phosphorylation allows GR to form a dimer with other transcription factors (TFs) or another GR[16-17] and translocate to the nucleus[15]. GRs can function as a homodimer or with other TFs in order to trans-activate or trans-repress genes. Within the nucleus, the ligand-bound GR homodimer is able to bind glucocorticoid response elements (GREs) that are present upstream of the promoter of a number of genes[18-19] or the homodimer can bind another TF in order to enhance or inhibit its transcription effect[20]. In fact, transcription can be altered by GRs in an estimated 1-2% of all genes[21].

It has been demonstrated that CORT is also able to induce rapid effects within minutes, a time frame not compatible with transcriptional effects of GR, via a putative membrane-bound GR (mGR). For instance, injection of the specific GR agonist dexamethasone to the paraventricular nucleus of the hypothalamus (PVN) is able to inhibit ACTH release in response to restraint stress within minutes[22]. Dexamethasone conjugated to BSA, which is membrane impermeable, is able to recapitulate this effect, giving further evidence for a mGR. Some debate does exist over the involvement of the classical GR in these rapid effects of CORT and dexamethasone. A possible yet-undetermined G-protein coupled receptor (GPCR) has been implicated as pituitary cell lines were able bind CORT and dexamethasone at the membrane with no apparent affinity for the GR antagonist RU486[23]. The binding of ligand in this study was blocked by pertussis toxin, which uncouples G-proteins from their GPCR[23]. However, GR antagonism has been shown to inhibit some rapid effects of CORT or dexamethasone[24]. In addition, possible mechanisms have been identified that could localize the classical GR to the membrane – such as the presence of a conserved palmitoylation site that has been shown to link the estrogen receptor to the membrane[25-26] and direct binding of GRs to caveolin[27].

GR-mediated Alterations in Synaptic Plasticity

A predominant effect of mGR activation is the recruitment of the endocannabinoid (eCB) system. Through the activation of PLC, GRs induce the production and retrograde release of the eCB 2-arachidonoylglycerol (2-AG) from the postsynaptic bouton[28]. 2-AG binds to the cannabinoid receptor (CB1), leading to a decrease in presynaptic neurotransmitter release[29]. In this way, mGRs can inhibit excitatory transmission[22,24] or inhibit GABAergic projections to glutamatergic neurons, thus disinhibiting excitatory transmission[30,31].

GRs have been implicated in alterations to synaptic plasticity and excitability. The hippocampus, in particular, has been studied extensively due to long-established electrophysiological recording techniques and high expression of both GRs and MRs. GRs are generally thought to reduce neuronal excitability within the hippocampus. For instance, in the CA1 region, activation of GRs mediates impairments in NMDA-dependent long-term potentiation (LTP) through a slow genomic mechanism[32-33], as well as facilitates metabotropic glutamate receptor-dependent long term depression (LTD) by lowering the threshold for LTD induction[34].

The Extended Amygdala

Although the involvement of GRs in many limbic areas has been examined[21,35], one region that has received relatively less attention is the extended amygdala. The extended amygdala consists of the shell of the nucleus accumbens (NAc-Sh), the central nucleus of the amygdala (CeA), and the bed nucleus of the stria terminalis (BNST)[36-38]. This region is situated at the crossroads of stress and reward circuitry and has thus been heavily implicated in the negative affect associated with stress disorders and withdrawal from drugs of abuse[39-41]. While the involvement of noradrenergic[42] and CRF signaling[43] within the extended amygdala in anxiety and addiction behaviors has been studied extensively, the role of GRs within this region is less clear. GRs within the extended amygdala are poised to alter synaptic plasticity and behavioral responses to stress and drugs of abuse. Given the prominent role of this region in stress response and HPA axis modulation, the high expression of GRs, and the proven ability of GR to alter synaptic transmission, the paucity of literature examining GRs within the extended amygdala is somewhat surprising. This review will explore current literature of GR-mediated effects within the extended amygdala, particularly in the context of anxiety and addiction behavior.

Nucleus Accumbens

GR-mediated Effects on Excitability

Within the NAc-Sh, GR activation has been associated with an increase in neuronal excitability and extracellular dopamine (DA) levels. It has long been known that the

NAc-Sh is more responsive to glucocorticoids than the NAc core region[44]. Recently, Campioni, et al.[45] demonstrated that the activation of GR leads to increased neuronal excitability in the NAc-Sh. The AMPA/NMDA ratio was increased in the shell following a cold water forced swim stress, and this effect was postulated to be GR-mediated, as it was mirrored with CORT application and abolished by RU486. Increased AMPAR miniature excitatory postsynaptic current (mEPSC) amplitude and reduced rectification of AMPA currents suggested that the increase in excitability was primarily due to an increase in the number of functional GluR2-containing AMPA receptors (AMPARs) present at the postsynaptic membrane[45]. In accordance, it has been demonstrated using cell culture models that long-term corticosterone application facilitates the lateral diffusion of AMPARs though a GR-mediated mechanism that can be blocked with a GR antagonist[46]. Because GR-facilitated integration of AMPARs to the post synaptic membrane is delayed and can be blocked by the protein synthesis inhibitor cycloheximide, it has been suggested that this effect is mediated through the genomic pathway[47]. The observation of increased AMPAR mEPSC amplitude in the NAc is mirrored by earlier work in the CA1 region of the hippocampus in which CORT or a selective GR agonist was able to enhance amplitude – but not frequency – of AMPAR mEPSCs[48]. The enhanced excitability within the NAc in response to GR activation could play a role in the increased drive to obtain a drug of abuse or in the ability of stress to evoke compulsive behaviors in addicted individuals.

GR Involvement in NAc Dopaminergic Signaling

Dopaminergic projections from the ventral tegmental area (VTA) to the NAc are crucial in the reward system[49], and it has been demonstrated that stressors are capable of initiating relapse to drug seeking in humans[50-51] and rodents[52-53]. Thus, the effect of stress on dopamine release within the NAc is an active area of research. Indeed, footshock stress is capable of increasing extracellular DA levels within the NAc shell in the rat, with no change in the NAc core[54]. This effect appears to be mediated through stress-induced CORT release, as adrenalectomy selectively lowers extracellular DA in the shell but not the core[44]. The DA spike observed in the NAc-Sh following stress or the administration of various drugs of abuse may be due in part to the activation of GRs. The hyperlocomotion and increase in extracellular NAc DA following systemic morphine administration can be attenuated by i.c.v. treatment with RU486[55]. In fact, direct infusion of RU486 to the NAc is capable of preventing conditioned place preference to morphine in rodents[56]. It has also been found that mice lacking GRs in D1 dopamine receptor-containing (dopaminoceptive) neurons showed decreased DA release in the NAc following cocaine administration[57]. These studies suggest that GRs have a central role in the release of DA within the NAc following drug administration.

The rise in extracellular DA within the NAc following stress or administration of drugs of abuse could be partially due to GRs in the VTA. Acute stress has been demonstrated to increase the AMPA/NMDA ratio in DA neurons within the VTA to a greater extent than acute administration of drugs of abuse[58], and this effect is blocked completely by RU486. Morphine, cocaine, nicotine, and forced swim stress impair the ability of GABAergic synapses to induce LTP onto VTA DA neurons. This leads to a disinhibition of these projections to the NAc, and increased DA release within the NAc[59]. The stress-induced impairment of GABAergic LTP is believed to be GR-mediated as it was attenuated by RU486. Further, direct infusion of CORT to the VTA is sufficient to induce NAc DA release, and this is effectively blocked by coapplication of RU486[60]. Because exposure to a stressor is capable of initiating DA release within the NAc through a GR-dependent mechanism, and because drugs of abuse have been demonstrated to induce a similar DA spike within the NAc, GRs within the NAc and regions projecting to the NAc are crucial for drug-seeking behaviors such as stress-induced reinstatement. Elevation in NAc DA following exposure to drugs of abuse is a key component in the early rewarding stages of drug addiction[39], thus xposure to a stressor after a long period of drug abstinence would cause a GR-mediated DA spike within the NAc that may be reminiscent of the rewarding effects of such early drug use. This could lead a previously addicted individual to return to their drug of choice in order to mediate the rewarding effects while simultaneously blocking the negative affect caused by the stressor.

Central Nucleus of the Amygdala

Regulation of the HPA Axis and GR Pharmacology:The CeA is well situated to contribute to the HPA axis response to a stressor. Electrical stimulation of the CeA leads to an HPA response with increased serum CORT[61]. Stimulation of GABAergic projections from the CeA to the BNST quiets BNST GABAergic projections to the PVN, thus leading to a disinhibition of the HPA response[62]. The CeA in particular has been implicated in the response to an acute stressor, and has been argued to mediate stimulus-specific fear-like behavior associated with such a stressor[63-64]. Indeed, ablation of the CeA completely eliminates cue-induced potentiation

of startle response to a footshock[65].

The CeA contains the densest expression of GR within the amygdala[11], potentially implicating GRs in the fear response mediated by the CeA. This has led some to examine the effects of GRs within the CeA on fear- and anxiety-like responses in rodents. Selective pharmacologic activation of GRs within the CeA elevates GR expression levels, increases anxiety-like behavior in the elevated plus maze (EPM), and increases the plasma CORT in response to the stress of exposure to the EPM[66]. Conversely, increased anxiety-like behavior on the EPM following implantation of a CORT pellet into the CeA can be blocked with co-administration of the GR antagonist RU486 into the CeA[67].

Genetic Deletion of GRs within the CeA

The development of a transgenic "floxed" mouse harboring loxP sites around exons 1 and 2 of the GR gene, NR3C1, has been an invaluable resource for determining region-specific involvement of GRs within the brain as the mouse exhibits a loss of GR expression in regions exposed to Cre-recombinase[68]. Lentivirally-mediated delivery of Cre-recombinase has allowed precise site-specific deletion of GR in this mouse line, and was recently utilized in order to examine the effect of GR deletion within the CeA (CeAGRKO) on anxiety- and fear-related behaviors[69-70]. The resulting 65% deletion of GRs within CeA neurons did not lead to alterations in locomotor activity or circulating plasma levels of CORT, and the apparent incongruity with the pharmacological HPA data described above may be attributable to the incomplete GR deletion observed in the present study. In accordance with the CeA's role in fear-like behavior, CeAGRKO mice exhibited impairment in both cue and contextual fear conditioning when compared to mice injected with lentiviral GFP[69]. Interestingly, the effect of GRs specifically within the CeA on fear-conditioning was further confirmed by mice with forebrain GR knockout (FBGRKO). These animals lack GRs in the cortex, hippocampus, BLA, and striatum but do not show GR disruption in the CeA or PVN[71]. FBGRKO mice do not demonstrate impairments in fear conditioning, indicating that the fear-like CeAGRKO phenotype is region-specific. Further, it was demonstrated that adrenalectomized mice show impairments in contextual fear conditioning, but have intact cue fear conditioning[72]. Thus, there may be mechanisms in place within other brain areas to account for global brain reductions in CORT signaling, as specific impairment of CeA GRs leads to a more robust fear conditioning phenotype than adrenalectomy. Thus, GRs within the CeA are capable of inducing HPA axis activation in response to an acutely stressful stimulus, which defies the classical role of GRs in the negative feedback of the HPA axis upon activation. Thus, elevated GR activation within this region could be postulated to cause chronically elevated CORT levels and future anxiety related disorders.

Bed Nucleus of the Stria Terminalis

Despite direct projections to the PVN and heavy GR expression, the roles of GRs within the BNST are very poorly understood. Dunn[73] demonstrated that electrical stimulation of the lateral aspect of the BNST decreased plasma CORT levels, presumably through activation of GABAergic projections to the PVN. Adrenalectomy decreases expression of CRF mRNA within the dorsolateral aspect of the BNST (dlBNST) and the CeA while increasing extracellular norepinephrine and DA in the dlBNST[74]. Our group has recently shown that chronic stress or systemic CORT administration increases anxiety-like behavior in mice and blunts LTP within the dlBNST[75]. It has long been known that chronic treatment with CORT downregulates GR function within the brain[76], leading to impaired negative feedback of the HPA axis. Thus, the inhibitory effect of CORT treatment on BNST plasticity may represent decreased GR function and reflect a role of GRs in the maintenance of excitability within the region. In support of this hypothesis, specific deletion of BNST GRs using the floxed GR mice described above exacerbated anxiety-like behavior in response to chronic stress as well decreasing locomotion in a stressful situation (EPM), mimicking the effects of chronic CORT administration described above (unpublished data). An important future study will examine alterations in excitability within the BNST of these mice, as well as the consequence of pharmacological manipulation of GRs on BNST excitability. We hypothesize that one role of GRs within the BNST is to maintain excitability in order to inhibit the HPA axis in response to a stressor. Thus, chronic stress exposure could downregulate GRs within the region and lead to hyperactivity of the HPA axis and associated conditions, such as anxiety-related disorders and addiction.

Conclusions

While GRs have been postulated to reduce excitability in other limbic regions such as the hippocampus[32-34], emerging literature seems to indicate that GRs in the extended amygdala strengthen excitability. GRs in this region likely mediate appropriate response to stressful events in healthy individuals, but dysregulation of GRs, through drug addiction or chronic stress for example, may trigger the development of maladaptive behaviors. . For instance, GR-mediated enhancement of glutamate response and extracel-

lular dopamine in the NAc might contribute to the salience of natural rewards under normal circumstances, but may lead to stress-induced relapse to addiction in individuals with altered extended amygdala circuitry as a result of previous drug use. Activation of CeA GRs causes anxiety-like responses and CORT release[66-67], whereas selective deletion of CeA GRs appears to alleviate fear-like behavior[69]. Thus, CeA GRs likely initiate an HPA response to a frightening stimulus by strengthening GABAergic projections to the BNST and disinbibiting the PVN. However, an individual with unusually high GR tone within the CeA would likely have a lowered threshold for fear-like responses and may be susceptible to anxiety disorders and addiction. Finally, GRs within the BNST may maintain excitability in order to inhibit the HPA axis following exposure to a stressor. However, in an individual that has undergone chronic stress, BNST GRs could be downregulated, and the ability of the BNST to inhibit the HPA axis would be impaired. This could result in anxiety disorders or stress-induced relapse to drug seeking, a behavior that is dependent upon the BNST[41]. GRs within the extended amygdala are important in mediating anxiety- or addiction-like responses to stress. Extensive further study of the effects of GRs within the region on plasticity and anxiety- and addiction-like behaviors will prove crucial to complete understanding of such maladaptive responses to stress.

References

1. Fink G, (2010). Stress Science: Neuroendocrinology, 1st Ed. San Diego: Academic Press.
2. Sareen J, Chartie M, Paulus MP and Stein MB (2006). Illicit drug use and anxiety disorders: findings from two community surveys. Psychiatry research, 142(1): 11-17.
3. Harris GW (1948). Neural control of the Pituitary Gland. Physiological Reviews, 28(2): 139-179.
4. Reul JM and de Kloet ER (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology, 117(6): 2505-2511.
5. Ellis FW (1966). Effect of Ethanol on Plasma Corticosterone Levels. Journal of Pharmacology and Experimental Therapeutics. 153(1): 121-127.
6. Levy AD, Li Q, Kerr JE., Rittenhouse PA, Milonas G, Cabrera TM, Battaglia G, Alvarez Sanz MC and Van de Kar LD(1991). Cocaine-induced elevation of plasma adrenocorticotropin hormone and corticosterone is mediated by serotonergic neurons. The Journal of pharmacology and experimental therapeutics, 259(2): 495-500.
7. Mello NK and Mendelson JH (1997). Cocaine's Effects on Neuroendocrine Systems: Clinical and Preclinical Studies. Pharmacology Biochemistry and Behavior, 57(3): 571-599.
8. Adinoff B, Ruether K, Krebaum S, Iranmanesh A and Williams MJ (2003). Increased salivary cortisol concentrations during chronic alcohol intoxication in a naturalistic clinical sample of men. Alcoholism, clinical and experimental research, 27(9): 1420-1427.
9. Mendelson JH, Sholar MB, Goletiani N, Siegel AJ and Mello NK (2006). Effects of Low and High Nicotine Cigarette Smoking on Mood States and the HPA Axis in Men. Neuropsychopharmacology, 30(9): 1751-1763.
10. Cole TJ, Blendy JA, Monaghan AP., Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K and Schultz G (1995). Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes & Development, 9(13): 1608-1621.
11. Morimoto M, Morita N, Ozawa H and Yokoyama K (1996). Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. Neuroscience Research, 26: 235-269.
12. Prager EM and Johnson LR (2009). Stress at the synapse: signal transduction mechanisms of adrenal steroids at neuronal membranes. Science signaling, 2(86): 1-10.
13. Smith DF and Toft DO (1993). Steroid receptors and their associated proteins. Molecular endocrinology, 7(1): 4-11.
14. Morishima Y, Murphy PJ, Li DP, Sanchez ER and Pratt WB (2000). Stepwise assembly of a glucocorticoid receptor.hsp90 heterocomplex resolves two sequential ATP-dependent events involving first hsp70 and then hsp90 in opening of the steroid binding pocket. The Journal of biological chemistry, 275(24): 18054-18060.
15. Wang Z, Frederick J and Garabedian MJ (2002). Deciphering the phosphorylation "code" of the glucocorticoid receptor in vivo. The Journal of biological chemistry, 277(29): 26573-26580.
16. Kassel O and Herrlich P (2007). Crosstalk between the glucocorticoid receptor and other transcription factors: molecular aspects. Molecular and cellular endocrinology, 275(1-2): 13-29.
17. Presman DM, Alvarez LD, Levi V, Eduardo S, Digman MA, Martí MA, Veleiro AS, Burton G and Pecci A (2010). Insights on glucocorticoid receptor activity modulation through the binding of rigid steroids. PloS one, 5(10): e13279
18. Chandler VL, Maler BA and Yamamoto KR (1983). DNA sequences bound specifically by glucocorticoid receptor in vitro render a heterologous promoter hormone responsive in vivo. Cell, 33(2): 489-499.
19. Becker PB, Gloss B, Schmid W, Strahle U and Schutz G (1986). In vivo protein-DNA interactions in a glucocorticoid response element require the presence of the hormone, Nature, 324: 686-688.
20. Schoneveld OJLM, Gaemers IC and Lamers WH (2004). Mechanisms of glucocorticoid signalling. Biochimica et biophysica acta, 1680(2): 114-128.
21. Joëls M, Krugers HJ, Lucassen PJ and Karst H (2009). Corticosteroid effects on cellular physiology of limbic cells. Brain research, 1293: 91-100.
22. Evanson NK, Tasker JG, Hill MN, Hillard CJ and Herman JP (2010). Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. Endocrinology, 151(10): 4811-4819.
23. Maier C, Rünzler D, Schindelar J, Grabner G, Waldhäusl W, Köhler G and Luger A (2005). G-protein-coupled glucocorticoid receptors on the pituitary cell membrane. Journal of cell science, 118: 3353-3361.
24. Karst H, Berger S, Erdmann G, Schütz G and Joëls M (2010). Metaplasticity of amygdalar responses to the stress hormone corticosterone. Proceedings of the National Academy of Sciences of the United States of America, 107(32): 14449-14454.

25. Pedram A, Razandi M, Sainson RCA, Kim JK, Hughes CC and Levin ER (2007). A conserved mechanism for steroid receptor translocation to the plasma membrane. The Journal of biological chemistry, 282(31): 22278-22288.
26. Groeneweg FL, Karst H, de Kloet ER and Joëls M (2011). Rapid non-genomic effects of corticosteroids and their role in the central stress response. The Journal of endocrinology, 209(2): 153-167.
27. Matthews L, Berry A, Ohanian V, Ohanian J, Garside H and Ray D (2008). Caveolin mediates rapid glucocorticoid effects and couples glucocorticoid action to the antiproliferative program. Molecular endocrinology, 22(6): 1320-1330.
28. Di S, Malcher-Lopes R, Halmos KC and Tasker JG (2003). Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. The Journal of Neuroscience 23(12): 4850-4857.
29. Kano M, Ohno-shosaku T, Hashimotodani Y and Uchigashima M (2009). Endocannabinoid-Mediated Control of Synaptic Transmission. Physiological Reviews, 89: 309-380.
30. Patel S, Kingsley PJ, Mackie K, Marnett LJ and Winder DG (2009). Repeated Homotypic Stress Elevates 2-Arachidonoylglycerol Levels and Enhances Short-Term Endocannabinoid Signaling at Inhibitory Synapses in Basolateral Amygdala. Neuropsychopharmacology, 34(13): 2699-2709.
31. Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TTY, Karatsoreos IN, Mackie K, Viau V, Pickel VM, McEwen BS, Liu Q, Gorzalka BB and Hillard CJ (2011). Recruitment of Prefrontal Cortical Endocannabinoid Signaling by Glucocorticoids Contributes to Termination of the Stress Response. Journal of Neuroscience, 31(29): 10506-10515.
32. Krugers HJ, Alfarez DN, Karst H, Parashkouhi K, van Gemert N and Joëls M (2005). Corticosterone shifts different forms of synaptic potentiation in opposite directions. Hippocampus, 15(6): 697-703.
33. Wiegert O, Pu Z, Shor S, Joëls M and Krugers H (2005). Glucocorticoid receptor activation selectively hampers N-methyl-D-aspartate receptor dependent hippocampal synaptic plasticity in vitro. Neuroscience, 135(2): 403-411.
34. Chaouloff F, Hémar A and Manzoni O (2008). Local facilitation of hippocampal metabotropic glutamate receptor-dependent long-term depression by corticosterone and dexamethasone. Psychoneuroendocrinology, 33(5): 686-691.
35. Sousa N, Cerqueira JJ and Almeida OFX (2008). Corticosteroid receptors and neuroplasticity. Brain research reviews, 57(2): 561-570.
36. Alheid G and Heimer L (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: The striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience, 27(1): 1-39.
37. Cassell MD, Freedman LJ and Shi C (1999). The intrinsic organization of the central extended amygdala. Annals of the New York Academy of Sciences, 877: 217-241.
38. Alheid GF (2003). Extended Amygdala and Basal Forebrain. Annals of the New York Academy of Sciences, 985: 185-205.
39. Koob GF and Volkow ND (2010). Neurocircuitry of addiction. Neuropsychopharmacology, 35(1): 217-238.
40. Yamada H and Bruijnzeel AW (2011). Stimulation of α2-adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. Neuropharmacology, 60(2-3): 303-311.
41. Briand LA, Vassoler FM, Pierce RC, Valentino RJ and Blendy JA (2010). Ventral tegmental afferents in stress-induced reinstatement: the role of cAMP response element-binding protein. The Journal of neuroscience, 30(48): 16149-16159.
42. Smith RJ and Aston-Jones G (2008). Noradrenergic transmission in the extended amygdala: role in increased drug-seeking and relapse during protracted drug abstinence. Brain structure & function, 213(1-2), 43-61.
43. Koob GF (2010). The role of CRF and CRF-related peptides in the dark side of addiction. Brain research, 1314: 3-14.
44. Barrot M, Marinelli M, Abrous DN, Rougé-Pont F, Le Moal M and Piazza PV (2000). The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. The European journal of neuroscience, 12(3): 973-979.
45. Campioni MR, Xu M and McGehee DS (2009). Stress-induced changes in nucleus accumbens glutamate synaptic plasticity. Journal of neurophysiology, 101(6): 3192-3198. The authors demonstrated the ability of stress and pharmacological GR activation to increase NAc-SH excitability through enhanced AMPA/NMDA ratio, mediated by recruitment of GluR2-containing AMPA receptors to the postsynaptic membrane.
46. Groc L, Choquet D and Chaouloff F (2008). The stress hormone corticosterone conditions AMPAR surface trafficking and synaptic potentiation. Nature neuroscience, 11(8), 868-870.
47. Martin S, Henley JM, Holman D, Zhou M, Wiegert O, van Spronsen M, Joels M, Hoogenraad CC and Krugers HJ (2009). Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity. PloS one, 4(3): e4714.
48. Karst H and Joëls M (2005). Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells. Journal of neurophysiology, 94(5): 3479-34786.
49. Willuhn I, Wanat MJ, Clark JJ and Phillips PEM (2010). Dopamine Signaling in the Nucleus Accumbens of Animals Self-Administering Drugs of Abuse. Current Topics of Behavioral Neuroscience, 3: 29-71.
50. Kosten TR, Rounsaville BJ and Kleber HD (1986). A 2.5 Year Follow-up of Depression, Life Crises, and Treatment Effects on Abstinence Among Opioid Addicts. Arch Gen Psychiatry, 43: 733-738.
51. Brown SA, Vik PV, Patterson TL, Grant I and Schuckit MA (1995). Stress, Vulnerability and Adult Alcohol Relapse. JSAD. 56(5): 538-545.
52. Erb S, Shaham Y and Stewart J (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology, 128(4): 408-412.
53. Shaham Y and Stewart J (1995). Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology, 119(3): 334-341.
54. Kalivas PW and Duffy P (1995). Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain research, 675(1-2): 325-328.
55. Marinelli M, Aouizerate B, Barrot M, Le Moal M and Piazza PV (1998). Dopamine-dependent responses to morphine depend on glucocorticoid receptors. Proceedings of the National Academy of Sciences of the United States of America, 95(13): 7742-7747.
56. Dong Z, Han H, Wang M, Xu L, Hao W and Cao J (2006). Morphine Conditioned Place Preference Depends on Glucocorticoid Receptors in Both Hippocampus and Nucleus Accumbens. Hippocampus, 16: 809-813.

This article demonstrates the essential role of NAc GRs in addiction-like drug-seeking behavior in rodents. Systemic administration of the GR antagonist RU486, as well as cannula delivered RU486 directly into the NAc or the hippocampus, blocked morphine conditioned place preference - a highly utilized model of drug-seeking - in rats.

57. Barik J, Parnaudeau S, Saint Amaux AL, Guiard BP, Golib Dzib JF, Bocquet O, Bailly A, Benecke A and Tronche F (2010). Glucocorticoid receptors in dopaminoceptive neurons, key for cocaine, are dispensable for molecular and behavioral morphine responses. Biological psychiatry, 68(3): 231-239.

58. Saal D, Dong Y, Bonci A and Malenka RC (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron, 37(4): 577-582.

59. Niehaus JL, Murali M and Kauer JA (2010). Drugs of abuse and stress impair LTP at inhibitory synapses in the ventral tegmental area. The European journal of neuroscience, 32(1): 108-117.

60. Tye SJ, Miller AD and Blaha CD (2009). Differential corticosteroid receptor regulation of mesoaccumbens dopamine efflux during the peak and nadir of the circadian rhythm: a molecular equilibrium in the midbrain? Synapse, 63(11): 982-990

61. Weidenfeld J, Itzik A and Feldman S (1997). Effect of glucocorticoids on the adrenocortical axis responses to electrical stimulation of the amygdala and the ventral noradrenergic bundle. Brain research, 754(1-2): 187-194.

62. Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC and Cullinan WE (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo–pituitary–adrenocortical responsiveness. Frontiers in Neuroendocrinology, 24(3): 151-180.

63. Davis M and Shi C (1999). The extended amygdala: are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? Annals of the New York Academy of Sciences, 877: 281-291.

64. Walker DL, Miles LA and Davis M (2009). Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. Progress in neuro-psychopharmacology & biological psychiatry, 33(8): 1291-1308.

65. Hitchcock J and Davis M (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. Behavioral neuroscience, 100(1): 11-22.

66. Weiser MJ, Foradori CD and Handa RJ (2010). Estrogen receptor beta activation prevents glucocorticoid receptor-dependent effects of the central nucleus of the amygdala on behavior and neuroendocrine function. Brain research, 1336: 78-88.

67. Myers B and Greenwood-Van Meerveld B (2007). Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. American journal of physiology. Gastrointestinal and liver physiology, 292(6): G1622-G1629.

68. Brewer JA, Khor B, Vogt SK, Muglia LM, Fujiwara H, Haegele KE, Sleckman BP and Muglia LJ (2003). T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. Nature medicine, 9(10): 1318-1322.

69. Kolber BJ, Roberts MS, Howell MP, Wozniak DF, Sands MS and Muglia LJ (2008). Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. Proceedings of the National Academy of Sciences of the United States of America, 105(33): 12004-12009.

The authors lentivirally administered Cre-recombinase into the CeA of mice harboring loxP sites around the GR gene in order to selectively delete GRs from the region, and saw impairments in context- and cue-conditioned fear behavior and reduced CRF expression in response to stress, demonstrating the involvement of GRs in these effects.

70. Arnett MG, Kolber BJ, Boyle MP and Muglia LJ (2011). Behavioral insights from mouse models of forebrain--and amygdala-specific glucocorticoid receptor genetic disruption. Molecular and cellular endocrinology, 336(1-2): 2-5.

71. Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y and Muglia LJ (2005). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proceedings of the National Academy of Sciences of the United States of America, 102(2): 473-478.

72. Pugh CR, Tremblay D, Fleshner M and Rudy JW (1997). A selective role for corticosterone in contextual-fear conditioning. Behavioral neuroscience, 111(3): 503-511.

73. Dunn JD (1987). Plasma corticosterone responses to electrical stimulation of the bed nucleus of the stria terminalis. Brain research, 407(2): 327-331.

74. Santibañez M, Gysling K and Forray MI (2005). Adrenalectomy decreases corticotropin-releasing hormone gene expression and increases noradrenaline and dopamine extracellular levels in the rat lateral bed nucleus of the stria terminalis. Journal of neuroscience research, 81(1): 140-152.

75. Conrad KL, Louderback KM, Gessner CP and Winder DG (2011). Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. Physiology & behavior, 104(2): 248-256.

Our paper demonstrates possible involvement of GRs in synaptic plasticity within the dlBNST as chronic stress-mediated anxiety-like behavior and blunting of LTP within the dlBNST was mirrored with chronic CORT administration.

76. Spencer RL, Miller AH, Stein M and McEwen BS (1991). Corticosterone regulation of type I and type II adrenal steroid receptors in brain, pituitary, and immune tissue. Brain research, 549(2): 236-246.

Further Information

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Auditory Cortical Processing in Primates and Rodents

Barbara M. J. O'Brien

Abstract

Auditory cortex is the first stage in cortical processing of auditory stimuli and is essential for the perception of sounds. Research of this area has increased in two closely related groups, rodents and non-human primates. However, no comparisons between these two popular animal models have been made to evaluate similarities and differences that could contribute to understanding how mammalian auditory cortex is involved in processing sounds. The most striking commonality among the two groups is evidence of serial and parallel processing. This review seeks to compare the physiology, anatomy, and histology of basic structures of monkey and rodent auditory cortex in order to understand the common roles of this region in these two groups.

Keywords

Auditory
Cortex
Thalamus
Primates
Rodents
Tonotopy
Anatomy

Hearing is an important component of experiencing the world we live in and interacting with our environment. At any given moment, an individual is exposed to many different sounds occurring simultaneously in time and from various locations and sources. It is the purpose of the central auditory system to interpret these sounds and filter out sounds that are irrelevant for the situation.

There are many structures in the brain that process auditory information, but auditory cortex is the first stage in cortical auditory processing and is necessary for perception of sounds[1]. Additionally, there are many areas of cerebral cortex involved in hearing, but other areas of cortex depend on auditory cortex for auditory input. Therefore, auditory cortex is an ideal place to research how sounds are coded by neural circuits, assigned meaning, and relayed to other areas so the individual can perceive sounds, associate them with emotions and memories, and make decisions about behavior. There are three major ways in which to define auditory cortical areas: (1) physiology, (2) anatomy or connectivity, and (3) histology.

In terms of physiology, different parameters can be used to describe how neurons in different fields behave in response to different stimuli being presented to the ear. These measurements often include response latency, spike rates, and properties of tuning curves. The most common tool used to differentiate subdivisions is by defining a reversal in *tonotopy*[a], which indicates the border of two adjacent areas.

a. **Tonotopy** is the topographic arrangement of frequency representation that is conserved along the lemniscal pathway of the central auditory system from the cochlea to auditory cortex.

In terms of anatomy and connectivity, areas can be defined based on their connectional patterns with thalamus and other parts of cortex. These pathways are most commonly examined using anatomical tracers. In terms of histology, tissue staining techniques can highlight differences in the architecture among various auditory areas. By combining these three techniques, each area of auditory cortex has a unique profile consisting of physiological, connectional, and architectural properties.

Non-human primate research has surged in the past ten years to focus on the neural mechanisms of processing and generation of species-specific vocalizations that may be similar to speech processing and generation in humans. Over the past two decades, rodents have become common models for researching the neural mechanisms of diseases and cortical plasticity. Rodents are the closest relatives of non-human primates, but little has been done to compare the two bodies of literature of these two popular models in auditory research. This review, therefore, seeks to compare the basic structures of monkey and rodent auditory cortex in order to understand the common roles of cortex in these two groups.

Core, belt, and parabelt of monkey auditory cortex

Non-human primates have become essential models for investigating neural components of speech by studying neural coding of species-specific vocalizations. The current primate model of auditory cortex was first suggested by Hackett et al[2] using histological techniques to define different regions that could be further subdivided into dif-

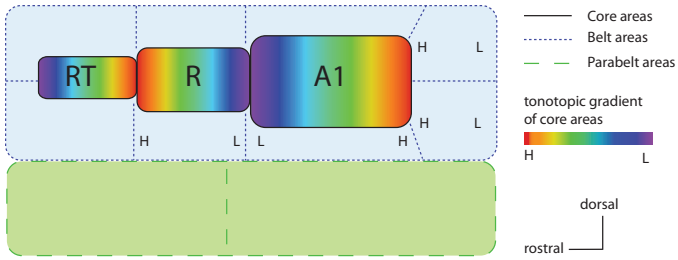

Figure 1. Schematic of primate auditory cortex. Core areas are shaded by tonotopic gradients. Subdivisions of belt and parabelt are indicated by dashed lines. Tonotopic gradients of belt areas are indicated by H (high) and L (low) frequency representation.

ferent areas based on physiological features and connection patterns. This model (Figure 1) describes three levels of processing, where each level occurs in a region that is further divided into separate areas. The first stage occurs in the "core" and consists of one primary and two primary-like areas; the second level is in the "belt" region of eight areas narrowly surrounding the core; the third region is the "parabelt," which has been divided into two general areas.

Physiological properties of primate auditory cortex. Early electrophysiolgical recordings of monkey auditory cortex utilized tuning curves to determine the best frequency of a neuron or a group of neurons in order to create a tonotopic map used to define fields within auditory cortex. Merzenich and Brugge[3] described several distinct areas, including primary auditory area (A1), an area rostral to A1 that they termed RL, and an area caudomedial to A1 called CM. These areas were further investigated in macaques by Morel et al[4], who proposed that A1 and RL, which they renamed R, were part of the core, while CM was part of the belt. A third core area was proposed by Morel and Kaas[5] in owl monkeys that was rostral to R called the rostrotemporal area (RT). The general consensus today is that these three areas – A1, R, and RT – make up the core region (Figure 1). Other tonotopic areas had been described as well, but they were later categorized as belt areas that receive tonotopic information from adjacent core areas.

In addition to being tonotopically organized and responding well to pure tones, the three core areas have been differentiated based on neuronal responses. Of the three areas, neurons in A1 have the shortest response latency[6-10] and the highest spike rates when stimuli were presented at the preferred sound level[7,9]. Neurons in area R have significantly longer minimum latencies, compared to A1[6-10] and have narrow intensity and frequency tuning compared to A1[8]. A smaller portion of neurons in RT respond well to pure tones, though the field overall was tonotopic. Neurons in RT also possessed a lower threshold for sound level, narrower bandwidth, and long minimum and peak response latencies. RT neurons were also found to have longer response duration compared to A1 but not to R, which may indicate that response duration increases among fields going caudal to rostral[9].

Belt areas have been shown to have different physiology than core areas. Neurons in the belt region are active when pure tones are presented to the ear, but tuning curves are broad[6,7,11]. Belt neurons also tend to have lower firing rates to both tones and noise[11] compared to responses in core neurons. Furthermore, neurons in the belt prefer increasingly complex stimuli[11-13] such as FM sweeps[14] or band-pass noise[6,10,15].

Overall, neurons in the core region have short response latencies, respond best to pure tones than to complex stimuli, and possess narrow tuning curves. Neurons in belt areas, however, have long latencies, possess broader tuning, and do not respond well to pure tones but rather seem to prefer complex stimuli. In this sense, it appears as though stimulus preference gets more complex from core to belt to parabelt, thus a general flow of information is set up in a hierarchical order from core to belt to parabelt. Parallel processing is also occurring among subdivisions within regions. One important study that illustrates this involved recording from two core areas A1 and R as well as a belt area CM while presenting either pure tones or broad-band noise clicks to the contralateral ear. After establishing tonotopic maps and that each area responded to both clicks and tones, the authors removed A1. After ablation of A1, the researchers recorded again from R and CM, which would be devoid of inputs from A1, and found that neurons in CM no longer responded well to pure tones, but maintained responses to clicks. Neurons in R, however, maintained responses to both pure tones and clicks. This study provides strong evidence of both serial and parallel processing in the auditory cortex. Parallel processing was evidenced by the fact that in the absence of A1, neurons in R maintained responses to both pure tones and clicks. The fact that CM lost its responses to pure tones is evidence that it receives tonal information from A1, showing not only that CM was a secondary level of processing but also that information flowed from a core area to a belt area[6].

Anatomy and histology of primate auditory cortex. Using anatomical tracers, connections between the auditory thalamus and different parts of auditory cortex have been described

and employed to define cortical regions. In general, the core region receives preferential input from the ventral division of the medial geniculate body (MGv), while belt and parabelt regions receive preferential input from the dorsal division (MGd). All regions receive inputs from the medial or magnocellular division (MGm)[16]. Thalaomocortical connections provide evidence of parallel processing because divisions within the thalamus project to multiple areas within a level of processing in cortex. Connections within auditory cortex have also been described. The belt region receives input from core; the parabelt region receives input from the belt; but parabelt does not receive input from core areas. This provides additional evidence of serial processing within auditory cortex, where information is passed from core to belt to parabelt, but not from core to parabelt[17-18].

The histological commonalities for sensory areas have been described in macaques and marmosets. The core region is heavily myelinated and has a thick, densely packed cell layer IV. Core areas also express dense staining of cytochrome oxidase (CO) which labels the metabolic enzymes in the cells, the vesicular glutamate transporter-2 (VGLuT2), and the calcium-binding protein Parvalbumin. This is likely because the core region is highly metabolic and active since it is the first stage of cortical processing. Within the core, the extents of these properties are less apparent going from rostral to caudal areas. However, these properties are still more common in the core than belt regions[2,18]. The cytoarchitecture between the core, belt, and parabelt regions also differs. Aside from the prominent layer IV, the core region possesses tightly packed columns of cells. The lateral belt contains similar columns spaced apart; layer IV is narrower; and prominent pyramidal cells can be seen in layer V. The parabelt layer III seems to be broader, and the columns appear to be more striking in appearance than in the belt region[18]. By combining these histological techniques and looking at the specific histological signatures of the various types of tissues, the three regions were further divided into three core areas, eight belt areas, and two parabelt areas. This differentiation among regions and areas provides additional evidence of parallel processing in the implied functional differences that come with histological differences.

Primary and secondary regions of rodent auditory cortex

Rodents have been used in auditory research as ideal models for plasticity and deafness or hearing disorders. Unlike primate auditory cortex, there is no general model for rodents. The bulk of basic research in the descriptions of auditory cortical fields has been well-described in guinea pigs, rats, mice, and gerbils, where there may be at least five

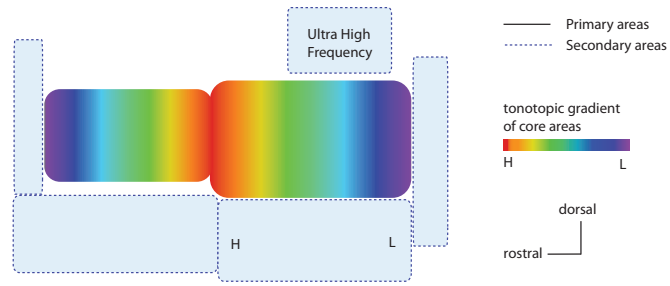

Figure 2. Schematic of a hypothetical rodent auditory cortex. Primary areas are shaded depicting tonotopic gradients. Example belt areas are indicated by dashed lines. Tonotopic gradients of belt areas are indicated by H (high) and L (low) frequency representations.

(mice[19] and rats[20]) and up to as many as seven (gerbils[21]) or eight (guinea pigs[22]) areas based on physiological distinctions and tonotopic reversals. This portion of the review will focus on the common properties described in the primary and secondary areas in these species. Figure 2 shows a general schematic of a hypothetical "typical" rodent.

Physiological properties of rodent auditory cortex. Of the rodents studied, at least two prominent adjacent tonotopic fields are found. These two primary fields show mirrored tonotopy and possess neurons with short response latencies and narrow tuning curves[19-24]. In addition to these two areas, another tonotopic primary area containing neurons with broad tuning curves and long latencies has been found in guinea pigs[22-23], gerbils[21], and rats[20]. These are characteristics similar to core areas in non-human primates. Also similar to primates, multiple core-like areas have been described, indicating parallel processing.

Common secondary areas have also been shown in these rodent models. One non-tonotopic area has been implicated as having neurons with short response latencies and broad tuning[21-22]. Another secondary area is described as tonotopic, but these neurons prefer more complex stimuli to pure tones[19,21-22]. Similarly, neurons in another area also have broad tuning and prefer complex stimuli, but tuning curves are consistently multi-peaked[19-20,22]. In rats[20] and gerbils[21] an additional secondary area has been demonstrated to contain neurons with variable responses. These areas are distinctly different but, in general, are characteristic of secondary areas.

In addition to characteristic primary and secondary areas, rodents may also have specialized fields. For example,

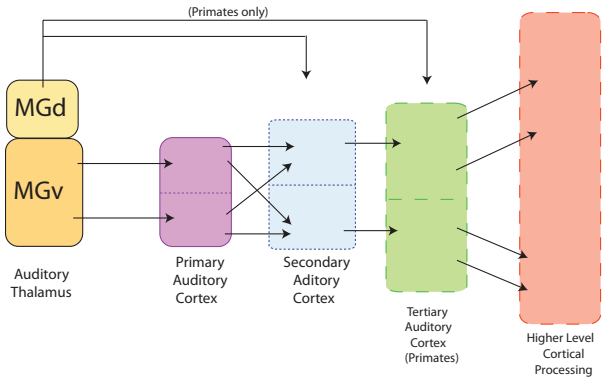

Figure 3. Schematic of serial and parallel processing in auditory cortex. Subdivisions of auditory cortex are indicated by dashed lines.

mice have a specialized ultrasonic field that possesses neurons that are active only when frequencies above 45kHz are present[19]. Similarly, rats[20] and gerbils[21] have an area with no clear tonotopy, but neurons in these regions prefer high frequencies.

Anatomy and histology of rodent auditory cortex. Among the physiologically defined primary areas, the densest projections come from the ventral division of the medial geniculate body (MGv) with sparse connections from the medial division (MGm)[20,25-26]. Some of the areas also receive sparse input from the dorsal division (MGd), but these are much less by comparison. Other primary areas receive preferential input from MGm rather than from MGv[25]. Physiologically defined secondary areas receive preferential input from MGd[26]. The specialized ultrasonic field in mice also has dense innervation from MGd, suggesting it may be a secondary level of processing[27]. The general trend of thalamic input to different levels of cortical processing is similar to primates, but the projection patterns are not necessarily as straightforward. For example, in gerbils, connections with the thalamus have shown that all cortical areas are connected with MGv, MGd, and MGm, but relative strengths of these connections differ among the areas. Primary areas are predominately innervated by MGv, but some are also predominately innervated by MGm. Similarly, secondary areas receive densest projections from MGd and MGv[28].

The topography of these projections has been examined, and it is evident that different parts of the divisions of the medial geniculate project to different areas of cortex, indicating parallel inputs. This is further evidence of parallel processing in auditory cortex whereby areas receive similar projections from the divisions of the thalamus in parallel. For example, in rats, the rostral portion of the MGv projects to the primary area A1; conversely, the caudal portion of the MGv projects to the ventral auditory field[20].

Unlike in non-human primates, the corticocortical connectivity patterns in rodent auditory cortex do not indicate a clear hierarchical processing. Rather, tracer injections into different fields of auditory cortex show that areas are highly interconnected ipsilaterally and contrallaterally. For connections between tonotopically organized fields, connections are generally between tonotopically matched frequency representations[29].

The histological evidence for auditory cortical areas in rodents has been described in guinea pigs and gerbils. Consistent with other primary sensory areas, the cytoarchitecture of the two most prominent primary areas of rodent auditory cortex are granular in nature[21]. Staining patters of cytochrome oxidase (CO) and myelin have been described for auditory cortical areas in guinea pigs. The primary areas contain the densest staining for CO and myelin. All other cortical areas showed low levels of CO staining. Similarly, primary areas show higher levels of myelination than secondary areas[22].

Concluding Remarks

In looking at the organization of auditory cortex in non-human primates and rodents, it is apparent that the two groups share a common feature of hierarchical processing from primary-like areas to secondary areas and parallel processing within these levels (Figure 3). Primates appear to have three distinct levels of serial processing while rodents only possess two. This may be attributed to demands of more complex communication such as species-specific vocalizations. It is worth noting, however, that the majority of conclusions on primate auditory cortex have come from studies of macaques and marmosets, which are highly evolved and possess specializations that may not generalize to all primates. In addition, the thalamocortical projections that define these areas in the primate model do not appear to be as strong of a marker for defining similar areas in rodents. Therefore, examining the cortical organization and thalamocortical connections of other non-human primates would further refine the model to describe all primates and what underlying principals occur in mammalian auditory cortex. In particular, looking to an animal model from the more primitive branch of primates would provide insight into the basics of primate auditory cortex across primates. Prosimian galagos (Otolemur garnettii) are a good candidate for this question. Understanding differences and similarities between galagos and the current model would allow

for more precise comparisons of primates to other species such as rodents. If the organization of galago auditory cortex is similar to that of the primate model, this would imply that the model could serve as a true template for all primate species because it would hold true for two drastically different primate groups. If the two are different, it would increase our knowledge of the role auditory cortex plays in processing sounds for different demands and environmental pressures and provide insight into the evolution of primate auditory cortex.

References

1. Heffner HE and Heffner RS (1990). Effect of bilateral auditory cortex lesions on sound localization in Japanese macaques. J Comp Neurol. 64 (3):915-931.

2 Hackett TA, Stepniewska I and Kaas JH (1998). Subdivisions of auditory cortex and ipsilateral cortical connections of the parabelt auditory cortex in macaque monkeys. J Comp Neurol. 394 (4):475-495.

Building on previous research, the authors use histology and anatomy to delineate different fields of auditory cortex in macaques. This paper proposes a model for primate auditory cortex that is widely accepted and used in current research.

3. Merzenich MM and Brugge JF (1973). Representation of the cochlear partition of the superior temporal plane in the macaque monkey. Brain Res. 50 (2):275-296.
4. Morel A, Garraghty PE, Kaas JH (1993). Tonotopic organization, architectonic fields, and connections of auditory cortex in macaque monkeys. J Comp Neurol. 335 (3):437-459.
5. Morel A and Kaas JH (1992). Subdivisions and connections of auditory cortex in owl monkeys. J Comp Neurol. 318 (1):27-63.

6. Rauschecker JP, Tian B, Pons T and Mishkin M (1997). Serial and parallel processing in rhesus monkey auditory cortex. J Comp Neurol. 382 (1):80-103.

This study provides evidence of serial and parallel processing in the auditory cortical network. This was shown by comparing responses of neurons in R and CM before and after ablating A1.

7. Recanzone GH (2000). Response profiles of auditory cortical neurons to tones and noise in behaving macaque monkeys. Hear Res. 150 (1-2):104-118.
8. Recanzone GH, Guard DC and Phan ML (2000). Frequency and intensity response properties of single neurons in the auditory cortex of the behaving macaque monkey. J Neurophysiol. 83 (4):2315-2331.
9. Bendor D and Wang X (2008). Neural response properties of primary, rostral, and rostrotemporal core fields in the auditory cortex of marmoset monkeys. J Neurophysiol. 100 (2): 888-906.
10. Kusmierek P and Rauschecker JP (2009). Functional specialization of medial auditory belt cortex in the alert rhesus monkey. J Neurophysiol. 102 (3):1606-1022.
11. Kajikawa Y, de La Mothe L, Blumell S and Hackett TA (2005). A comparison of neuron response properties in areas A1 and CM of the marmoset monkey auditory cortex: tones and broadband noise. J Neurophysiol. 93 (1):22-34.
12. Rauschecker JP, Tian B and Hauser M (1995). Processing of complex sounds in the macaque nonprimary auditory cortex. Science. 268 (5207):111-114.
13. Tian B, Reser D, Durham A, Kustov A and Rauschecker JP (2001). Functional specialization in rhesus monkey auditory cortex. Science. 292 (5515):290-293.
14. Tian B and Rauschecker JP (2004). Processing of frequency-modulated sounds in the lateral auditory belt cortex of the rhesus monkey. J Neurophysiol. 92 (5):2993-3013.
15. Rauschecker JP and Tian B (2004). Processing of band-passed noise in the lateral auditory belt cortex of the rhesus monkey. J Neurophysiol. 91 (6):2578-2589.
16. de la Mothe LA, Blumell S, Kajikawa Y and Hackett TA (2006). Thalamic connections of the auditory cortex in marmoset monkeys: core and medial belt regions. J Comp Neurol. 496 (1):72-96.
17. Hackett TA, Stepniewska I and Kaas JH (1999). Prefrontal connections of the parabelt auditory cortex in macaque monkeys. Brain Res. 817 (1-2):45-58.
18. de la Mothe LA, Blumell S, Kajikawa Y and Hackett TA (2006). Cortical connections of the auditory cortex in marmoset monkeys: core and medial belt regions. J Comp Neurol. 496 (1):27-71.
19. Stiebler I, Neulist R, Fichtel I and Ehret G (1997). The auditory cortex of the house mouse: left-right differences, tonotopic organization and quantitative analysis of frequency representation. J Comp Physiol A. 181 (6):559-571.

20. Polley DB, Read HL, Storace DA and Merzenich MM (2007). Multiparametric auditory receptive field organization across five cortical fields in the albino rat. J Neurophysiol. 97 (5):3621-3638.

This paper uses multiple assessments of neuronal response properties to describe five separate areas of rat auditory cortex. Anatomy is also used to provide evidence that the original description of A1 was actually a composite of two separate fields.

21. Thomas H, Tillein J, Heil P and Scheich H (1993). Functional organization of auditory cortex in the Mongolian gerbil (Meriones unguiculatus). I. Electrophysiological mapping of frequency representation and distinction of fields. Eur J Neurosci. 5 (7):882-897.

22. Wallace MN, Rutkowski RG and Palmer AR (2000). Identification and localisation of auditory areas in guinea pig cortex. Exp Brain Res. 132 (4):445-456.

This study uses electrophysiological mapping to differentiate the areas of auditory cortex in gerbils. The authors also show histological differences between physiologically defined areas.

23. Redies H, Sieben U and Creutzfeldt OD (1989). Functional subdivisions in the auditory cortex of the guinea pig. J Comp Neurol. 282 (4):473-488.
24. Harrison RV, Kakigi A, Hirakawa H, Harel N and Mount RJ (1996). Tonotopic mapping in auditory cortex of the chinchilla. Hear Res. 100 (1-2):157-163.
25. Redies H, Brandner S and Creutzfeldt OD (1989). Anatomy of the auditory thalamocortical system of the guinea pig. J Comp Neurol. 282 (4):489-511.
26. Llano DA and Sherman SM (2008). Evidence for nonreciprocal organization of the mouse thalamocortical-corticothalamic projection systems. J Comp Neurol. 507 (2):1209-1227.
27. Hofstetter KM and Ehret G (1992). The auditory cortex of the mouse: connections of the ultrasonic field. J Comp Neurol. 323 (3):370-386.
28. Budinger E, Heil P and Scheich H (2000). Functional organization of auditory cortex in the Mongolian gerbil (Meriones unguiculatus). IV. Connections with anatomically characterized subcortical

structures. Eur J Neurosci. 12 (7):2452-2474.
29. Budinger E, Heil P and Scheich H (2000). Functional organization of auditory cortex in the Mongolian gerbil (Meriones unguiculatus). IV. Anatomical subdivisions and corticocortical connections. Eur J Neurosci. 12 (7):2425-2451.

Mitochondrial Signaling through PTEN-Inducible Putative Kinase-1 (PINK1) in Response to Ischemia: Lessons from Familial Parkinson's Disease

Amy Palubinsky

Abstract

While much is known about the signaling events elicited in response to stroke, the role of mitochondrial signaling following an ischemic event has only recently begun to be investigated. Increasing evidence demonstrates mitochondrial dysfunction in numerous neurological disorders including Parkinson's disease (PD), Alzheimer's disease, Huntington's disease, autism spectrum disorders and stroke, which suggests that understanding the components of mitochondrial signaling in these disorders may uncover conserved signaling molecules at the level of the mitochondria. The recent identification of genetic mutations that result in the development of familial forms of PD and affect mitochondrially-associated proteins, such as the stress-associated kinase PTEN-Inducible putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, have provided new and interesting insights into mitochondrial signaling pathways in response to stress. Studies regarding the PINK1 pathway suggest a role for this protein in mitochondrial quality control via initiation of the selective autophagic removal of damaged mitochondria or mitophagy. Given that mitophagic processing has been shown to occur in a number of different stress paradigms suggests that PINK1 may act as global sensor of stress, recognizing damaged mitochondria in a number of pathological settings including that of neuronal ischemia. This review aims to introduce evidence for a link between PD and stroke namely through conserved PINK1 signaling at the level of the mitochondria.

Keywords

Ischemic stroke
excitotoxicity
mitophagy
PINK1
Parkin
CHIP

Stroke: The Basics

Stroke is the world's second leading cause of mortality, accounting for over 6,000,000 deaths annually[2]. Moreover, stroke is the leading cause of long-term adult disability[3] and accounts for nearly 70 billion dollars per year in direct costs such as healthcare, prescriptions and rehabilitation, as well as, indirect costs such as those that accrue from loss of workforce and therefore loss of economic efficiency[4]. Ischemic stroke encompasses 85% of stroke cases and is defined as an occurrence in which a reduction in blood flow results in alterations in normal cellular function[2,5]. The major risk factors for ischemic stroke include age, diabetes, and hypertension— all of which are represented though family history and genetics[6]. While these risk factors account for a significant portion of strokes, there is no explanation as to why some patients with similar risk profiles incur strokes while others do not[7]. Currently the estimated lifetime risk for stroke lies between 8 and 10%[2], yet both preemptive treatments for high-risk patients , as well as therapies that could reduce neuronal damage following a stroke remain elusive[4].

Ischemic Stroke: Pathophysiological Mechanisms

A blocked cerebral vessel results in decreased supply of oxygen and glucose to an area or areas of the brain that usually rely on blood flow from the occluded vessel. These events trigger the initiation of what is often referred to as an excitotoxic cascade wherein the loss of oxygen and glucose leads to a subsequent loss of energy in the form of ATP. As such, neurons in this region no longer have the proper substrates to carry out oxidative phosphorylation and instead are forced to switch to anaerobic respiration. Without ATP, numerous energy-dependent membrane pumps become dysregulated, the neuron becomes depolarized and an influx of calcium ions (Ca^{2+}) results[8]. Because ATP-dependent ion pumps can no longer remove the Ca^{2+} from the cell, intracellular calcium becomes much higher than physiological levels. This increased intracellular calcium initiates two major events: activation of enzymes, proteases and endonucleases that further disrupt the cellular membrane and the release of the major excitatory neurotransmitter, glutamate. The presence of excessive amounts of glutamate in the synaptic cleft causes stimulation of both AMPA receptors and Ca^{2+} permeable NMDA receptors on neighboring neurons, evoking even more Ca^{2+} to enter these already damaged neurons. The resulting overexcitation leads to the generation of free

radicals, specifically reactive oxygen species (ROS[a]), as well as further glutamate release. In addition, phospholipases continue to be activated and ,consequently, excessive membrane damage and non-regulated movement of ions into and out of the cell occurs. The overall result of excess intracellular calcium, excess glutamate release, the generation of free radicals and ROS, in addition to the breakdown of the cell's outer membrane is termed neuronal excitotoxicity[b] and often results in neuronal cell death[9].

Ischemic Stroke: Understanding the Role of Mitochondria

An ischemic event in the brain is particularly devastating due to this organ's high metabolic demand and, therefore, its major reliance on mitochondria[10]. The excitotoxic cascade is initiated in large part by loss of ATP generation within mitochondria, which are the site of greater than 90% of ROS generation— yet another major consequence of ischemia. In addition to their role in maintaining cellular energetics, mitochondria are also intimately involved in the regulation of cellular ion homeostasis and are particularly well known for their role in caspase-dependent apoptosis, often the end result of ischemic events. Given the connection between mitochondria and the events involved in the excitotoxic cascade, identifying the molecules and molecular complexes involved in the ischemic response, as well as understanding their mechanisms of action at the level of these organelles, is of utmost importance.

Mitochondrial signaling in response to stress has gained much attention in relation to diseased states of the brain. In fact, many recent articles have focused on the role of abnormal or dysregulated mitochondria in neurodegenerative diseases including Parkinson's Disease (PD), Alzheimer's Disease (AD) and typical ageing processes[10,11]. In this context, major strides have been made in identifying genetic mutations in mitochondrial proteins in the PD field. Because there are a limited number of molecules available to participate in mitochondrial signaling, it is plausible that crucial insight can be gained from the Parkinson's Disease literature regarding mitochondrial responses to stress.

Parkinson's Disease: Basic Information and an Introduction to Genetic Mutations

Currently, Parkinson's disease is estimated to effect 6 million people worldwide, although many undiagnosed cases are probable. PD is therefore noted as the most common age-related neurodegenerative movement disorder (WHO). PD patients typically present with bradykinesia, resting tremor, muscular rigidity and postural instability, as well as, major cellular hallmarks such as the presence of cytoplasmic Lewy bodies and neuronal cell loss specifically within the substantia nigra pars compacta[12]. The majority of PD cases are sporadic, exhibiting no genetic inheritance; however, 5% of PD cases are familial[12]. Recently, genetic links have been discovered in autosomal recessive forms of PD and include mutations in the PARK2[c] and PARK6[d] genes[12-15]. One of the most interesting findings regarding single mutations in either PARK2 or PARK6 is that mitochondrial turnover is dysregulated[16].

PINK1 and Parkin: Involvement in Mitochondrial Quality Control and Mitophagy

PINK1 is a ubiquitously expressed, 63kDa protein that is encoded by the PARK6 gene. It has a N-terminal mitochondrial targeting sequence (MTS) that is inserted into the outer mitochondrial membrane (OMM), as well as a C-terminal kinase domain that faces the cytosol. When directed to healthy mitochondria via its MTS, PINK1 inserts into the OMM and is immediately cleaved by mitochondrial proteases and released into the cytosol where it is delivered to the proteasome for degradation[17-19]. Ongoing studies that focus on the mechanisms of PINK1 cleavage have identified a number of mitochondrial proteases that cleave PINK1. Examples of these proteases include presenilin-associated rhomboid-like protein (PARL) and matrix metalloproteinase (MMP), both of which have been shown to generate markedly different PINK1 cleavage products. Such studies are key as they may uncover novel signaling roles of the various PINK1 cleavage products. Furthermore, when mitochondria is injured or depolarized, PINK1 not only becomes stabilized but also accumulates in the OMM due to the inhibition of mitochondrial proteases[19]. In such cases, PINK1 then acts to recruit the 53kDa, cytoplasmic, E3 ubiquitin ligase, Parkin, to damaged mitochondria[18]. Extensive studies by Matsuda, et al have demonstrated that while in the cytosol, the ubiquitin ligase activity of Parkin is repressed; however, once stabilized at the mitochon-

a. **Reactive Oxygen Species (ROS)**: Normal byproducts of mitochondrial metabolism that increase dramatically during times of cellular stress.

b. **Excitotoxicity**: A pathological process during which neurons are severely damaged due to excessive stimulation by neurotransmitters often resulting in neuronal cell death.

c. **PARK2**: Gene encoding the E3 ubiquitin ligase, Parkin, found to be mutated in 50% of autosomal recessive forms of PD as well as 10-15% of sporadic PD cases.

d. **PARK6**: Gene encoding the stress-associated kinase, PINK1, found to be mutated in some cases of familial Parkinson's disease.

dria, via recruitment and interaction with PINK1, its enzymatic activity is unmasked[18]. Activated Parkin has been shown to ubiquitinate protein substrates of mitochondria with reduced membrane potential following treatment with the mitochondrial uncoupling agent, carbonyl cyanide m-chlorophenylhydrazone (CCCP)[18]. Furthermore, using mouse embryonic fibroblasts (MEFs) from PINK1 wildtype (WT) and PINK1 knockout (KO) mice, it has been demonstrated that only WT MEFs are able to recruit Parkin to mitochondria following CCCP treatment and, following this recruitment, damaged mitochondria are cleared from the cells[18]. The disappearance of mitochondria following CCCP treatment was also noted by another group when they compared HeLa cells lacking Parkin to HeLa cells expressing Parkin. This study found that 48 hours following treatment, Parkin expressing HeLa cells had no remaining detectable mitochondria as assessed by three independent mitochondrial markers[20]. Additionally, following administration of CCCP the knockdown of an essential mammalian autophagy protein, autophagy-related protein 7 (Atg7), in Parkin-expressing HeLa cells demonstrated a loss of damaged mitochondrial clearance[20, 21].

Together, these data support a hypothesis whereby PINK1 accumulation and stabilization within the OMM of a damaged, depolarized mitochondria leads to the recruitment of Parkin from the cytosol to these organelles where its unmasked E3 ligase activity, results in the ubiquitination of mitochondrial substrates and, in turn, orchestrates the selective autophagy of damaged mitochondria, otherwise known as mitophagy[e*] (**Figure 1**).

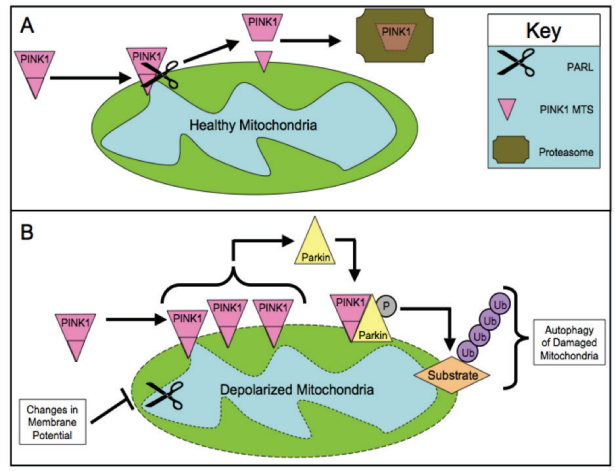

Figure 1: PINK1 plays a key role in determining healthy versus damaged mitochondria. Healthy mitochondria (A) undergo constitutive turnover of PINK1 via the proteasome. Damaged, depolarized mitochondria (B) accumulate PINK1 in their OMM which leads to the subsequent recruitment of other molecular players necessary for autophagy of unhealthy mitochondria also known as mitophagy (adapted from [1]).

Ischemic Stroke: Mitophagic Responses and the Role of E3 Ligases

Autophagy commonly refers to the bulk degradation of the cytoplasm and organelles in order to regulate intracellular homeostasis and numerous groups have reported changes in autophagy in response to different types of *in vivo* and *in vitro* stress paradigms including ischemic models (Reviewed in[22]). Recently, we have become aware of more selective forms of autophagy that target specific organelles for degradation such as mitochondria. In an elaborate set of experiments, Narenda et al., demonstrated for the first time that Parkin is a regulator of the selective mitophagic response[20]. Another E3 ligase, carboxy-terminus of HSC70 interacting protein (CHIP), has been shown to enhance Parkin activity under normal circumstances and to compensate for loss of Parkin activity in cases of Parkin mutation[23]. Interestingly, Stankowski et al., have shown that CHIP expression levels are increased in post mortem human brain tissue samples from patients that had suffered from either a transient ischemic attack (TIA) or a stroke. In addition, they found that CHIP is also upregulated in response to oxygen and glucose deprivation (OGD) in an *in vitro* model of primary rat cortical neurons[24]. The noted interactions between CHIP and Parkin are interesting in light of the emerging role of CHIP in ischemia. Given the previously discussed data supporting PINK1 involvement in the recruitment of Parkin and subsequent mitophagic processing, a closer investigation into the possible role of the PINK1 pathway in response to ischemia and in relation to CHIP is warranted.

Ischemic Stroke: Involvement of PINK1 Signaling

Thus far only one study has been published with regards to a role for PINK1 in cerebral ischemic models. This study demonstrates a decrease in PINK1 expression in primary cortical neuronal cultures 24 hours following 2 hours of OGD[25]; however, the cells utilized were only cultured for 12 days *in vitro* (DIV) while the literature supports that mature NMDA receptors expressing the necessary subunits to respond to excitotoxicity are not developed until at least

e. **Mitophagy**: The selective, autophagic degradation of damaged mitochondria that can occur as a means to regulate mitochondrial quality control under normal cellular conditions or in response to cellular stressors.

DIV14[26]. In addition, given that PINK1 is immediately targeted to mitochondria via its MTS and that the previously discussed studies find few remaining damaged mitochondria by 24 hours following a stress due to mitophagic clearance[18, 20], it may be that earlier time points following OGD need to be examined. Accordingly, a study of ischemia in spinal cord neurons demonstrated, via Western blot and immunocytochemistry, increases in PINK1 expression in response to this stress within 8 hours[27].

Given the immense amount of new evidence supporting a role for PINK1 and Parkin in general mitochondrial quality control mechanisms[28,29], the heavy reliance of neurons on mitochondrial support and the increasingly noted affect of mitochondrial dysfunction in neurodegenerative diseases, more detailed investigations into the role of mitochondrial signaling in response to ischemic events may uncover novel therapeutic targets for the treatment of stroke.

Conclusion:

Stroke and Parkinson's disease are common neurodegenerative disorders affecting millions of patients worldwide. Although science has made great strides in identifying the molecular mechanisms and pathways involved in these disorders, therapeutic intervention remains elusive. In recent years, the role of PINK1 signaling at the level of the mitochondria in response to stress has come to the forefront of research studies as we now recognize common themes regarding mitochondria and stress signaling across such disorders. As we continue to search for therapeutics for these neurological diseases, interesting new insights into the relationship between general mitochondrial dynamics and neurodegeneration have come to light. In addition, many conserved pathways and molecules have been uncovered, including but not limited to, those that involve: PINK1, Parkin and CHIP. The purpose of this review was to not only introduce these relatively new molecular players, but also to remind us of what can be learned from similar but different fields of science and to stimulate further interest and investigation of potential therapeutic targets across these fields towards a common goal.

References:

1. Matsuda N and Tanaka K (2010). Uncovering the roles of PINK1 and parkin in mitophagy. Autophagy. 6 (7): 952-954.
2. Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM and Arumugam TV (2011). Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener. 6 (1): 11.
3. Razvi SS and Bone I (2006). Single gene disorders causing ischaemic stroke. J Neurol. 253 (6): 685-700.
4. Stankowski JN and Gupta R (2011). Therapeutic targets for neuroprotection in acute ischemic stroke: lost in translation? Antioxid Redox Signal. 14 (10): 1841-1851.
5. Mattson MP, Duan W, Pedersen WA and Culmsee C (2001). Neurodegenerative disorders and ischemic brain diseases. Apoptosis. 6 (1-2): 69-81.
6. Markus HS (2010). Unraveling the genetics of ischaemic stroke. PLoS Med. 7 (3): e1000225.
7. Sacco RL, Benjamin EJ, Broderick JP, Dyken M, Easton JD, Feinberg WM, Goldstein LB, Gorelick PB, Howard G, Kittner SJ, Manolio TA, Whisnant JP and Wolf PA (1997). American Heart Association Prevention Conference. IV. Prevention and Rehabilitation of Stroke. Risk factors. Stroke. 28 (7): 1507-1517.
8. Iadecola C and Anrather J (2011). The immunology of stroke: from mechanisms to translation. Nat Med. 17 (7): 796-808.
9. Hazell AS (2007). Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies. Neurochem Int. 50 (7-8): 941-953.
10. Chan DC (2006). Mitochondria: dynamic organelles in disease, aging, and development. Cell. 125 (7): 1241-1252.
11. Navarro A and Boveris A (2010). Brain mitochondrial dysfunction in aging, neurodegeneration, and Parkinson's disease. Front Aging Neurosci. 2
12. Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M, Albanese A and Wood NW (2001). Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet. 68 (4): 895-900.
13. Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G and Wood NW (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science. 304 (5674): 1158-1160. This paper identifies mutations in the PARK6 gene that encodes PINK1 as a cause of familial PD.
14. Matsumine H, Saito M, Shimoda-Matsubayashi S, Tanaka H, Ishikawa A, Nakagawa-Hattori Y, Yokochi M, Kobayashi T, Igarashi S, Takano H, Sanpei K, Koike R, Mori H, Kondo T, Mizutani Y, Schaffer AA, Yamamura Y, Nakamura S, Kuzuhara S, Tsuji S and Mizuno Y (1997). Localization of a gene for an autosomal recessive form of juvenile Parkinsonism to chromosome 6q25.2-27. Am J Hum Genet. 60 (3): 588-596.
15. Valente EM, Brancati F, Ferraris A, Graham EA, Davis MB, Breteler MM, Gasser T, Bonifati V, Bentivoglio AR, De Michele G, Durr A, Cortelli P, Wassilowsky D, Harhangi BS, Rawal N, Caputo V, Filla A, Meco G, Oostra BA, Brice A, Albanese A, Dallapiccola B and Wood NW (2002). PARK6-linked parkinsonism occurs in several European families. Ann Neurol. 51 (1): 14-18.
16. Vives-Bauza C, de Vries RL, Tocilescu M and Przedborski S (2010). PINK1/Parkin direct mitochondria to autophagy. Autophagy. 6 (2): 315-316.
17. Meissner C, Lorenz H, Weihofen A, Selkoe DJ and Lemberg MK (2011). The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. J Neurochem. 117 (5): 856-867.
18. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu

M, Hattori N and Tanaka K (2010). PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol. 189 (2): 211-221. This study identifies the mechanism by which PINK1 delineates between healthy and depolarized mitochondria.

19. Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP and Youle RJ (2010). Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol. 191 (5): 933-942.

20. Narendra D, Tanaka A, Suen DF and Youle RJ (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol. 183 (5): 795-803. This is the first study to demonstrate that Parkin plays a role in autophagic processing of damaged mitochondria.

21. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K and Chiba T (2005). Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol. 169 (3): 425-434.

22. Smith CM, Chen Y, Sullivan ML, Kochanek PM and Clark RS (2011). Autophagy in acute brain injury: feast, famine, or folly? Neurobiol Dis. 43 (1): 52-59.

23. Imai Y, Soda M, Hatakeyama S, Akagi T, Hashikawa T, Nakayama KI and Takahashi R (2002). CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. Mol Cell. 10 (1): 55-67.

24. Stankowski JN, Zeiger SL, Cohen EL, DeFranco DB, Cai J and McLaughlin B (2011). C-terminus of heat shock cognate 70 interacting protein increases following stroke and impairs survival against acute oxidative stress. Antioxid Redox Signal. 14 (10): 1787-1801. This paper demonstrates for the first time that expression of the E3 ligase CHIP is upregulated in postmortem human brain tissue following TIA or stroke and in response to ischemia in vitro.

25. Shan Y, Liu B, Li L, Chang N, Li L, Wang H, Wang D, Feng H, Cheung C, Liao M, Cui T, Sugita S and Wan Q (2009). Regulation of PINK1 by NR2B-containing NMDA receptors in ischemic neuronal injury. J Neurochem. 111 (5): 1149-1160.

26. Sinor JD, Boeckman FA and Aizenman E (1997). Intrinsic redox properties of N-methyl-D-aspartate receptor can determine the developmental expression of excitotoxicity in rat cortical neurons in vitro. Brain Res. 747 (2): 297-303.

27. Sakurai M, Kawamura T, Nishimura H, Suzuki H, Tezuka F and Abe K (2009). Induction of Parkinson disease-related proteins in motor neurons after transient spinal cord ischemia in rabbits. J Cereb Blood Flow Metab. 29 (4): 752-758.

28. Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, Miller D, Maric D, Cedazo-Minguez A and Cookson MR (2011). DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet. 20 (1): 40-50.

29. Narendra DP and Youle RJ (2011). Targeting mitochondrial dysfunction: role for PINK1 and Parkin in mitochondrial quality control. Antioxid Redox Signal. 14 (10): 1929-1938.

CANDIDATE
REVIEWS

TRPV1 and the Intrinsic Neuronal Response to stress

Nicholas J. Ward

Abstract

By gating cation entry into cells, the TRP superfamily of ion channels aid in signal transduction of various stimuli. One particular TRP channel, TRPV1, activates upon exposure to certain noxious stimuli such as heat, low pH, and pressure. Although first characterized as a channel critical to nociception, TRPV1 is now known to participate in such diverse activities as mediating synaptic plasticity, initiating and regulating filopodia, aiding axonal guidance and migration, and participating in the neuronal stress response. Evidence from the literature, reviewed here, suggests that TRPV1 may promote neuronal survival under stress. Using glaucomatous neurodegeneration as a model of neuronal stress, a potential role of TRPV1-mediated neuroprotection is outlined here. Reasoning for this protective role draws upon TRPV1-/- data and the demonstrated abilities of TRPV1 to sensitize and translocate to the membrane in response to stressors, to localize to synapses, and to maintain synaptic structures via potentiating excitatory synaptic activity.

Keywords

TRP channels
TRPV1
Neuronal stress response
Neurotoxicity
Neuroprotection

TRPV1: A multifunctional TRP channel

Transient receptor potential (TRP) channels represent a diverse superfamily of proteins that gate cation entry into cells. Functional characteristics of TRP channels are so dissimilar that these proteins comprise six subfamilies grouped solely by amino acid homology rather than by function[1]. In mammals, these subfamilies include 28 different TRPs: canonical (TRPC1-7), vanilloid (TRPV1-6), melastatin (TRPM1-8), ankyrin (TRPA1), polycystin (TRPP1-3) and mucolipin (TRPML1-3)[2]. First characterized in the Drosophila phototransduction cascade[3], TRP channels are situated in the cell membrane, which positions them to transduce extracellular sensory information to the intracellular space. TRP channel subunits all possess six putative transmembrane (TM) domains with a stretch of hydrophobic amino acids between TM5 and TM6 that serves as a pore region (**Figure 1**). When these subunits tetramerize, they form a pore permeable to monovalent and divalent cations. Upon activation, TRP channels mediate Ca^{2+} flux across membranes, resulting in an increase in $[Ca^{2+}]_i$. Perturbations of neuronal Ca^{2+} signaling are a hallmark of neurodegenerative disease, thus it is particularly important to understand how TRP channels functionally influence neuropathic mechanisms[4].

One subfamily of TRP channels, the vanilloid TRPs (TRPVs) derive their name from the vanillyl functional group found on some of their ligands. Of the TRPVs, TRPV1 was the first discovered and remains the best characterized[5]. TRPV1 was first discovered based on its activation by the pungent component of chili peppers, capsaicin[6]. TRPV1 transduces information regarding other noxious stimuli, including heat (>42 °C temperatures), low pH (<6.0), and pressure[6, 7]. Within the peripheral nervous system, TRPV1 channels are expressed in primary sensory afferent fibers, allowing peripheral pain information to reach the central nervous system[6]. Accordingly, TRPV1 knockout mice exhibit a reduced pain response, which makes this channel of particular interest as a target for pain and hyperalgesia therapeutics[8, 9].

TRPV1 activity depends upon the sensitization state of the channel, which can be influenced by cellular signaling cascades. Phosphorylated TRPV1 represents a channel state that is more sensitive to activation[10]. Protein kinase A (PKA)[11], protein kinase C (PKC)[12], protein kinase D (PKD)[13], cyclin-dependent kinase 5 (Cdk5)[14] and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[15] all sensitize TRPV1 by phosphorylation at serine, threonine, or tyrosine residues (**Figure 1**). This sensitization can be reversed by protein phosphatase 2B (calcineurin), which employs a Ca^{2+}-dependent phosphatase activity during this desensitization process[5]. When assessing TRPV1 function within injury or pathology, it is important to consider these modulatory effects by protein kinases and phosphatases.

Although TRPV1 was first characterized as a molecular detector of noxious stimuli, the discovery of widespread TRPV1 expression throughout the brain suggested

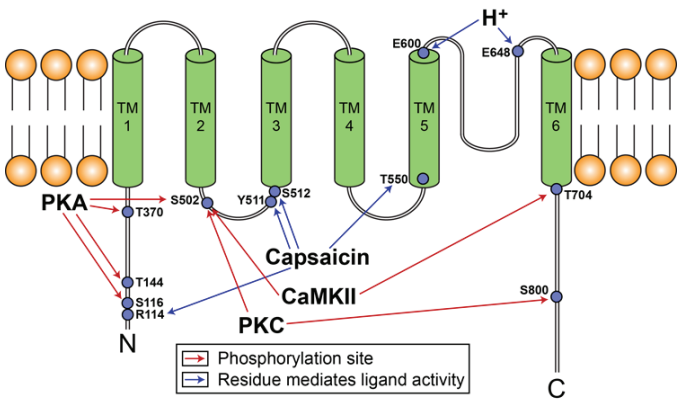

Figure 1. TRPV1 channel structure and functionally important residues. TRPV1 is a membranous ion channel characterized by intracellular N- and C-termini, 6 transmembrane (TM) domains, and a cation-permeable pore region between TM5 and TM6. Protein kinases phosphorylate specific residues (red arrows) in order to sensitize TRPV1 to ligand interactions (blue arrows). Figure constructed from reviewed information[16].

that the channel may possess additional functions beyond nociception[17]. It was proposed that there must be a class of endogenous ligands (endovanilloids) that regulated this signaling[18]. This class of ligands exists, and includes endocannabinoids such as anandamide and N-arachidonoyl-dopamine (NADA), lipoxygenase products, as well as endogenous inhibitors like PIP_2. The endocannabinoids are particularly interesting because anandamide and anandamide-like structures can often act at both TRPV1 and the cannabinoid (CB1 and CB2) receptors[10]. These promiscuous interactions indicate that there may be some interplay between the cannabinoid system and TRPV1.

Functions of TRPV1 in the central nervous system

Although TRPV1 is well-characterized with respect to pain perception at the periphery, data regarding its function within the CNS is limited. Examination of TRPV1 knockout mice revealed a reduction in anxiety, conditioned fear responses, long-term potentiation (LTP) in the hippocampus, and long-term depression (LTD) in the dentate gyrus[19, 20]. These alterations in behavior and neurophysiology suggest the relevance of TRPV1 to synaptic plasticity and neuronal networks. Within the CNS, TRPV1 activation in dorsolateral periaqueductal gray neurons increases neuronal activity by potentiating input from glutamatergic synapses[21]. Despite TRPV1's involvement in modulating synaptic transmission, it was not known if TRPV1 itself was located in synaptic terminals. Recently, it was determined that TRPV1 is present in synaptic structures by colocalization with pre- and post-synaptic markers as well as presence within biochemical fractions of synaptosomes and the post-synaptic density[22].

TRPV1 functions in developmental aspects of the CNS, such as regulation of the neuronal growth cones and filopodia. These TRPV1-positive filopodia contain synaptic vesicular and scaffolding proteins, thus it is likely that TRPV1 plays a role in synapse formation[23, 24]. These studies were complemented by another developmental study which indicated that TRPV1 mediates LTD in the developing superior colliculus via the depression of glutamatergic retinocollicular synapses[25]. Altogether, these examples show that TRPV1 is involved in plasticity and activity of synapses.

TRPV1 and the neuronal stress response

TRPV1 expression and localization in neurons is affected by injurious stressors and pathology. Increases in TRPV1 protein were observed in models of neuronal injury such as lingual nerve injury[26], chronic constriction injury[27], and gentamicin-induced ototoxicity[28]. In human tissue, increases in TRPV1 protein levels were found in aged and photoaged skin and its associated nerve fibers[29], as well as in tissue collected from patients with traumatic and diabetic neuropathy[30].

In multiple instances, TRPV1 has been implicated in physiological stress responses. Injured cells release ATP into the extracellular space, which can bind metabotropic ATP receptors[31]. These ATP receptors in turn sensitize TRPV1 via PKC-dependent phosphorylation[32]. Additionally, neurodegenerative diseases often have a sustained neuroinflammatory component that contributes to pathology[33]. Proinflammatory chemokines bind G protein-coupled receptors, which can cause downstream sensitization of TRPV1 by PKC[34]. Another proinflammatory mediator, nerve growth factor (NGF), promotes an increase in membrane current carried by TRPV1 by increasing the number of TRPV1 channels inserted in the membrane[35]. NGF binds the TrkA receptor, which activates a signaling cascade that ultimately phosphorylates TRPV1 at tyrosine residue Y200 via Src kinase. Tyrosine phosphorylation is involved in trafficking ion channels[36] and receptors[37], and is responsible for increasing the number of TRPV1 channels at the membrane following NGF binding[35]. These examples show that neuronal stressors can affect TRPV1 both by sensitizing the channel to activation as well as increasing levels of TRPV1 at the membrane, where it enhances current.

TRPV1: functionally neurotoxic or neuroprotective?

For many years, it has been known that capsaicin treatment causes degeneration of primary sensory neurons[38] as well as many neurons of the central nervous system[39]. Both in vivo and in vitro data gathered from mesencephalic dopaminergic neurons indicate that direct activation of TRPV1 with capsaicin or the endogenous ligand anandamide mediates cell death[40]. Such treatment produces a large increase in $[Ca^{2+}]_i$, subsequent mitochondrial damage, and cell death. However, the pathway mediated by anandamide may not actually act through TRPV1 due to the inability of TRPV1 antagonist capsazepine to prevent anandamide-induced cell death[41]. This is particularly important to consider in relation to cellular signaling occurring in neuropathy—anandamide may contribute to neurotoxicity independently of TRPV1. Although direct activation of TRPV1 via capsaicin is neurotoxic, there is some evidence that TRPV1 can be functionally neuroprotective. In a global ischemia model, TRPV1 antagonist capsazepine was able to block the neuroprotective effects of CB1 receptor antagonist rimonabant in CA1 hippocampal neurons[42]. The ability of capsazepine to block neuroprotection in this case suggests that TRPV1 at least partially mediates the neuroprotective effects of rimonabant[43]. This data concerning neurotoxic versus neuroprotective functions must be considered with the understanding that perturbing neurons with TRPV1 agonists and antagonists (some of which are not endogenous) does not necessarily represent functions that actually occur in stressed or degenerating neurons in vivo. While evidence supports neurotoxic and neuroprotective roles of TRPV1, it is of primary importance to understand that channel function is dictated by the neuronal signaling milieu. This signaling inevitably varies between classes of neurons as well as between different injury and disease states.

TRPV1 function in retinal ganglion cells: potential neuroprotection

The role of TRPV1 in neuronal survival remains controversial, especially with respect to disease and injury states in vivo. Glaucoma, an irreversible optic neuropathy, presents an especially interesting system in which to study TRPV1. In glaucoma, intraocular pressure (IOP) is the primary modifiable risk factor[44], so many animal models of this neurodegenerative disease require inducing elevated IOP[45, 46]. It is known that this channel contributes to pressure-induced changes in Ca^{2+} signaling in retinal ganglion cells (RGCs)[47] and retinal microglia[7]. Preliminary data from TRPV1 knockout mice suggests a neuroprotective role of TRPV1 against pressure-induced neurodegeneration of RGCs (Ward - unpublished data). These TRPV1-/- mice, when subjected to IOP elevation, exhibited increased optic nerve pathology when compared to wildtype controls. This is unusual, given that pressure is known to activate TRPV1 in RGCs, and that such Ca^{2+} influx can cause neurotoxicity. It seems logical that loss of TRPV1 would render RGCs less susceptible to pressure-induced death in vivo; however, this would not be the case if TRPV1 activation is actually neuroprotective. The potential for a TRP channel to exhibit neuroprotective activity in retinal injury is not unprecedented, as a recent study of retinal ischemia/reperfusion injury indicated that TRPC6 is protective[48].

Our working hypothesis is that TRPV1 functions as an intrinsic stress responder that slows down RGC degeneration by increasing excitatory activity at RGC synapses. In the DBA/2 mouse model of glaucoma, RGCs experiencing degeneration regularly exhibit dendrites with decreased complexity that lack higher-order branching, an indication of dendritic pruning[49]. Likewise, it is known that synaptic activity is a crucial factor in long-term synapse maintenance[50]. Increased TRPV1 activity at RGC synapses may counter the dendritic pruning seen in glaucoma, as retention of synapses requires maintenance of synaptic activity. In fact, eyes with elevated IOP exhibit increased levels of TRPV1 in the inner plexiform layer (IPL) of the retina[47] (**Figure 2**), which supports the idea of increased synapse potentiation in response to stress. The IPL includes extensive synaptic connections between RGC dendrites and bipolar cells, so this observed localization to the RGC dendrites may involve potentiation of synaptic connections under IOP stress.

As described in this review, TRPV1 exhibits a functional profile that fits with this working hypothesis. First, TRPV1 exhibits an intrinsic stress response that often includes increased levels of channel expression in injured and degenerating neurons[26-30]. Second, under stressed conditions, TRPV1 is sensitized by phosphorylation and relocalization to the membrane, where it increases membrane currents[32, 34, 35]. Third, within neuronal networks in the CNS, TRPV1 is known to modulate synaptic plasticity[19, 20] and to potentiate input from glutamatergic synapses[21]. Finally, the potential for TRPV1-mediated neuroprotection is supported by our preliminary data, where TRPV1-/- mice exhibit reduced RGC survival despite elevated IOP. Altogether, these functions indicate that TRPV1 may exhibit neuroprotective activity in glaucoma.

This hypothesis specifically addresses a potential TRPV1-mediated mechanism for intrinsic neuroprotection. Examination of TRPV1 function outside the neuron itself may provide even more information regarding how this channel mediates RGC survival. For example, retinal

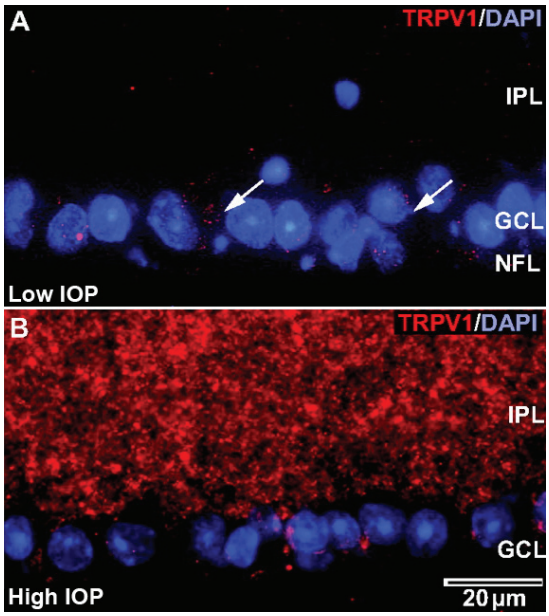

Figure 2. TRPV1 expression increases in RGC dendrites with elevated IOP. A. Immunolabeling for TRPV1 in a 6 month DBA/2J mouse retina from an eye with normal IOP. TRPV1 localizes primarily to the ganglion cell layer (GCL). B. TRPV1 immunolabeling increases in an age-matched retina with elevated IOP. Labeling persists in the GCL and increases in the inner plexiform layer (IPL), where RGC dendrites ramify. Figure modified for use with permission from author[47].

microglia exhibit pressure-dependent release of IL-6, a cytokine that is protective against pressure-induced RGC death[51]. Specific antagonism of TRPV1 revealed that this release was partially mediated by TRPV1-induced Ca^{2+} influx[7]. It is therefore likely that TRPV1-mediated neuroprotection is not simply intrinsic to RGCs, but may also involve glial cells.

Conclusions

Transduction of stimuli from the extracellular environment is a critical component of the neuronal response to stressors. The responses of TRPV1 to stress, reviewed here, indicate a potential role of TRPV1 in neuroprotection. TRPV1 activation is known to potentiate glutamatergic synapses, thus relocalization of TRPV1 to RGC dendrites may be involved in slowing the progression of dendritic pruning in glaucoma. Neurodegenerative diseases such as glaucoma do not push neurons unidirectionally toward death without a response from intrinsic cellular mechanisms that counter dysfunction. It is therefore important to characterize intrinsic stress responders such as TRPV1 in order to assess the potential for therapeutic interventions.

REFERENCES

1. Clapham DE (2003). TRP channels as cellular sensors. Nature. 426 (6966): 517-524.
2. Ramsey IS, Delling M and Clapham DE (2006). An introduction to TRP channels. Annu Rev Physiol. 68: 619-647.
3. Montell C and Rubin GM (1989). Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. Neuron. 2 (4): 1313-1323.
4. Marambaud P, Dreses-Werringloer U and Vingtdeux V (2009). Calcium signaling in neurodegeneration. Mol Neurodegener. 4: 20.
5. Vennekens R, Owsianik G and Nilius B (2008). Vanilloid transient receptor potential cation channels: an overview. Curr Pharm Des. 14 (1): 18-31.
6. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD and Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 389 (6653): 816-824.
7. Sappington RM and Calkins DJ (2008). Contribution of TRPV1 to microglia-derived IL-6 and NFkappaB translocation with elevated hydrostatic pressure. Invest Ophthalmol Vis Sci. 49 (7): 3004-3017.
8. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI and Julius D (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science. 288 (5464): 306-313.
9. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A and Sheardown SA (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature. 405 (6783): 183-187.
10. Toth A, Blumberg PM and Boczan J (2009). Anandamide and the vanilloid receptor (TRPV1). Vitam Horm. 81: 389-419.
11. Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS and Gereau RWt (2002). cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron. 35 (4): 721-731.
12. Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS and Gereau RWt (2003). Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci U S A. 100 (21): 12480-12485.
13. Wang Y, Kedei N, Wang M, Wang QJ, Huppler AR, Toth A, Tran R and Blumberg PM (2004). Interaction between protein kinase Cmu and the vanilloid receptor type 1. J Biol Chem. 279 (51): 53674-53682.
14. Pareek TK, Keller J, Kesavapany S, Agarwal N, Kuner R, Pant HC, Iadarola MJ, Brady RO and Kulkarni AB (2007). Cyclin-dependent kinase 5 modulates nociceptive signaling through direct phosphorylation of transient receptor potential vanilloid 1. Proc Natl Acad Sci U S A. 104 (2): 660-665.
15. Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H and Oh U (2004). Phosphorylation of vanilloid receptor 1 by Ca2+/calmodulin-dependent kinase II regulates its vanilloid binding. J Biol Chem. 279 (8): 7048-7054.
16. Jara-Oseguera A, Simon SA and Rosenbaum T (2008). TRPV1: on the road to pain relief. Curr Mol Pharmacol. 1 (3): 255-269.

17. Toth A, Boczan J, Kedei N, Lizanecz E, Bagi Z, Papp Z, Edes I, Csiba L and Blumberg PM (2005). Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. Brain Res Mol Brain Res. 135 (1-2): 162-168.

18. Szallasi A and Di Marzo V (2000). New perspectives on enigmatic vanilloid receptors. Trends .Neurosci. 23 (10): 491-497.

19. Marsch R, Foeller E, Rammes G, Bunck M, Kossl M, Holsboer F, Zieglgansberger W, Landgraf R, Lutz B and Wotjak CT (2007). Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J Neurosci. 27 (4): 832-839. This paper demonstrates that TRPV1 is important in regulating synaptic plasticity via long term potentiation.

20. Chavez AE, Chiu CQ and Castillo PE (2010). TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci. 13 (12): 1511-1518.

21. Xing J and Li J (2007). TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. J Neurophysiol. 97 (1): 503-511.

22. Goswami C, Rademacher N, Smalla KH, Kalscheuer V, Ropers HH, Gundelfinger ED and Hucho T (2010). TRPV1 acts as a synaptic protein and regulates vesicle recycling. J Cell Sci. 123 (Pt 12): 2045-2057. This paper provides the first evidence of TRPV1 localization to synaptic structures, thus implicating the channel in synaptic processes.

23. Goswami C, Schmidt H and Hucho F (2007). TRPV1 at nerve endings regulates growth cone morphology and movement through cytoskeleton reorganization. FEBS J. 274 (3): 760-772.

24. Goswami C (2010). Structural and functional regulation of growth cone, filopodia and synaptic sites by TRPV1. Commun Integr Biol. 3 (6): 614-618.

25. Maione S, Cristino L, Migliozzi AL, Georgiou AL, Starowicz K, Salt TE and Di Marzo V (2009). TRPV1 channels control synaptic plasticity in the developing superior colliculus. J Physiol. 587 (Pt 11): 2521-2535.

26. Biggs JE, Yates JM, Loescher AR, Clayton NM, Boissonade FM and Robinson PP (2007). Changes in vanilloid receptor 1 (TRPV1) expression following lingual nerve injury. Eur J Pain. 11 (2): 192-201.

27. Kanai Y, Nakazato E, Fujiuchi A, Hara T and Imai A (2005). Involvement of an increased spinal TRPV1 sensitization through its up-regulation in mechanical allodynia of CCI rats. Neuropharmacology. 49 (7): 977-984.

28. Ishibashi T, Takumida M, Akagi N, Hirakawa K and Anniko M (2009). Changes in transient receptor potential vanilloid (TRPV) 1, 2, 3 and 4 expression in mouse inner ear following gentamicin challenge. Acta Otolaryngol. 129 (2): 116-126.

29. Lee YM, Kim YK and Chung JH (2009). Increased expression of TRPV1 channel in intrinsically aged and photoaged human skin in vivo. Exp Dermatol. 18 (5): 431-436.

30. Facer P, Casula MA, Smith GD, Benham CD, Chessell IP, Bountra C, Sinisi M, Birch R and Anand P (2007). Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. BMC Neurol. 7: 11.

31. Mitchell CH, Lu W, Hu H, Zhang X, Reigada D and Zhang M (2009). The P2X(7) receptor in retinal ganglion cells: A neuronal model of pressure-induced damage and protection by a shifting purinergic balance. Purinergic Signal. 5 (2): 241-249.

32. Tominaga M, Wada M and Masu M (2001). Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci U S A. 98 (12): 6951-6956.

33. Glass CK, Saijo K, Winner B, Marchetto MC and Gage FH (2010). Mechanisms underlying inflammation in neurodegeneration. Cell. 140 (6): 918-934.

34. Zhang N, Inan S, Cowan A, Sun R, Wang JM, Rogers TJ, Caterina M and Oppenheim JJ (2005). A proinflammatory chemokine, CCL3, sensitizes the heat- and capsaicin-gated ion channel TRPV1. Proc Natl Acad Sci U S A. 102 (12): 4536-4541.

35. Zhang X, Huang J and McNaughton PA (2005). NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J. 24 (24): 4211-4223.

36. Wang WH, Lin DH and Sterling H (2002). Regulation of ROMK channels by protein tyrosine kinase and tyrosine phosphatase. Trends Cardiovasc Med. 12 (3): 138-142.

37. Hayashi T and Huganir RL (2004). Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. J Neurosci. 24 (27): 6152-6160.

38. Jancso G, Kiraly E and Jancso-Gabor A (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature. 270 (5639): 741-743.

39. Ritter S and Dinh TT (1990). Capsaicin-induced neuronal degeneration in the brain and retina of preweanling rats. J Comp Neurol. 296 (3): 447-461.

40. Kim SR, Lee DY, Chung ES, Oh UT, Kim SU and Jin BK (2005). Transient receptor potential vanilloid subtype 1 mediates cell death of mesencephalic dopaminergic neurons in vivo and in vitro. J Neurosci. 25 (3): 662-671.

41. Sarker KP and Maruyama I (2003). Anandamide induces cell death independently of cannabinoid receptors or vanilloid receptor 1: possible involvement of lipid rafts. Cell Mol Life Sci. 60 (6): 1200-1208.

42. Pegorini S, Braida D, Verzoni C, Guerini-Rocco C, Consalez GG, Croci L and Sala M (2005). Capsaicin exhibits neuroprotective effects in a model of transient global cerebral ischemia in Mongolian gerbils. Br J Pharmacol. 144 (5): 727-735.

43. Pegorini S, Zani A, Braida D, Guerini-Rocco C and Sala M (2006). Vanilloid VR1 receptor is involved in rimonabant-induced neuroprotection. Br J Pharmacol. 147 (5): 552-559.

44. Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK, 2nd, Wilson MR and Kass MA (2002). The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. Arch Ophthalmol. 120 (6): 714-720; discussion 829-730.

45. Sappington RM, Carlson BJ, Crish SD and Calkins DJ (2010). The microbead occlusion model: a paradigm for induced ocular hypertension in rats and mice. Invest Ophthalmol Vis Sci. 51 (1): 207-216.

46. Pang IH and Clark AF (2007). Rodent models for glaucoma retinopathy and optic neuropathy. J Glaucoma. 16 (5): 483-505.

47. Sappington RM, Sidorova T, Long DJ and Calkins DJ (2009). TRPV1: contribution to retinal ganglion cell apoptosis and increased intracellular Ca2+ with exposure to hydrostatic pressure. Invest Ophthalmol Vis Sci. 50 (2): 717-728. This paper presents evidence that TRPV1 modulates RGC survival in vitro in response to hydrostatic pressure.

48. Wang X, Teng L, Li A, Ge J, Laties AM and

Zhang X (2010). TRPC6 channel protects retinal ganglion cells in a rat model of retinal ischemia/reperfusion-induced cell death. Invest Ophthalmol Vis Sci. 51 (11): 5751-5758. This paper demonstrates that TRP channels can mediate RGC neuroprotection in retinal injury.

49. Jakobs TC, Libby RT, Ben Y, John SW and Masland RH (2005). Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. J Cell Biol. 171 (2): 313-325.

50. Lin YC and Koleske AJ (2010). Mechanisms of synapse and dendrite maintenance and their disruption in psychiatric and neurodegenerative disorders. Annu Rev Neurosci. 33: 349-378.

51. Sappington RM, Chan M and Calkins DJ (2006). Interleukin-6 protects retinal ganglion cells from pressure-induced death. Invest Ophthalmol Vis Sci. 47 (7): 2932-2942.

FURTHER INFORMATION

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CANDIDATE
REVIEWS

Glucagon-like Peptide-1 is Much More Than an Incretin

Erin E. Watt

Abstract

This review will discuss glucagon-like peptide-1 (GLP-1) in both the periphery and the brain; GLP-1 analogues as treatments for type-2 diabetes mellitus (T2DM) and obesity; and examine the recent findings about GLP-1 signaling and striatal dopamine (DA) homeostasis. The published literature was reviewed, with an emphasis on recent publications investigating GLP-1's actions beyond its typical incretin role, such as novel signaling pathways in the brain. In patients with T2DM, GLP-1 levels are reduced, but GLP-1 receptor (GLP-1R) signaling remains intact. Therefore, GLP-1R agonists are ideal therapies for T2DM. Furthermore, GLP-1R signaling reduces appetite, decreases fat mass, promotes cardiovascular protection, and can modulate striatal DA homeostasis, making GLP-1R agonists an ideal therapy for obesity. GLP-1R agonists can cross the blood-brain barrier, while GLP-1R's in the brain contribute to the reduction in appetite and decrease in fat mass shown in GLP-1R agonist studies. Recent studies show that GLP-1R signaling may also regulate striatal DA homeostasis, which is known to be dysfunctional in obesity, further implicating GLP-1R agonists as powerful obesogenic therapies.

Keywords

Glucagon-like peptide-1
Incretin
Obesity
Type-2 diabetes mellitus
Dopamine
Reward

Introduction

The identification of glucagon-like peptide-1 (GLP-1) as a potent incretin (an insulinotropic gut hormone), along with the development of non-hydrolysable forms of this incretin, has revolutionized the treatment of diabetes mellitus type-2 (T2DM). The physiological effects of GLP-1 have been extensively studied in the periphery due to its important clinical application in the treatment of T2DM. Clinical studies have revealed that, in addition to its ability to enhance glucose-stimulated insulin secretion, GLP-1 analogues are able to curb appetite (hypophagia)[1] and have a protective effect on cardiovascular function[2]. GLP-1's hypophagic properties have led many researchers to explore its actions in appetite-regulating regions of the brain. Evidence indicates that GLP-1 acts directly in the brain to control appetite and energy balance[3]; this evidence, in addition to data suggesting cardiovascular protection (a known comorbidity with obesity[2]), has led investigators to view GLP-1 as a potential therapeutic for obesity.

Obesity is a medical condition in which there is a positive energy balance (calories taken in are greater than calories expended). Brain regions, such as the hypothalamus, tightly regulate this important homeostatic process. However, reward pathways can override the hypothalamic signals, leading to compulsive overeating, obesity, and T2DM[5]. In obesity, there are deficits in reward pathways, such as dysregulated striatal dopamine (DA) signaling. These deficits are similar to what is seen in other substance use disorders[6]. Recent evidence shows that GLP-1 can regulate striatal DA through a novel, transynaptic signaling mechanism, involving nitric oxide (NO)[4], potentially making it an even more powerful therapy for obesity. This review will discuss what is known about GLP-1 in both the periphery and the brain; GLP-1 as a treatment for T2DM and obesity; and examine the recent findings about GLP-1 signaling and striatal DA homeostasis.

1. GLP-1 in the Periphery

1.1 Source

GLP-1 (7-36-NH_2 or 7-37) is a peptide derived from the 180-amino acid prohormone preproglucagon (PPG) encoded in the proglucagon gene[7]. This prohormone contains the sequences of several small peptide hormones, such as GLP-1, glucagon-like peptide-2, and glucagon. Prohormone convertases (PCs) that process PPG are localized to specific tissues, allowing for targeted production of PPG products. For example, glucagon is produced in the pancreas by PC2, while GLP-1 (1-37) is produced in the gut by PC1/3[8, 9]. In the gut, after PPG has been cleaved to create GLP-1 (1-

36-NH_2 or 1-37), it undergoes further processing by the removal of six amino acids from the amino terminus, generating the mature GLP-1 (7-36-NH_2 or 7-37)[10]. This mature form (most commonly 7-36-NH_2) can activate the GLP-1 receptors (GLP-1R) located in GLP-1's target tissues.

1.2 Secretion, Action & Metabolism

GLP-1 is an incretin, a substance secreted from the gut to enhance oral glucose-stimulated insulin release (**Figure 1**). After a meal, GLP-1 is secreted from the small intestine. The specific gastrointestinal cells that process and secrete mature GLP-1 are L-cells found in the distal ileum and colon[11, 12]. Upon ingestion of a meal, L-cells release GLP-1 in two phases: an early phase (10-15 minutes post prandial[a]) and a later phase (30-60 minutes post prandial)[13]. The early phase is stimulated by gastrin-releasing peptide, acetylcholine, gastric inhibitory peptide (the other known incretin) and the vagus nerve[14-16], whereas the later phase is induced by direct nutrient sensing on the apical surface of the L-cells[17]. GLP-1 has a half-life of only 2 minutes; once secreted, it is inactivated by the serine exopeptidase, dipeptidyl peptidase-4 (DPP-4), which hydrolyses peptides at serines with prolines or alanines in the penultimate positions[18]. GLP-1's quick biphasic response to a meal and rapid clearance by DPP-4 allows GLP-1 to enhance glucose-dependent insulin release at exactly the right time for the appropriate amount of time[11, 12]. Other than the enhancement of glucose-dependent insulin secretion, GLP-1 also decreases glucagon secretion[19]; increases insulin sensitivity in both α-cells and β-cells[20]; increases β-cell mass and insulin gene expression[21]; inhibits stomach acid production; slows gastric emptying[22]; and indirectly increases insulin sensitivity in muscle, liver and adipose tissue[23]. All of the effects mediated by GLP-1 are critical for the body to properly metabolize a meal. Each direct action of GLP-1 described above occurs when GLP-1 binds to its receptor, GLP-1R, which will be discussed in more detail below.

1.3 Receptors

The GLP-1R is a seven transmembrane, heterotrimeric, G-protein coupled receptor (GPCR)[24]. In order for this receptor it to be fully functional, it must be glycosylated[25]. The GLP-1 binding domain is the N-terminal region of the GLP-1R. The third intracellular loop of this GPCR is critical for G-protein coupling. The GLP-1R can couple with many G-proteins including $G\alpha_s$, $G\alpha_q$, $G\alpha_i$, and $G\alpha_o$[26]. This GPCR has been most extensively studied in the context of enhancing glucose-dependent insulin secretion post prandial in the pancreatic β-cell. In these cells it is known to be $G\alpha_S$-coupled. Upon activation of GLP-1R in the pancreas, $G\alpha_S$ dissociates from GLP-1R and stimulates membrane-anchored adenylate cyclase (AC). AC catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). As this reaction continues, high levels of cAMP accumulate and can activate protein kinase A (PKA), among other intracellular messengers, having the combined effect of increasing intracellular calcium (Ca^{2+}) levels and depolarizing the membrane. Activating PKA, increasing intracellular Ca^{2+} concentrations, and depolarizing the membrane all lead to enhancement of glucose-dependent insulin secretion[11, 17, 27]. In pancreatic islet cell lines, the GLP-1R localizes to lipid rafts[b]; the subcellular localization, trafficking, and signaling is dependent on its interaction with caveolin-1[c]. In these same cell lines, there is evidence of rapid desensitization and internalization of the GLP-1R, which is dependent on phosphorylation of certain residues on this GPCR. However, some *in vivo* studies of chronic GLP-1R stimulation show no such sensitization[28].

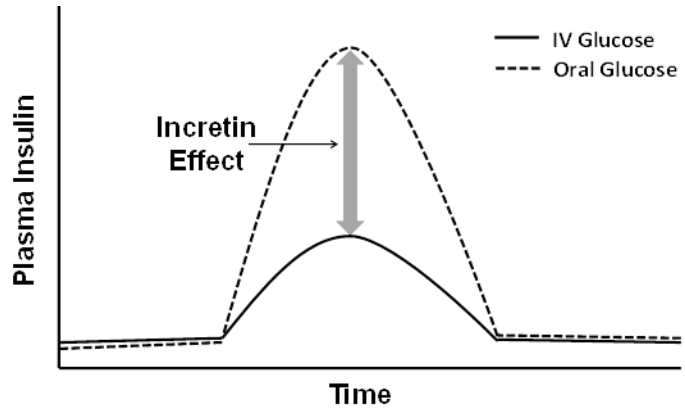

Figure 1: The incretin effect accounts for 50-70% of insulin secretion after oral glucose ingestion. GLP-1 is an incretin.

Structurally identical GLP-1R's are expressed in many areas of the body other than the pancreas, including the heart, stomach, and numerous brain regions[29]. In clinical trials, GLP-1 analogues have been shown to exert a protective effect on cardiovascular function, prompting researchers to explore GLP-1R activity in vasculature endothelial cells. It was found that GLP-1R signaling increased phosphorylation (thereby increasing activity) of endothelial

a. After a meal

b. Glycolipoprotein microdomains responsible for centralizing signaling molecules needed for specific signaling pathways and trafficking of membrane receptors and transporters

c. Integral membrane protein that mediates receptor-independent endocytosis

nitric oxide synthase (eNOS), and decreased inflammation through inhibiting expression of genes encoding inflammatory molecules[2]. The specific cellular mechanisms as to how GLP-1R modulates phosphorylation of eNOS and inflammatory gene expression are still unknown and are under further investigation. Activation of the GLP-1R in the brain will be discussed in more detail below.

2. GLP-1 Analogues as T2DM Drugs

2.1 GLP-1R Agonist

GLP-1 plays an important role in maintaining proper glucose homeostasis in the body[29]. In patients with T2DM, GLP-1 secretion is reduced while GLP-1R signaling remains intact[30], making the GLP-1R a logical pharmaceutical target for the treatment of T2DM. The administration of the native form of GLP-1 must be given continually by intravenous therapy (IV) in order to be effective because of DPP-4-mediated rapid enzymatic degradation of GLP-1, making it an infeasible solution[23]. Fortunately, non-hydrolysable analogues of GLP-1 have since been created. There are currently two GLP-1R agonists available for the treatment of T2DM: exenatide (Byetta, Eli Lilly) and liraglutide (Victoza, Novo Nordisk). Both are synthetic analogues of the GLP-1 peptide that act as agonists at the GLP-1R and have been modified to be resistant to enzymatic cleavage by DPP-4[30]. Exenatide is a mimetic of a naturally occurring peptide hormone found in the saliva of Hela monsters. It has 53% sequence identity to native GLP-1 but lacks the DPP-4 cleavage site[31]. As a therapeutic, it must be given twice daily; when given along with other oral anti-diabetic drugs (such as metformin), exenatide is efficient at maintaining glycemic control in patients with T2DM[1]. Furthermore, it has been reported that exenatide promotes β-cell proliferation[32] and protects β-cells from apoptosis[33]. In clinical trials, it has been reported that exenatide reduces food intake and promotes weight loss[1]. Meanwhile, liraglutide has 97% sequence homology with native GLP-1[34]. What makes this peptide resistant to enzymatic degradation is the addition of a C16 fatty acid side chain that can reversibly bind albumin, prolonging its activity to over 24 hours. Much like exenatide, liraglutide (given along with metformin) is effective in maintaining glycemic control and aiding in weight loss[35]. These GLP-1R agonists have recently been shown to have protective cardiovascular action[2]. Since there is a high comorbidity of obesity and cardiovascular disease, this further supports that GLP-1R agonists are appropriate for treating obesity as well as T2DM.

2.2 DPP-4 Inhibitors

Another class of GLP-1 drugs used to treat T2DM is the DPP-4 inhibitors. DPP-4, as described above, is a serine protease that is responsible for the degradation of GLP-1. DPP-4 inhibitors increase endogenous levels of native GLP-1 by inhibiting the enzyme responsible for its degradation. There are four approved DPP-4 inhibitors on the market: sitagliptin, vildagliptin, saxagliptin, and alogliptin. All of these drugs are small molecules and can be taken orally. They also have good efficacy and have been proven to be very safe with few side effects, but are not as popular as GLP-1R agonists[36].

3. GLP-1 in the Brain

3.1 Source

PPG is expressed in two brain regions, the nucleus of the solitary tract (NTS) and the olfactory bulb. PPG expression in the olfactory bulb is contained in interneurons, signifying that the only known GLP-1 projections in the brain originate from the NTS[37]. mRNA expression of PC1/3 coincide with regions of the NTS that have been shown to express both PPG and GLP-1, indicating that PPG is similarly processed in the NTS as to what has been shown in the periphery[28, 38, 39]. Through retrograde tracing, immunoreactive staining of PPG, and GLP-1 specific antibodies, investigators have determined that NTS neurons containing GLP-1 most densely project to the hypothalamus[40]. The specific regions of the hypothalamus receiving these projections are important for food intake and the regulation of energy balance[41]. No other known GLP-1 projections have been discovered at this time.

3.2 Receptors

Although GLP-1 projections mainly target the hypothalamus, the GLP-1R is found throughout the brain and is structurally identical to GLP-1R's in the periphery. GLP-1R agonists easily cross the blood brain barrier[42]. Therefore, the study of GLP-1R signaling in other brain regions is critical. There have been extensive studies exploring which brain regions express the GLP-1R. One of the most comprehensive studies was done using *in situ* hybridization to show that GLP-1R-expressing cells are found in the olfactory bulb, cortex, striatum, amygdala, hippocampus, bed nucleus of the stria terminalas, hypothalamus, thalamus, medulla and many other specific nuclei[37]. As previously mentioned, GLP-1R is structurally identical throughout, but the G-protein coupling and subsequent signaling of GLP-1R in

each region has not yet been elucidated.

3.3 Studies of GLP-1 and the Brain

Some of the earliest studies of GLP-1 in the brain showed that intracerebro-ventricular (ICV) injection of GLP-1 could reduce food intake[3] and body weight in rats. This effect was specific to GLP-1R signaling because GLP-1R antagonists could block these effects[43]. This indicates that at least some of the anorexic effects of GLP-1R agonists given peripherally could be due to actions on GLP-1R's in the brain. More recently, investigators have shown that knocking down PPG in the NTS or blockade of GLP-1R's with an antagonist in the hypothalamus of rats leads to hyperphagia and fat accumulation[44]. This is the best recent evidence that GLP-1R signaling in the brain, specifically in the hypothalamus, is critical for maintaining energy balance.

Some investigators have found common pathologies between Alzheimer's disease (AD) and T2DM, prompting researchers to explore GLP-1R signaling in the hippocampus and in mouse models of AD. In hippocampal neuron cultures, it was discovered that GLP-1R activation is neuroprotective against glutamatergic excitotoxicity[45]. In mouse models of AD, GLP-1R signaling was able to prevent the formation of β-amyloid plaques[46]. These studies, among others, show that GLP-1R signaling is much more complex than previously thought.

The important role of DA signaling in obesity has become increasingly apparent. GLP-1R's are expressed in the striatum, an important brain region for reward[37]. Dr. Aurelio Galli and colleagues at Vanderbilt University have recently discovered that GLP-1 can regulate trafficking of the DA transporter (DAT) in the striatum. DAT is an important component of the machinery used to maintain proper DA homeostasis and clearly instrumental in reward pathways, as many drugs of abuse target DAT. The molecular mechanism by which GLP-1R signaling can modulate DAT is entirely novel from what is currently known about GLP-1R signaling and is the first account of GLP-1R signaling in the striatum. The pathway these investigators propose entails the employment of the diffusible transynaptic messenger, nitric oxide (NO), to regulate surface levels of DAT protein. They have shown that the GLP-1R is not on DA terminals in the striatum, but rather on a subpopulation of neuronal NO synthase (nNOS, the enzyme that produces NO in neurons)-producing interneurons. In acute rat striatal slices, they demonstrate that GLP-1 acts by reducing the activity of nNOS via phosphorylating nNOS at serine 847 (an inhibitory site on nNOS), thereby increasing surface levels of DAT on nearby DA terminals[4] (Figure 2).

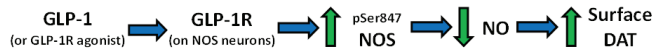

Figure 2: Schematic of proposed GLP-1R signaling in the striatum by Erreger et. al, 2011

The regulation of monoamine transporters by NO[47] and GLP-1R coupling to NO[2] is not completely novel, yet these experiments illustrate that GLP-1R signaling has even more versatility than previously imagined. The implications of GLP-1R signaling regulating DA homeostasis in the striatum, independent of known mechanisms for regulating DAT, are that GLP-1 may play a role in regulating the reward component of food intake (striatal DA), not just the homeostatic (hypothalamus) or peripheral (pancreas) roles it is already known to have. If this is the case, GLP-1 may be a potential therapy for other substance use disorders in which dysregulated striatal DA is also present.

Summary & Future Directions

GLP-1 was first discovered as an incretin but has since proven to have other vital roles. GLP-1R agonist and therapies that increase endogenous levels of GLP-1 (DPP-4 inhibitors) are powerful pharmaceutical agents used to treat T2DM. GLP-1R signaling has been studied in several regions of the periphery and brain. While the amino acid structure of the GLP-1R is identical in all regions of the body, its signaling is versatile. Studies to further elucidate the molecular mechanisms behind GLP-1R coupling to NO in the cardio-vasculature and striatum will be essential in maximizing the use of the powerful GLP-1R agonists.

In the United States today, about one third of the population is obese[48], and over 1.5 million new cases of T2DM are diagnosed per year[49]. The leading cause of T2DM is obesity, a disease of positive energy balance that is most commonly the outcome of compulsive overeating and lack of physical activity. Homeostatic pathways in brain regions, such as the hypothalamus, have been extensively studied in hopes of finding therapeutics for obesity[5]. Thus far, this has not been a successful route for the treatment of obesity. More recently, it has been demonstrated that reward pathways, specifically striatal DA, are altered in obese subjects and that therapies that target striatal DA would be beneficial in the treatment of obesity[6]. GLP-1 treats dysglycemia; regulates food intake; protects against cardiovascular disease in T2DM and obesity; and now potentially rescues dysregulated DA signaling seen in obesity (Figure 3).

References

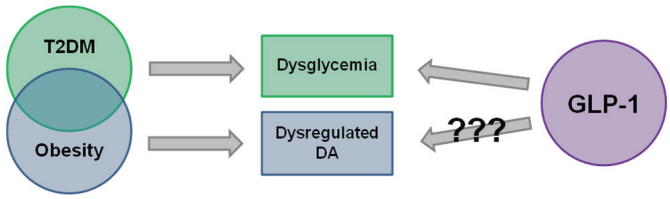

Figure 3: Obesity and T2DM are comorbid disorders. In T2DM there is dysglycemia, and in obesity there is dysregulated DA homeostasis. GLP-1 agonists are used to treat dysglycemia in T2DM, and new evidence suggests that GLP-1 analogues can treat dysfunction in DA signaling in the striatum[4].

1. DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS and Baron AD (2005). Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. Diabetes Care. 28 (5): 1092-1100.

2. Hattori Y, Jojima T, Tomizawa A, Satoh H, Hattori S, Kasai K and Hayashi T (2010). A glucagon-like peptide-1 (GLP-1) analogue, liraglutide, upregulates nitric oxide production and exerts anti-inflammatory action in endothelial cells. Diabetologia. 53 (10): 2256-2263.

This article is essential because it describes a novel GLP-1R signaling pathway from what is shown in the rest of the periphery. It also supports GLP-1R coupling to NO.

3. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J and Bloom SR (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. Nature. 379 (6560): 69-72.

4. Erreger K, Davis AR, Robertson SD, Watt EE, Rogers GJ, Matthies HJG, Saunders C, Vaughan RA, West AR, Greig NH, Niswender KD and Galli A (2011). Glucagon-like peptide-1 (GLP-1) regulates the dopamine transporter in the striatum by a signaling mechanism mediated by nitric oxide synthase. Society for Neuroscience.

5. Daws LC, Avison MJ, Robertson SD, Niswender KD, Galli A and Saunders C (2011). Insulin signaling and addiction. Neuropharmacology.

6. Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N and Fowler JS (2001). Brain dopamine and obesity. Lancet. 357 (9253): 354-357.

This article is essential because it describes dysregulated striatal DA homeostasis observed in obesity supporting the need for obesogenic therapies to target brain DA.

7. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L and Habener JF (1986). Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. J Biol Chem. 261 (25): 11880-11889.

8. Rouille Y, Martin S and Steiner DF (1995). Differential processing of proglucagon by the subtilisin-like prohormone convertases PC2 and PC3 to generate either glucagon or glucagon-like peptide. J Biol Chem. 270 (44): 26488-26496.

9. Rouille Y, Kantengwa S, Irminger JC and Halban PA (1997). Role of the prohormone convertase PC3 in the processing of proglucagon to glucagon-like peptide 1. J Biol Chem. 272 (52): 32810-32816.

10. Mojsov S (1992). Structural requirements for biological activity of glucagon-like peptide-I. Int J Pept Protein Res. 40 (3-4): 333-343.

11. Mojsov S, Weir GC and Habener JF (1987). Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest. 79 (2): 616-619.

12. Kreymann B, Williams G, Ghatei MA and Bloom SR (1987). Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet. 2 (8571): 1300-1304.

13. Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R and Goke B (1995). Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion. 56 (2): 117-126.

14. Balks HJ, Holst JJ, von zur Muhlen A and Brabant G (1997). Rapid oscillations in plasma glucagon-like peptide-1 (GLP-1) in humans: cholinergic control of GLP-1 secretion via muscarinic receptors. J Clin Endocrinol Metab. 82 (3): 786-790.

15. Rocca AS and Brubaker PL (1999). Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. Endocrinology. 140 (4): 1687-1694.

16. Anini Y and Brubaker PL (2003). Muscarinic receptors control glucagon-like peptide 1 secretion by human endocrine L cells. Endocrinology. 144 (7): 3244-3250.

17. Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ and Gribble FM (2008). Glucose sensing in L cells: a primary cell study. Cell Metab. 8 (6): 532-539.

18. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B and Holst JJ (1995). Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes. 44 (9): 1126-1131.

19. Heller RS, Kieffer TJ and Habener JF (1997). Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. Diabetes. 46 (5): 785-791.

20. Holz GGt, Kuhtreiber WM and Habener JF (1993). Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). Nature. 361 (6410): 362-365.

21. Drucker DJ, Philippe J, Mojsov S, Chick WL and Habener JF (1987). Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci U S A. 84 (10): 3434-3438.

22. Nauck MA, Kemmeries G, Holst JJ and Meier JJ (2011). Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes. 60 (5): 1561-1565.

23. Zander M, Madsbad S, Madsen JL and Holst JJ (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet. 359 (9309): 824-830.

24. Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B and Drucker DJ (2003). International Union of Pharmacology. XXXV. The glucagon receptor family. Pharmacol Rev. 55 (1): 167-194.

25. Goke R, Just R, Lankat-Buttgereit B and Goke B (1994). Glycosylation of the GLP-1 receptor is a prerequisite for regular receptor function. Peptides. 15 (4): 675-681.

26. Hallbrink M, Holmqvist T, Olsson M, Ostenson CG, Efendic S and Langel U (2001). Different domains in the third intracellular loop of the GLP-1 receptor are responsible for Galpha(s) and Galpha(i)/Galpha(o) activation. Biochim Biophys Acta. 1546 (1): 79-

86.
27. Holst JJ, Orskov C, Nielsen OV and Schwartz TW (1987). Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett. 211 (2): 169-174.
28. Baggio LL, Kim JG and Drucker DJ (2004). Chronic exposure to GLP-1R agonists promotes homologous GLP-1 receptor desensitization in vitro but does not attenuate GLP-1R-dependent glucose homeostasis in vivo. Diabetes. 53 Suppl 3: S205-214.
29. Baggio LL and Drucker DJ (2007). Biology of incretins: GLP-1 and GIP. Gastroenterology. 132 (6): 2131-2157.
30. Garber AJ (2011). Long-acting glucagon-like peptide 1 receptor agonists: a review of their efficacy and tolerability. Diabetes Care. 34 Suppl 2: S279-284.
31. Furman BL (2010). The development of Byetta (exenatide) from the venom of the Gila monster as an anti-diabetic agent. Toxicon.
32. Buteau J, Foisy S, Joly E and Prentki M (2003). Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes. 52 (1): 124-132.
33. Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U and Perfetti R (2002). Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology. 143 (11): 4397-4408.
34. Russell-Jones D (2009). Molecular, pharmacological and clinical aspects of liraglutide, a once-daily human GLP-1 analogue. Mol Cell Endocrinol. 297 (1-2): 137-140.
35. Nauck M, Frid A, Hermansen K, Shah NS, Tankova T, Mitha IH, Zdravkovic M, During M and Matthews DR (2009). Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes: the LEAD (liraglutide effect and action in diabetes)-2 study. Diabetes Care. 32 (1): 84-90.
36. Deacon CF (2011). Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes Obes Metab. 13 (1): 7-18.
37. Merchenthaler I, Lane M and Shughrue P (1999). Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. J Comp Neurol. 403 (2): 261-280.
38. Schafer MK, Day R, Cullinan WE, Chretien M, Seidah NG and Watson SJ (1993). Gene expression of prohormone and proprotein convertases in the rat CNS: a comparative in situ hybridization analysis. J Neurosci. 13 (3): 1258-1279.
39. Larsen PJ, Tang-Christensen M, Holst JJ and Orskov C (1997). Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience. 77 (1): 257-270.
40. Vrang N, Hansen M, Larsen PJ and Tang-Christensen M (2007). Characterization of brainstem preproglucagon projections to the paraventricular and dorsomedial hypothalamic nuclei. Brain Res. 1149: 118-126.
This article is essential because it describes GLP-1 projections in the brain which have been difficult to elucidate.
41. Jin SL, Han VK, Simmons JG, Towle AC, Lauder JM and Lund PK (1988). Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. J Comp Neurol. 271 (4): 519-532.
42. Hamilton A, Patterson S, Porter D, Gault VA and Holscher C (2011). Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. J Neurosci Res. 89 (4): 481-489.
43. Meeran K, O'Shea D, Edwards CM, Turton MD, Heath MM, Gunn I, Abusnana S, Rossi M, Small CJ, Goldstone AP, Taylor GM, Sunter D, Steere J, Choi SJ, Ghatei MA and Bloom SR (1999). Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. Endocrinology. 140 (1): 244-250.
44. Barrera JG, Jones KR, Herman JP, D'Alessio DA, Woods SC and Seeley RJ (2011). Hyperphagia and increased fat accumulation in two models of chronic CNS glucagon-like peptide-1 loss of function. J Neurosci. 31 (10): 3904-3913.
This article is essential because it shows that native central GLP-1 plays a critical role in food intake and body weight.
45. Perry T, Haughey NJ, Mattson MP, Egan JM and Greig NH (2002). Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. J Pharmacol Exp Ther. 302 (3): 881-888.
46. Gengler S, McClean PL, McCurtin R, Gault VA and Holscher C (2010). Val(8)GLP-1 rescues synaptic plasticity and reduces dense core plaques in APP/PS1 mice. Neurobiol Aging.
47. Kiss JP (2000). Role of nitric oxide in the regulation of monoaminergic neurotransmission. Brain Res Bull. 52 (6): 459-466.
48. Friedman JM (2009). OBESITY Causes and control of excess body fat. Nature. 459 (7245): 340-342.
49. Bogaert YE and Schrier RW (2011). Into the future: prevention of diabetes. Contrib Nephrol. 170: 256-263.

CANDIDATE
REVIEWS

Glucocorticoid Receptor Mediated Stress Signaling in the Prefrontal Cortex

Alonzo Whyte

Abstract

Stress reflects physiological or psychological displacement from homeostasis. In mammals, stressors activate the hypothalamic-pituitary-adrenal axis (HPA axis). HPA axis activation provides the nervous system with the required signals to respond to the stressor. In the brain, the response to HPA-axis activation is largely mediated by the glucocorticoid receptor (GR). GR orchestrates the transcriptional changes required for long term adaptation to the stressor in addition to ending the stress response via a negative-feedback circuit. This adaptation and feedback includes modulation of brain regions implicated in cognition and emotion. One of these brain regions is the prefrontal cortex (PFC). Acute and chronic stress are both known to affect PFC regulation of cognitive processes. Elucidating how the GR influences processing in the PFC is important for understanding the stress response; however, the mechanisms remain incompletely defined. This review presents current knowledge on the PFC and GRs as well as areas for future investigation into the PFC-GR interaction in regulation of cognition and emotion.

Keywords

Glucocorticoid receptor
Stress
Prefrontal cortex
Cognition
Emotion

The glucocorticoid stress response

The stress response. Stress reflects physiological or psychological displacement from homeostasis [1,2]. Encountering a "stressor" (either physical or psychological) can result in adaptive physiological changes known as the stress response. In mammals the stress response activates the hypothalamic-pituitary-adrenal (HPA) axis. The purpose of HPA axis activation is to maximize the energy resources needed to drive the response to the stressor. Neurons in the hypothalamus secrete corticotrophin releasing hormone (CRH) onto the anterior pituitary gland. CRH then binds to its receptor resulting in the release of adrenocorticotropin releasing hormone (ACTH) from the pituitary gland. In response to ACTH the adrenal cortex releases membrane permeable glucocorticoids (GCs) into the bloodstream. Once released from the adrenal gland GCs bind to either mineralocorticoid receptors (MRs) or glucocorticoid receptors (GRs) throughout the brain. Both MR and GR are transcription factors that normally reside in the cytosol. MRs have a high binding affinity for GCs and they are normally bound by the basal plasma GC levels making them less available for activation via stress induced rises in GC. However, GRs have a lower affinity for the ligand, and thus remain relatively unbound at basal GC levels. Since the MR's are already bound when GC levels rise, the stress response is believed to be primarily mediated by GRs[3,4]. GR is ubiquitously expressed; however, brain regions such as the PFC (see Fig. 1) contain a higher density of GR. Denser expression may be an indicator of the receptors importance in the PFC, particularly in the stress response.

The role of prefrontal cortex in the stress response

Prefrontal cortex function, anatomy, and homology. A large body of evidence implicates the PFC in the stress response. The PFC regulates cognitive and emotional processes by integrating past information from long term storage with current information before deciding on and initiating the optimal response via **top-down regulation**[a]. The ability to process past and present information has been coined "working memory"[6]. While the PFC is not the only brain region where cognitive functions are processed, a majority of human imaging studies indicate the PFC as the main site of working memory processing and decision making[7].

There is consensus that although the homologous regions in lower level primates are not as developed as those of humans, the type of information being processed is similar. For example, macaque monkeys trained to perform a modified version of the **Wisconsin Card Sorting Task**[b] (WCST) display PFC activation similar to that exhibited

a. **Top-down regulation**: Control of lower order process by higher order regions.

b. **Wisconson Card Sorting Task**: a classic rule based test of attention in which changes to the "rules" must be recognized by the participant. The participants score is determined by how long it takes them to learn the new rule.

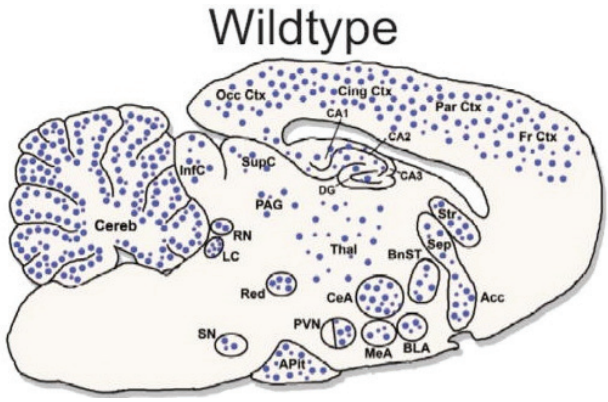

Figure 1. Regions of dense GR expression. Acc-nucleus accumbens; APit-anterior pituitary gland, BLA- basolateral nucleus of the amygdala; BnST- bed nucleus of the stria terminalis; CA1, CA2, CA3- hippocampal areas CA to CA3; InfC- inferior colliculus; LC- locus coeruleus; CeA- central nucleus of the amygdala; Cerb-cerebellum; Cing Ctx- cingulate cortex; DG- dentate gyrus; Fr Ctx- frontal cortex; PAG- periaqueductal gray; Par Ctx- parietal cortex; PVN- parventricular hypothalamic nucleus; Red- Red nucleus; Rn- raphe nuclei; Sep- septum; SupC- superior colliculus; SN- substantia nigra; Stri- striatum; Thal- thalamus. (Reproduced with permission[5])

in human controls[8]. For rodents however, the concept of a homologous PFC region has only recently begun to be accepted. By comparing the anatomical connectivity of the rodent and primate PFC areas, researchers have determined subdivisions in rodents that exhibit similar projection patterns[9]. Specifically, the granular medial portion of the rodent PFC has three distinct subregions which have the same connectivity as the primate PFC. The anterior cingulate cortex (ACC) is in the dorsal subdivision of the region and its projections are known to result in occulomotor movements. The ventral subdivision contains the prelimbic (PL) and infralimbic (IL) cortices which are implicated in cognitive and emotional regulation based on their connectivity with the amygdala, **mediodorsal thalamus**[c], **reunions nuclei**[d], and other **limbic system**[e] related structures[9,10]. While the ACC is considered part of the homologous PFC structure, this review will focus on the role of PL and IL in the stress response (each of which differentially regulate neuronal processes through their unique connectivity).

Early studies examined the functions of PL and IL by selectively lesioning one of the regions and examining the effects in stress-related behavioral assays. Lesions to PL diminish performance in tasks that involve delays (thus requiring functioning working memory) increase anxiety-like behaviors[11]. PL lesions do not however, affect performance on non-delayed tasks. Lesions to IL have the opposite effect on anxiety-related behaviors. Further distinctions between the two regions have been found using **fear conditioning**[f] **and extinction**[g] paradigms. Researchers found that inactivation of PL resulted in an inability for rats to express fear to stimuli that were previously paired with a shock[12]. However, PL inactivation did not diminish the response to innately feared stimuli. Further investigation revealed that IL inactivation impairs the ability to undergo extinction acquisition and develop an extinction memory[13]. IL has also been found to be important in the development of the stress resiliency that arises from environmental enrichment (EE)[14]. Lesions to IL prior to EE (although not after) prevented rats from developing the superior positive behavioral responses to chronic stress that were developed in control (but EE exposed) rats. Further differences between PL and IL have been demonstrated for autonomic responses. Under basal conditions, inactivation of neither PL nor IL produces cardiovascular changes[15]. However, when rats are administered restraint stress, rats with PL inactivation exhibit an elevated heart rate, while rats with IL inactivation exhibit a diminished response (compared to controls). It is possible that these PL- and IL- mediated stress-induced changes in behavior and physiology are regulated by GR activation.

The role of GR in the stress response

Activation of GR. When GC binds to GR the receptor undergoes a conformational change which results in its translocation to the nucleus. Once translocated, GR can affect the intracellular environment either as a dimer or a monomer. As a dimer it can bind the **glucocorticoid response element**[h] located on the promoter region of target genes leading to

c. **Mediodorsal thalamus**: Nucleus which plays a major role in relaying information from limbic regions to association cortices.

d. **Reunions nuclei**: Thalamic nucleus that relays signals from them PFC and the hippocampus

e. **Limbic system**: Network of brain regions that process and regulate cognition, memory and emotion -related stimuli

f. **Fear conditioning**: Learning paradigm in which a neutral stimulus is paired with a feared stimulus. Acquisition occurs when the neutral stimulus becomes feared.

g. **Fear extinction**: Learning paradigm in which the response a conditioned feared stimulus is learned to no longer be predictive of a noxious stimulus.

h. **Glucocorticoid response element**: region of a gene that activates its transcription when it is bound by GR.

transactivation[i] or **transrepression**[j][16,17]. As a monomer it can downregulate transcription via transrepression[18,19]. At numerous sites throughout the brain, binding of GCs to GRs facilitates adaptive changes to the stressor and restores the stress response to baseline[5] through negative-feedback on the HPA axis. Global and region specific manipulations of GR have been used to experimentally dissect the role of GR in the stress response.

Targeting GR in animal models. GR is encoded in the Nr3c1 gene. Of Nr3c1's 9 exons, exon 2 is the main transcriptional activation domain, and exons 3 and 4 are responsible for homodimerization and DNA binding[20]. Nr3c1 is ubiquitously expressed throughout the central and peripheral nervous system and early investigations revealed that prenatal global deletion of GR resulted in perinatal death as a result of GRs role in the periphery[21]. The first non-lethal deletion methods were developed in 1998[22]. One involved a point mutation in the DNA binding domain, and the other utilized the **cre-lox system**[k] and flanked Exon 3 with LoxP sites. In 2003, another method for non-lethal deletion of Nr3c1 was demonstrated[23]. In this model exon 2 of Nr3c1 was flanked with LoxP sites (Fig. 2). The cre-inducible methods allowed for local deletions dependent on where the cre enzyme was expressed. These cre-inducible methods of generating GR knockouts (GRKOs) along with several other methods have allowed for investigations into the function of the receptor.

Initial Studies on physiological effects GR activation. The initial animal models used to study the role of GR in the stress response involved gross expression of **antisense**[l] GR mRNA, gross expression of GR protein that lacked the DNA binding domain, or conditional knockouts (KO) using a region-specific promoter. Studies involving the antisense GR mRNA expression revealed depression-related cognitive deficits[24]. Mice with point mutations to Nr3c1 that prevented the formation of GR homodimers and DNA binding display diminished spatial memory capacity[25]. Using the cre inducible model (see Fig. 2), a forebrain GRKO mouse model was developed. Investigation of this model revealed a depression and despair phenotype[26, 27]. However, across most of these studies conflicting results concerning the interpretation of the anxiety-phenotype were reported with many of the mice exhibiting reduced anxiety-phenotypes in some behavior paradigms and heightened anxiety responses in others[3]. The confusion around the exact effect may result from the recently discovered fact that GR activation has different effects based on which region of the brain it is activated in and the conditions underlying the activation. Recent studies have begun to target GR in specific brain regions to elucidate its many roles.

Region specific GR studies. A majority of the region specific GR research has been performed in the hippocampus. Electrophysiological experiments have revealed that GR activation enhances **miniature excitatory postsynaptic currents**[m] (mEPSCs) in CA1 pyramidal neurons[28] for 2-4 hours post-administration. Increased amplitude of mEPSCs at the postsynaptic terminal of GR activated cells results in enhanced signaling, a correlate of **long term potentation**[n] (LTP). However GR also plays a role in decreasing the responsiveness of a cell. **Long term depression**[o] (LTD) in CA1 was found to be dependent on GR activation[29]. Under conditions of low synaptic input post-stress GR works to diminish the cell's responsiveness to incoming signals, as opposed to placing the synapse in a ready to receive state. How can GR accomplish both of these seemingly contradictory actions in CA1? **AMPA receptors**[p] (AMPARs) are mediators of both LTD and LTP. GR activation results in increased trafficking of AMPARs to the postsynaptic terminal[30]. If the synapse is receiving basal levels of input then this increase in AMPARs allows for increased synaptic efficacy. In addition to increasing surface AMPARs, GR activation decreases the threshold by which **NMDA receptors**[q] (NMDARs) initiate NMDAR dependent AMPAR endocytosis[31]. The decrease

i. **Transactivation**: biological process that results in increased rate the target genes expression

j. **Transprepression**: biological process that results in the decreased rate the target genes expression.

k. **Cre-lox system**: A molecular tool used to regulate gene transcription. In the presence of cre recombinase, DNA located between inserted lox P sites is excised.

l. **Antisense**: mRNA that contains the complementary sequence. The antisense mRNA binds the endogenous RNA, blocking translation.

m. **Miniature excitatory postsynaptic currents**: positively charged flow of ions in the absence of presynaptic depolarization.

n. **Long term potentiation**: Prolonged enhancement in signal transmission resulting for simultaneous stimulation of connected neurons

o. **Long term depression**: Prolonged reduction in signal transmission. It can be induced by numerous signal strengths dependent on the brain region the neurons are located in.

p. **AMPA receptors**: ionotropic glutamate receptor that allows cation intracellular influx. It is responsible for fast signaling at the synapse.

q. **NMDA receptors**: ionotropic glutamate receptor that allows cation intracellular influx. Its opening is also voltage dependent thus only signals above a threshold will activate the receptor.

in surface AMPARs is concomitant with LTD as the terminal is less responsive to incoming signals. Thus GR is able to generate a synaptic environment that optimizes either LTD or LTP depending on the nature of the incoming pre-synaptic signals. It is known that GC signaling leads to decreased viability of CA1 neurons[32]. With GR mediating both of these processes what would occur following a stressor if GR was not regulating the environment? Twenty-four hours following a traumatic brain injury, rats administered a GR antagonist show no loss of CA1 pyramidal neurons while control mice showed losses of ~30%[33]. Therefore GR is likely responsible for cell death in CA1 neurons following chronic stress. Thus, in the absence of synaptic input, GR activation bypasses LTD instead functioning to assist in the elimination of the inactive neuron.

GR research done in other limbic brain regions revealed that responses to GCs vary for each region[34]. Research into the effects of stress on **dentate gyrus**[r] (DG) pyramidal neurons revealed no change in calcium currents in response to 20 min GC exposure, while CA1 pyramidal neurons exhibited increased currents in response to the same stimulus[35]. GRs are present in high density in both the DG and CA1 indicating that it was not a difference in GR expression levels that caused the different responses in the DG and CA1. Rather, it was a difference in the stimulus induced expression of protein that resulted in the effect. While GR activation in CA1 neurons results in increased calcium channel Cav1.2 expression, in the DG GR does not upregulate transcription of that channel. In the basolateral amygdala (BLA), GR was found to enhance neuronal excitability over several hours, unlike the short term enhancement demonstrated in CA1[36]. In addition, under conditions of chronic stress, BLA GRs have the opposite effect on neuronal excitability. These physiologic responses to GR activation likely underlie behavioral responses. Kolber and colleagues found that deletion of CeA GRs results in decreased cFos expression and diminished fear conditioning[37]. The findings of GRs effects in these regions have important implications for its possible role in the PFC.

Known Role of GR in the PFC. Early investigations to elucidate the role of GR in the PFC revealed it to be a negative-feedback site. Dioro and colleagues found that lesions to the PFC result in elevated plasma GC levels in rats exposed to a 20 minute restraint stress[38]. Compared to the control group, the PFC lesioned rats exhibited a diminished ability to reduce the stress-induced rise in GC. A later study examined the effects of chronic stress on GR expression in the PFC. Rats were exposed to 4 weeks of chronic stress resulting in significant reductions in total GR mRNA expression compared to non-stressed controls[39]. Although overall mRNA was reduced, the researchers reported significant increases in nuclear GR and reductions in cytosolic GR.

Within the last decade, researchers have begun to examine the effects of GR activation on PFC structure, transcription, and function. Rats exposed to repeated restraint stress exhibit reduction in apical dendritic spine density, as well as apical dendritic length in PFC neurons[40,41]. Consistent with this finding, previous research has shown that chronic activation of GR (via **dexamethasone**[s]) results in behavioral dysfunction in working memory as well as atrophy and neuron loss in **layer II/III**[t] of PFC[42]. PFC neuronal expression of CRH mRNA has been shown to negatively correlate with chronic PFC GR activation[43]. Meng and colleagues were able to demonstrate a direct recruitment of the CRH promoter by GR and propose it as the mechanism by which activated GR reduces CRH mRNA expression. Similar to its actions in CA1, acute GR activation enhances the amplitude of NMDAR and AMPAR **excitatory postsynaptic currents**[u] in response to glutamate[44]. GR potentiation of those responses is responsible for the working memory enhancement associated with acute stress. However, chronic stress has the opposite effect on working memory. The working memory deficit in chronically stressed rats is correlated with diminished plasticity in hippocampal-PFC synapses, possibly through a similar NMDAR-AMPAR-GR regulated molecular signaling pathway[45].

Summary and future directions

The role of GR in the stress response has been heavily investigated. These studies have revealed that the long-lasting behavioral and cellular changes that result from GR activation are region specific[34]. It is possible that region specific GR mechanisms are responsible for proper regulation of each limbic system nucleus[13]. GR-mediated molecular pathways are currently being investigated and results demonstrate the involvement of a multitude of proteins including ERK1/2, MSK[46], and EGR1[47], which are which are not only regulated by classic **genomic**[v] GR activity, but also by rapid non-genomic GR mechanisms. For example, GR-

r. **Dentate gyrus**: Part of the hippocampal formation

s. **Dexamethasone**: a potent synthetic glucocorticoid receptor agonist

t. **Layer II/III**: largely responsible for intercortical signaling

u. **Excitatory postsynaptic currents**: influx of cations resulting from presynaptic depolarization.

v. **Genomic**: classical regulation through gene transcription (ie GR binding to GRE)

dependent **epigenetic**[w] modifications are being uncovered providing a mechanism by which GR can affect the cellular environment in a matter of minutes[46,48]. Ongoing research will determine how GR activation in the PFC can result in enhanced functioning, as well as how dysregulation of GR signaling leads to impairments in working memory such as those exhibited in many psychiatric illnesses associated with PFC dysfunction[49, 50].

References

1. Joels M and Baram TZ (2009). The neuro-symphony of stress. Nat. Rev. Neuro. 10: 459-466.
2. de Kloet ER, Joels M and Holsboer F (2005). Stress and the brain: From adaptation to disease. Nat. Rev. Neuro. 6: 463-475.
3. Kolber BJ, Wieczorek L and Muglia LJ (2008). HPA axis dysregulation and behavioral analysis of mouse mutants with altered GR or MR function. Stress. 11(5): 321-338.
4. Holsboer F and Ising, M (2010). Stress hormone regulation: Biological role and translation into therapy. Annu Rev Psychol. 61: 81-109.
5. Kolber BJ, (2009). Defining brain region-specific glucocorticoid action during stress by conditional gene disruption in mice. Brain Research 1293: 85-90.
6. Baddley A (2003). Working memory: Looking back and looking forward. Nat. Rev. Neuro. 4: 829-839.
7. Duncan J and Owen A (2000). Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends in Neuroscience. 23: 475-483.
8. Nakahara K, Hayashi T, Konishi S and Miyashita Y (2002). Functional MRI of macaque monkeys performing a cognitive set-shifting task. Science. 295: 1532-1536.
9. Vertes RP (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse. 51:32-58.
10. Vertes RP (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience. 142:1-20.
11. Jinks AL and McGregor IS (1997). Modulation of anxiety-related behaviors following lesions of the prelimbic and infralimbic cortex in the rat. Brain Research. 772: 181-190.
12. Corcoran KA and Quirk GJ (2007). Activity in prelimbic cortex is necessary for the expression of learned but not innate fears. J. Neurosci. 27(4): 840-844.
This paper shows a distinct role for the PFC in fear learning. In addition, the researchers distinguish between the expressions of different types of fear.
13. Sierra-Mercado D, Padilla-Coreano N and Quirk GJ (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology. 36: 529-538.
14. Lehmann ML and Herkenham M (2011). Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. J Neurosci. 31(16): 6159-6173.
15. Tavares RF, Correa FMA and Resstel LBM. (2009). Opposite role of infralimbic and prelimbic cortex in the tachycardiac response evoked by acute restraint stress in rats. Journal of Neuroscience Research. 87: 2601-2607.
16. Dahlman-Wright K., Wright A, Gustafsson J and Carlstedt-Duke J (1991). Interaction of the glucocorticoid receptor DNA-binding domain with DNA as a dimer is mediated by a short segment of five amino acids. J Bio Chem. 266(5): 3107-3112.
17. Schoneveld OJLM, Gaemers IC and Lamers WH (2004). Mechanisms of glucocorticoid signaling. Biochimica et Biphysica Acta. 1680: 114-128.
18. Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schutz G (1998). DNA binding of the glucocorticoid receptor is not essential for survival. Cell. 93: 531-541.
19. Tuckermann JP, Reichardt HM, Arribas R, Richterm KH, Schutz, G and Angel P (1999). The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1 dependent genes in skin. J Cell Bio. 147(7): 1365-1370.
20. Mittelstadt PR and Ashwell JD (2003). Disruption of glucocorticoid receptor exon 2 yields a ligand-responsive C-terminal fragment that regulates gene expression. Mol Endocr. 17(8): 1534-1542.
21. Cole TJ, Blendy JA, Monaghan A, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K and Schutz G (1995). Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes & Development. 9: 1608-1621.
22. Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R and Schutz, G. (1998). Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nature Genetic. 23, 99-103.
23. Brewer JA, Khor B, Vogt SK, Muglia LM, Fujiwara H, Haegle KE, Sleckman BP and Muglia LJ (2003) T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. Nature Medicine. 9(10): 1318-1322
24. Montkowski A, Barden N, Wotjak C, Stec I, Ganster J, Meaney M, Engelmann M, Reul JMHM, Landgraf R, Holsboer F (1995). Long-term antidepressant treatment reduces behavioral deficits in transgenic mice with impaired glucocorticoid receptor function. Journal of Neuroendocrinology. 7: 841-845.
25. Oitzl M, Reichardt H, Joels M and de Kloet ER (2001). Point mutation in the mouse glucocorticoid receptor preventing DNA binding impairs spatial memory. Proc. Natl. Acad. Sci. 98(22): 12790-12795.
26. Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y and Muglia LJ (2004). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proc. Natl. Acad. Sci. 102(2): 473-478.
27. Boyle MP, Kolber BJ, Vogt SK, Wozniak DF and Muglia LJ (2006). Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. J. Neurosci. 27(7): 1971-1978.
28. Karst H and Joels M (2005) Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal neurons. J Neurophysiol. 94: 3479-3486.
29. Xu L, Holscher C, Anwyl R and Rowan MJ (1998). Glucocorticoid receptor and protein/RNA synthesis-dependent mechanisms underlie the control of synaptic plasticity by stress. Proc. Natl. Acad. Sci. 92: 3204-3208.
30. Martin S, Henley JM, Holman D, Zhou M, Wiegert O, van

w. **Epigenetic**: alterations in gene expression without modifying the genes DNA sequence.

Spronsen M, Joels M, Hoogenraad CC and Krugers HJ (2008). Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity. PLoS One. 4(3): e4714.

31. Coussens CM, Kerr DS and Abraham WL (1997). Glucocorticoid receptor activation lowers the threshold for NMDA-receptor-dependent homosynaptic long-term depression in the hippocampus through activation of voltage-dependent calcium channels. J Neurophys. 78: 1-9.

32. Sapolsky RM, Packan DR and Vale WW (1988). Glucocorticoid toxicity in the hippocampus: in vitro demonstration. Brain Research. 453: 367-371.

33. McCullers DL, Sullivan PG, Scheff SW and Herman JP (2002). Mifepristone protects CA1 hippocampal neurons following traumatic brain injury in rat. Neuroscience. 109(2): 219-230.

34. Joels M (2011). Impact of glucocorticoids on brain function: Relevance for mood disorders. Psychoneuroendocrinology. 36: 406-414.

35. van Gemert NG, Carvalho DM, Karst H, van der Laan S, Zhang M, Meijer OC, Hell JW and Joels M (2003). Dissociation between rat hippocampal CA1 and dentate gyrus cells in their response to corticosterone: Effects on calcium channel protein and current. Endocrinology. 150(10): 4615-4624.

36. Karst H, Berger S, Erdmann G, Schutz G and Joels M (2010). Metaplasticity of amygdalar responses to the stress hormone corticosterone. Proc. Natl. Acad. Sci. Early Edition.
This paper demonstrates how GR can regulate the neuronal environment dependent on the recent stress history. In addition, the researches explain how GR effects are unique for each nucleus based on the required function of the region.

37. Kolber BJ, Roberts MS, Howell MP, Wozniak DF, Sands MS, Muglia LJ (2008). Central amgydala glucocorticoid receptor action promotes fear associated CRH activation and conditioning. Proc. Natl. Acad. Sci. 105 (33), 12004-12009.
This paper is important as the methodological basis for nuclei specific GR targeting. Also, significant effects of GR deletion in a single nucleus are demonstrated.

38. Diorio D, Viau V and Meaney MJ (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of the hypothalamic-pituitary-adrenal responses to stress.J Neurosci. 13(9): 3839-3847.

39. Mizoguchi K, Ishige A, Aburada M and Tabira T (2003). Chronic stress attenuates glucocorticoid negative feedback: Involvement of the prefrontal cortex and hippocampus. Neuroscience. 119, 887-897.

40. Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR, McEwen BS and Morrison JH (2006). Repeated stress induces dendritic spine loss in rat medial prefrontal cortex. Cerebral Cortex. 16: 313-320.

41. Cerqueira JJ, Taipa R, Uylings HB, Almeida OF and Sousa N (2007). Specific configuration of dendritic degeneration in pyramidal neurons of the medial prefrontal cortex induced by differing corticosteroid regimens. Cerebral Cortex. 17: 1998-2006.

42. Cerqueira JJ, Pego JM, Taipa R, Bessa JM, Almeida OF and Sousa N (2005). Morphological correlates of corticosteroid-induced changes in prefrontal cortex dependent behaviors. J Neurosci. 25(34): 7792-7800.

43. Meng Q, Chen X, Tong D, Zhou J (2011). Stress and glucocorticoids regulated corticotrophin releasing factor in rat prefrontal cortex. Molecular and Cellular Endocrinology. Article in press.

44. Yuen EY, Liu W, Karatsoreos IN, Feng J, McEwen BS, Yan Z (2009). Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory. Proc. Natl. Acad. Sci. 106(33):14075-14079.

45. Cerqueira JJ, Mailliet F, Almeida OF, Jay TM and Sousa N (2007). The prefrontal cortex as a key target of the maladaptive response to stress. J Neurosci. 27(11): 2781-2787.

46. Gutierrez-Mecinas M, Trollope AF, Collins A, Morfett H, Hesketh SA, Kersante F and Reul JM (2011). Long lasting behavioral responses to stress involve a direct interaction of glucocorticoid receptor with ERK1/2-MSK1-Elk-1 signaling. Proc. Natl. Acad. Sci. Early Edition.
Reveals an interaction between GR and the ERK 1/2 signaling pathway, which is relevant for behavior and memory. The researchers also demonstrate that the interaction occurs on the order of several minutes indicating a nongenomic mechanism.

47. Revest J, Blasi FD, Kitchener P, Rouge-Pont F, Desmedt A, Turiault M, Tronche F and Piazza PV (2005). The MAPK pathway and Egr-1 mediate stress-related behavioral effects of glucocorticoids. Nat Neuro. 8(5): 664-672.

48. Mifsud KR, Gutierrez-Mecinas M, Trollope AF, Collins A, Saunderson EA and Reul JM (2011). Epigentic mechanisms in stress and adaptation. Brain, Behavior, and Immunity. Article in press.

49. Rauch SL, Shin LM, Segal E, Pitman RK, Carson MA, McMullin K, Whalen PJ and Makris N (2003). Selectively reduced regional cortical volumes in post-traumatic stress disorder. Neuroreport. 14(7): 913-916.

50. Drevets WC, Price JL, Simpson JR, Todd RD, Reich T, Vannier M and Raichle ME (1997). Subgenual prefrontal cortex abnormalities in mood disorders. Nature. 386: 824-827.