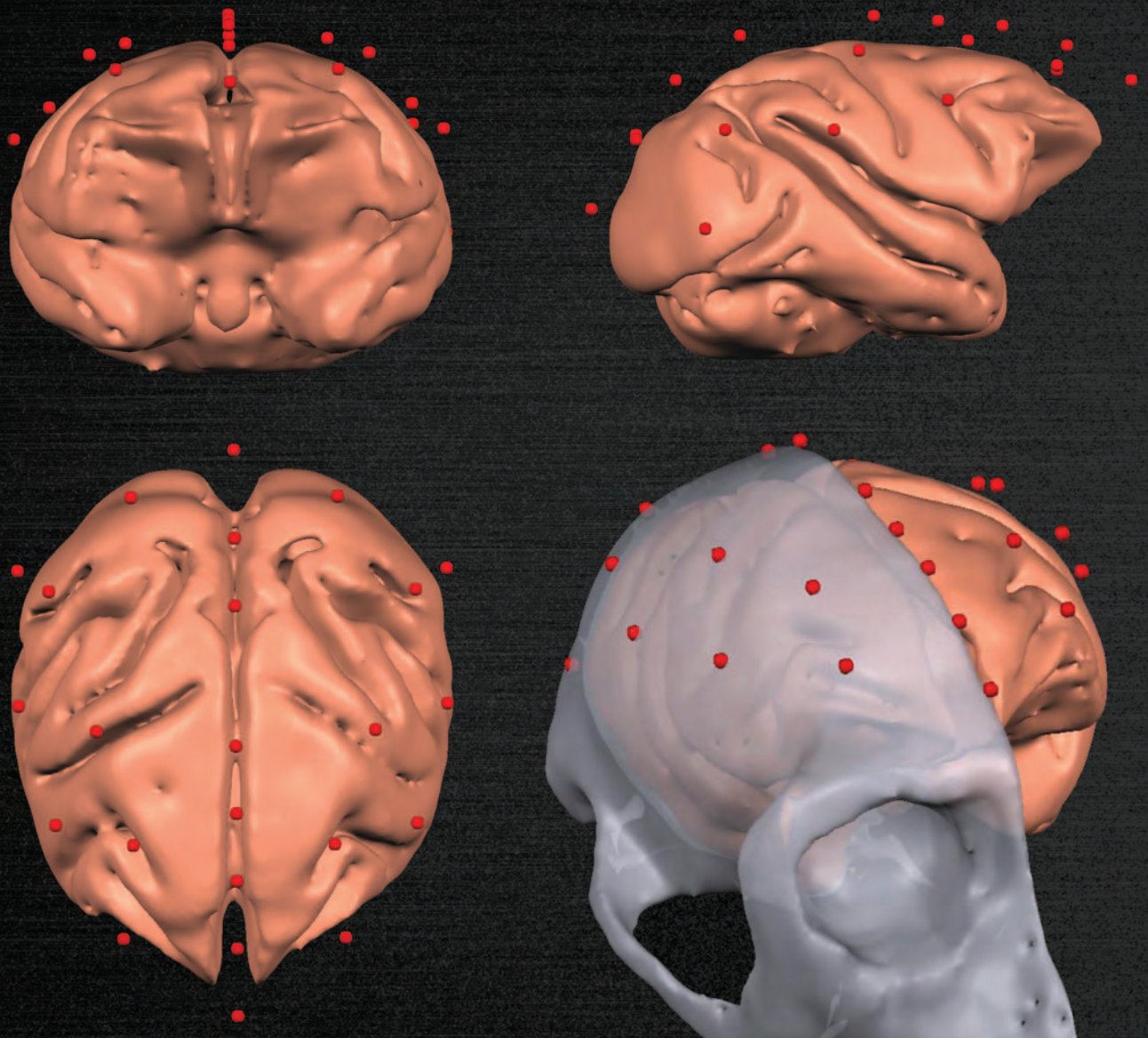
VANDERBILT REVIEWS | NEUROSCIENCE

VRN

Vanderbilt Reviews Neuroscience



Volume 2 | 2010

Vanderbilt Brain Institute

VANDERBILT REVIEWS | NEUROSCIENCE Volume 2 | 2010

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CONTENTS

VRN starts trouble and gets a makeover at 1-year-old

The release of the inaugural volume of *Vanderbilt Reviews Neuroscience* was widely hailed by the Vanderbilt University Neuroscience community as a success—in some cases surprising people in regards to the quality of the work presented, and how the journal was constructed. I was personally quite pleased with the results and, for the most part, how it was received.

Its publication did, however, raise some interesting concerns within the community that—as with any new venture—were difficult to predict. One of the first concerns was about the role of dissertation advisors in the authorship and publication of the doctoral qualifying review articles. It has long been the policy of the Neuroscience Graduate Program that the Phase I qualifying review was to be written by the student alone—with no input from the advisor. However, when I was first reformatting these reviews for the journal, I not only included the advisor's name on publication, but also made the advisor the corresponding author. I didn't think much of this arrangement, since it is somewhat conventional, but then I received an email from a student questioning why his advisor was the corresponding author and not him. At first, I made the mistake of feeling that this concern was trivial, but it turns out that this student was not alone. I discussed the issue briefly with Mark Wallace, and decided to make students the contact for the review while leaving the advisor as the final author.

This move sparked some debate among the faculty on two fronts: first, if the students were expected to write the entire review by themselves without any input from their mentors, should the advisors be listed as authors at all? Second, is this policy of faculty non-interference even good for the program and its trainees? To the former concerns, *VRN* has changed its policy on authorship: the reader will find that research advisors are not listed as authors in this volume. While this reflects the situation surrounding most of the reviews, I am aware of some that were indeed edited by the trainee's advisor after s/he passed for the purposes of publication in this journal. I feel that this new policy will need to be updated as VBI policy changes. To the latter concern, the Vanderbilt Brain Institute and the committees advising it on the Neuroscience Graduate Program have begun to reconsider the policy of non-interference. Since I was on the Curriculum Committee in 2006, I've been trying to argue to the powers-that-be that the whole point of this review is to make students better writers. Of course, the advisor can't be allowed to write the review for the student, but how is the trainee supposed to learn good writing if those that know how to write effectively can't critique it? It is the view of the *VRN* editorial board that this policy needs to be changed for the good of the program and its students.

A second major criticism of the journal was its policy on publication. Upon founding the journal, we toyed with the idea of actually registering and indexing this journal and its contents so that it is searchable on PubMed and the like in order to instill enthusiasm among the students. This idea was met with heavy criticism almost immediately. As I said at the time, I would hope that since the details on the project have still not been worked out—even among those involved—that nobody would have a strong opinion one way or another. That's rather shooting first and asking questions later—a methodology to which I do not ascribe.

A third major issue arising from the publication of the VRN was a concern for actual control of the journal. While it was not my intention at all, I was the sole editor of the 2009 volume. Understandably, more eyes on a publication are preferable. This year, two associate editors have signed-on (Mariam Eapen and Caleb Doll) to train for the top spot. However, the concerns were for a lack of faculty intervention in the publication process. The formation of a Faculty Review Board has been suggested by one of the neuroscience committees to augment the work and decisions being made by the editorial board. While I do not object to this idea, it must be clear that this journal is to be run primarily by the trainees, and the Editor-in-Chief must always be a trainee for the following reasons: 1. faculty have a tendency to make good ideas "requirements" of the program (i.e. forum, foundations, etc.) and make already established requirements more difficult; 2. as with most people, faculty tend to care more about their own work than work done for what they may view as a publication of little consequence. In contrast, the trainees will always look to lighten the load for fellow students in a qualifying process that is (whether the Program likes to admit it or not) already four grueling parts. The trainees will also care more about this journal than the faculty because it is for many their first real publication. This care and dedication to student and journal are what will keep the publication fresh and anything but "of little consequence."

Some changes the reader may find this year are the inclusion of reviews from various other, unpublished qualifying classes (2004-07) in addition to the entire 2009 qualifying class. This initiative was undertaken to recognize the best of what *VRN* missed in the past. Furthermore, the *VRN* website (http://vrn.vanderbilt.edu) is finally up-and-running thanks to the generous efforts of Aaron Nidiffer, who also designed Mark Wallace's lab website and redesigned the VBI site, which was sorely needed. Mr. Nidiffer also designed the logo that graces the cover of this volume.

On a personal note, I will be stepping down as Editor-in-Chief with the publication of this volume. While I plan to continue contributing to this endeavor, Mariam Eapen and Caleb Doll will take the *VRN* reins and keep it fresh in the years to come.

C. M. Ciarleglio, Ph.D. `09
Editor-in-Chief
c.ciarleglio@vanderbilt.edu

Vanderbilt Brain Institute
U1205 Medical Center North
Nashville, TN 37232
(615) 936-3736
vrn@vanderbilt.edu

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

What to Expect

In this volume of *Vanderbilt Reviews Neuroscience*, the qualifying class of 2009 reviews the literature on topics from the molecular underpinnings of schizophrenia to a characterization of $GABA_A$ receptors; from the use of *C. elegans* as a model system for psychoactive drugs to the use of crocodilians in the study of mechanoreception. For its second volume ever, *VRN* 2010 stands to be just as strong an issue as last year, and demonstrate the breadth and depth of the Graduate Program as it spans the molecular to the mind.

As can be expected from a strong program, many high-impact papers were published in late 2009 and early 2010. These are highlighted by our exceptional team of writers in the popular "Research Highlights" section. For the second year in a row, Cohen *et al*. offered an exceptional study in which the team of researchers compared the efficacy of EEG to other, more invasive electrophysiological techniques (p. 5); Treadway *et al*. demonstrated a role for motivation in rewarding tasks (p. 9); Wu *et al.* published a study in *Nature Neuroscience* which identified a group of glial cell precursors that serve as developmental trash-collectors through the expression of two receptors (p. 7).

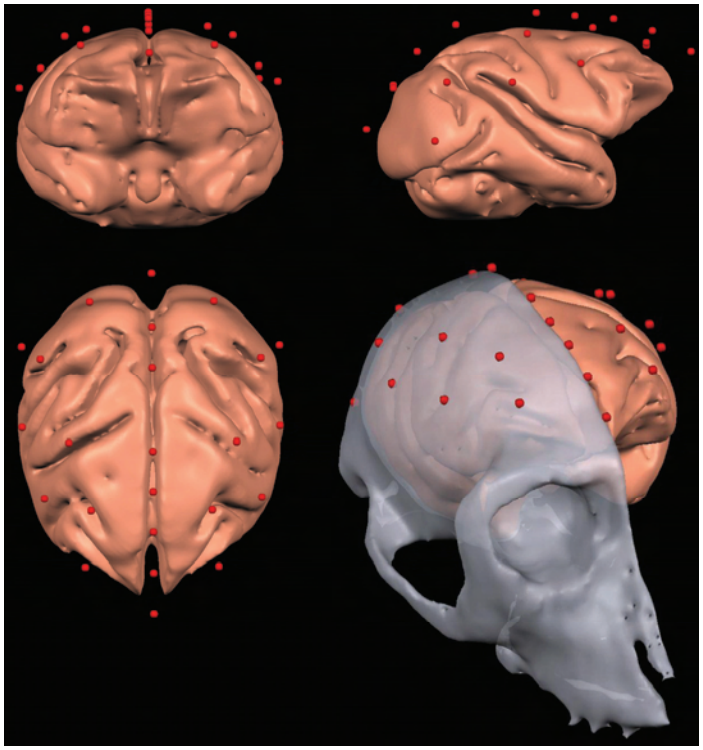
Neurotoxicity was a big topic at Vanderbilt University this past year, as was the ever-popular dopamine. Williams *et al*. demonstrated that mutant huntingtin, the notorious protein responsible for Huntington's Disease, may actually provide a neuroprotective effect against manganese toxicity (p. 8). In the same vein, Stanwood *et al*. showed that manganese exposure may change the cytoarchitecture of dopamine neurons in the substantia nigra, possibly contributing to Parkinsonian symptoms (p. 8). On the systems side, multisensory integration was big (p. 6), as was novelty, detection and recognition (pp. 7-8).

Peruse this volume at your leisure. The Contents (p. 1) should be clear and easy to follow. As always, feel free to contact us with suggestions; we're always up for making this journal better…

C. M. Ciarleglio
C. A. Doll
M. Eapen

ON THE COVER…

Anatomical reconstruction of a rhesus macaque skull and brain based on a structural magnetic resonance image. Red dots indicate stereotaxic locations of electrodes implanted on the surface of the skull beneath the scalp and muscle tissue to measure the electroencephalogram (EEG) . The goal of this research is to compare directly human and monkey EEG and event-related potentials (Woodman et al. 2007). This surgical method allows accurate comparisons between EEGs by ensuring that the underlying electrical signals propagate through similar layers of tissue and bone in both species. The electrode locations mimic the international 10-20 electrode coordinate system used in human EEG research (Jasper, 1958). First, a template of the adapted macaque 10-20 system was created by measuring electrode distances on a model skull. Second, stereotaxic positions of each proposed electrode location were recorded with accuracy in tens of millimeters, along with the locations of several anatomical landmarks. Third, during surgical implantation, the same anatomical landmarks were located allowing the electrode template to be rescaled to fit the size of the subjects skull. Finally, the anatomical landmarks measured during the procedure were located in a MRI taken prior to the operation, and used to guide virtual placement of electrodes based on surgical records. The voltages recorded from these electrodes while monkeys perform cognitively demanding tasks will allow researchers in Jeffrey Schall and Geoff Woodman's laboratories to construct scalp surface potential maps and perform dipole source localization necessary to understand how intracranial measures recorded from monkeys relate to extracranial measures recorded from humans.



-David Godlove

A Searching Hierarchy

"The advantage of animal models is the ability to invasively explore the underlying biology and physiology of disease and behavior that are, for obvious ethical reasons, otherwise prohibitive in humans."

Electroencephalography (EEG) is one of the most popular functional imaging techniques in psychology, neurology, and neuroscience. Compared to methods such as magnetic resonance imaging (MRI) and positron emission tomography (PET), EEG has unparalleled advantages in temporal resolution and cost. The blaring weakness of EEG is the inverse problem which leads to a deficiency in spatial resolution. Cohen and colleagues tackle this impasse in their recent *Journal of Neurophysiology* report. The researchers combined measurements of intracranial single-neuron spikes, local field potentials (LFPs), and event-related potentials (ERPs) from the macaque frontal eye field (FEF) to investigate the source of the human N2pc, an attention-related ERP component.

A visual search task was employed where the monkeys made a saccade to a target (**L** or **T**) among a group of 1, 3, or 7 distractors (**T** or **L**). The target selection time was measured by three spatially distinct electrophysiological techniques; microelectrodes recorded single-neuron spikes and LFPs in the FEF while skull electrodes recorded m-N2pc ERP components over extrastriate visual cortex, hypothesized to be the macaque homologue to the human N2pc. Selection times were consistently faster in single-neuron spikes than LFPs, and LFP selection times were consistently faster than m-N2pc selection times. The selection times in all three techniques increased in parallel with the number of distractors, which is consistent with increased saccade response times. In a majority of test pairs there was a trial-by-trial correlation between the amplitude of FEF LFPs and the amplitude of extrastriate ERPs. These results supported the authors' hypothesis that "feedback from FEF contributes to the generation of the m-N2pc component."

There is often a gap between scientific studies performed in humans and those performed in animal models. The advantage of animal models is the ability to invasively explore the underlying biology and physiology of disease and behavior that are, for obvious ethical reasons, otherwise prohibitive in humans. Here, Cohen *et al.* used two invasive electrophysiological techniques (single-neuron spike recording and LFP) commonly used in animal studies in parallel with one noninvasive electrophysiological technique (ERP) that is frequently used in human studies. These techniques span the spatial scale and together provide a better understanding of the origin of monkey selective visual attention that can in turn be directly utilized in human research. The combination of methodologies provides exceptional temporal and spatial resolution to overcome the limited spatial resolution inherent to EEG scalp recordings. In doing so, the authors succeed in bridging the gap between human and nonhuman primate electro-physiology. One expects to see more investigators employing similar multimodal approaches to further our understanding of the neural bases of noninvasive technologies.

Original Research Article:
JY Cohen, RP Heitz, JD Schall and GF Woodman (2009). On the origin of event-related potentials indexing covert attentional selection during visual search. *J Neurophysiol.* **102** (4): 2375-2386.

Stress, Plasticity and Abuse

The bed nucleus of the stria terminalis (BNST), a key nucleus within the "extended amygdala", is anatomically poised in both the reward and stress circuitry. Given this unique position, the BNST has become a focal point of studies interested in exploring the link between anxiety and addiction. In this nucleus, long-term depression (LTD) plays a critical role in monitoring excitatory influence over circuits which impact cognition and emotional behavior. Previous evidence suggested that both noradrenergic and glutamatergic initiated G_q-coupled signaling in the BNST converge on a final common mechanism for LTD maintenance. McElligott *et al.* provide evidence to the contrary. While norepinephrine induced LTD via α1-adrenergic receptors (α1-AR) occurs in the same neurons as glutamate-induced LTD (mGluR5-LTD), α1-AR mediated LTD transpires exclusively through the downregulation of calcium permeable AMPA receptors. mGluR5-dependent LTD, however, does not involve the regulation of calcium permeable AMPA receptors. Importantly, these differences are emphasized by the fact that the persistence of these two distinct forms of LTD can be impacted differentially by environmental challenges. For example, α1-AR dependent LTD is disrupted by stress and is resistant to disruption by cocaine. While, on the other hand, mGluR5 dependent LTD is susceptible to cocaine-induced disruptions but is resilient to stress challenges. The authors extend these findings by further demonstrating that the α1-AR dependent LTD can also be diminished by chronic ethanol exposure. Together, these data suggest that in the BNST noradrenergic- and glutamate- activated G_q-coupled initiated signaling is delicately tuned to specific stimuli. Thus, these distinct pathways allow for fine differential control of glutamate synapse efficacy in response to stress.

Original Research Article:
ZA McElligott, JR Klug, **WP Nobis**, S Patel, BA Grueter, TL Kash and DG Winder (2010). Distinct forms of G_q-receptor-dependent plasticity of excitatory transmission in the BNST are differentially affected by stress. *PNAS.* **107** (5): 2271-2276.

A tale of two senses—a look at the confluence and malleability of multisensory stimuli

"…perceptual training paradigms can create lasting changes in a person's judgment of perceived simultaneity between visual and auditory events."

In our daily activities, whether it is driving down the road, playing a sport or enjoying a meal at our favorite restaurant, the brain is constantly inundated with multisensory information that it shrewdly processes to comprehend every sensory experience. Just how information from the multiple senses is integrated in the brain to produce a cohesive perceptual experience is intriguing. In particular, the temporal dynamics of the individual sensory information has to uniquely fall within a certain binding window in order for the brain to perceive it as a stimulus from the same environmental event. A better understanding of how binding of cross-modal cues takes place in relation to this temporal window is imperative, especially in the clinical realm where an enlargement of this window has been reported for various neurobiological disorders like dyslexia and autism. Research scientists at Vanderbilt University recently investigated the temporal characteristic and malleability of this multisensory binding window by using an auditory and visual stimulus pair in two perceptual training paradigms.

Powers *et al.* first tested normal adults on a 2-alternative forced choice (2-AFC) paradigm where participants had to make a simultaneity judgment about whether the occurrence of a visual and auditory stimulus was "simultaneous" or "non-simultaneous". This assessment phase was followed by a simultaneity judgment training phase where the participants were given feedback about whether their judgment was correct. A subset of participants was requested to return one week later for another follow up assessment phase. The authors were successfully able to use this 2-AFC paradigm to define and characterize a multisensory temporal binding window profile. Moreover, they found that the training received during the 2-AFC task actually narrowed this multisensory binding window suggesting the flexibility of neural processes to adapt to feedback during training. The follow up assessment conducted one week after the training resulted in the maintenance of this temporal binding window suggesting the stability of brain processes to maintain a learned perceptual experience. However, the modifiable nature of this temporal binding window was not observed for passive exposure to identical stimuli that did not require a simultaneity judgment with feedback.

While these results showed an interesting and unique finding, one question that still remained unanswered was whether the flexible nature of this temporal binding window was a result of inherent cognitive biases. To further explore the nature of this multisensory temporal binding window and rule out the possibility that participants had a cognitive bias towards one particular multisensory task, Powers and colleagues ran a second experiment, a two-interval forced choice(2-IFC) perceptual paradigm. This paradigm was similar to the 2-AFC in that they used exactly the same stimuli, however, the participants were presented with two visual-auditory pairs, one that was simultaneously presented and the other that was not simultaneously presented. Participants were instructed to indicate which of the two presentations contained the simultaneous multisensory stimulus. This assessment session was then followed by a training phase where the participants were given feedback as to the accuracy of their responses after each trial. A subset of these participants also returned one week later to do a similar simultaneity judgment assessment.

The authors found similar results to those seen in the 2-AFC. The perceptual training in the 2-IFC training task resulted in a significant narrowing of the multisensory temporal binding window. Moreover, these modified binding widow changes seemed to remain stable even after the one week follow-up assessment. Powers and colleagues keenly observed that there was no significant difference in the degree and time course of window narrowing across the two different paradigms suggesting that the neural mechanisms underlying the malleability of this binding window is similar across different experimental contingencies.

So what does this tell us about human multisensory perceptual experiences? This study has successfully shown that perceptual training paradigms can create lasting changes in a person's judgment of perceived simultaneity between visual and auditory events. More notably, the perceptual discrimination abilities prompted by training is not a result of simple exposure to passive stimuli, but the result of feedback on the accuracy of the simultaneity judgment. Seminal studies have shown significant reorganization of cortical space when a constrained set of stimuli were used early in development. However, a passive exposure to the same set of stimuli did not produce any notable behavioral

IN BRIEF…

BAC-driven miRNA gene expression knockdown

KA Garbett, S Horvath, PJ Ebert, MJ Schmidt, K Lwin, **A Mitchell**, P Levitt and K Mirnics (2010). Novel animal models for studying complex brain disorders: BAC-driven *mi*RNA-mediated *in vivo* silencing of gene expression. *Mol. Psychiatry*. doi: 10.1038/mp.2010.1

Animal models of disease represent one of the most powerful methods of analyzing the pathophysiological mechanisms of genetic disorders. However, the development of such models is often time-consuming, complex, and carries nonspecific caveats, such as the imprecise deletion of a gene of choice. Using bacterial artificial chromosomes, cell-type specific promoters, a standard reporter, and a microRNA mechanism for gene silencing, Garbett *et al*. present a powerful mechanism to specifically reduce gene expression *in vivo*. As microRNAs are of small size, they anticipate that this new method could simultaneously silence multiple genes in a cell-type specific manner. Accordingly, these transgenic mice would allow exquisite precision in determining the effects of a given set of genes on the presentation of disease.

Pain Pathways: Neuropeptide Y may be targeted to relieve pain

RG Wiley, **LL Lemons** and RH Kline IV (2009). Neuropeptide Y receptor-expressing dorsal horn neurons: role in nocifensive reflex responses to heat and formalin. *Neuroscience*. **161** (1): 139-147.

There is an endless list of reasons why individuals seek treatment for pain, but the molecular mechanisms that underlie pain perception are unclear. Wiley *et al*. demonstrate how Neuropeptide Y (NPY) and its receptor Y1 (Y1R) function in the rodent spinal cord to mediate nociception. After intrathecal injection of saporin toxin conjugated to NPY to selectively kill Y1R-expressing neurons in the dorsal horn of the spinal cord, rats displayed an increased latency to withdraw their paws from noxious hot stimuli. The rodents also had a significant decrease in nocifensive behavior when presented with the hot stimuli or when injected with formalin in the plantar region of the hind paw, as measured by licking and guarding events. This toxin-based approach allows researchers to selectively examine groups of neurons involved in the perception of pain and tease apart each group's contribution. These studies could prove to have a significant impact on the field of pain research and may provide researchers with some insight into alternative approaches to treat pain.

What's unusual about that? Neural substrates for the detection of novel, unusual stimuli

Blackford JU, Buckholtz JW, **Avery SN**, Zald DH (2010). A unique role for the human amygdala in novelty detection. *Neuroimage*. 50(3):1188-93.

Novelty detection is an important trait in perceiving and responding to our environment. In particular novel, yet unusual or uncommon stimuli that are behaviorally salient can engage specific neural mechanisms involved in emotional learning and memory. In this study, the authors used functional magnetic resonance imaging to observe blood oxygen level dependent (BOLD) responses in the human amygdala and hippocampus when they presented participants with novel, common stimuli (e.g., chair, clock, tree) versus novel, unusual stimuli (e.g., Prague Dancing House, futuristic skyscraper, leafy sea dragon). Blackford and colleagues found that novel, common stimuli showed robust BOLD activation in both the amygdala and the hippocampus. However, only the amygdala showed a preferential activation for the novel, unusual stimuli, compared to the novel common stimuli. These results lead the authors to speculate that within the novelty detection circuit, the amygdala plays a distinct role in uniquely responding to a specific category of stimuli, namely those that are novel and unusual.

change or neural reorganization. Powers and colleagues found significant effects in the temporal dynamics of the multisensory binding window after only one day of training, with stable effects observed even after one week. This alludes to the fact that the brain is capable of adapting quickly to new environmental situations and long term memory consolidation may play a role in strengthening learned traits. Thus results from this study would support the idea that the pairing of a sensory stimulus with behavioral salience (like feedback in this study) is crucial for sensory reorganization of adult cortical space.

It is intriguing to consider where and how the plasticity associated with this temporal binding window is modulated. In humans, recent neuroimaging studies reported a large, dynamic network of areas including the insular cortex, posterior parietal and superior temporal cortices, all critically involved in the perception of audiovisual stimuli. The neuronal oscillations among different cortical populations have also been shown to play a potential role in multisensory processing and temporal binding. At a more cellular level, the temporal tuning profile of multisensory neurons has been associated with adult plasticity in various sensory systems, with basal cholinergic signals acting as an instructive cue. This indicates that the synchronous role of both cortical and subcortical mechanisms may be responsible for temporal plasticity in multisensory systems.

The present work by Powers and colleagues and future extensions of similar studies holds particular clinical significance, especially in designing tailored intervention strategies for disorders such as dyslexia, autism and schizophrenia where altered multisensory temporal processes have been observed.

Original Research Article:
AR Powers III, AR Hillock and MT Wallace (2009). Perceptual training narrows the temporal window of multisensory binding. *J Neurosci*. **29** (39): 12265-12274.

Development: birth, life, death, cleanup, repeat.

The development of a nervous system entails several obvious processes such as the proliferation of cells, the elaboration of dendrites, or the wiring of functional axonal circuits, yet it is now becoming clear that the less publicized (and slightly more sinister) mechanisms of programmed cell death and debris clearance are a vital component of nervous growth. For example, in the mouse dorsal root ganglia (DRG) over 50% of sensory neurons undergo

IN BRIEF…

Manganese, a friend or foe to dopaminergic neurons?

GD Stanwood, **DB Leitch**, V Savchenko, J Wu, VA Fitsanakis, DJ Anderson, **JN Stankowski**, M Aschner, B McLaughlin (2009). Manganese exposure is cytotoxic and alters dopaminergic and GABAergic neurons within the basal ganglia. *J Neurochem*. **110** (1): 378-89.

Manganese is a naturally occurring element essential for the proper metabolism of amino acids, protein and lipids. It is crucial for maintaining proper cellular functioning including maintenance of redox states, facilitating protein conformation, modulating ion and energy homeostasis and overall signal transduction in neurons. Over exposure to manganese can result in various irreversible neurological phenomenon such as motor symptoms similar to that of Parkinsons, dystonia and gastrointestinal tract dysfunction. In this study, the authors explored the neurotxic potential of manganese in dopaminergic neurons *in vivo* in mice, looking especially at the vulnerability of nigrostrial pathways. They found that manganese chloride exposure, even at subtoxic doses changed the neuronal cytoskeleton of dopaminergic neurons. When the manganese treatment was extended to a period of 30days, a 20% reduction in TH-posiive neurons was observed in the substantia nigra pars compacta (SNpc), quantified by a widespread reduction in SNpc cell numbers through Nissil body staining. Parts of the basal ganglia including the striatum were also affected during treatment, showing sensitivity to nigrostriatal pathways. This study provides chronicles the timely repercussions of acute and chronic manganese exposure and provides an explanation for the motor manifestations observed from manganese intoxification.

How quickly can you detect a face? Temporal dynamics within neural constructs involved in the detection of familiar and novel faces

Blackford JU, **Avery SN**, Shelton RC, Zald DH. (2009). Amygdala temporal dynamics: temperamental differences in the timing of amygdala response to familiar and novel faces. *BMC Neurosci*. 10:145.

Inhibited temperament involves a predisposition of individuals to respond to new people, places or things with wariness or avoidance behavior, a characteristic trait known to be associated with increased risk for social anxiety disorder and major depression. Within the human brain, functional magnetic resonance imaging (fMRI) has shown the magnitude of blood oxygen level dependent (BOLD) responses in the amygdala for novel stimuli to be associated with inhibited temperament. The authors in this study used an event related fMRI paradigm to investigate the temporal dynamics (latency, duration and peak) of the BOLD response to novel stimuli within the amygdala. They presented both novel and familiar faces to both inhibited and uninhibited temperament populations. Results indicated that the amygdala in inhibited participants responded faster to novel faces than familiar faces. In addition, they found that the inhibited participants showed both a longer and greater amygdala response to all faces in comparison to the uninhibited population, even though there were no differences in the peak BOLD response. Blackford and colleagues speculate that this temporal computational bias for novel stimuli within the amygdala may lead to greater neophobic responses and could allude to a plausible mechanism for the development of social anxiety.

apoptosis during embryogenesis. The rapid and controlled clearance of these dead cells is vital, as non-ingested cells can generate inflammation and detrimental immune responses.

In a recent *Nature Neuroscience* article, a team led by Bruce Carter recognizes a specialized glial cell, satellite glial cell precursors (SGC), in the clearance of dead neurons in the dorsal root ganglia of embryonic mice. Previously, it was unclear which cells were responsible for apoptotic clearance, with default responsibility handed to macrophages. However, through confocal and electron microscopy and *in vitro* assays, the group demonstrates that the vast majority of apoptotic neurons are associated or engulfed within SGC's, highlighting an unrecognized role for glial cells in phagocytosis.

In addition, the group provided a potential molecular mechanism for engulfment of dead cells. Jedi-1 and MEGF10, homologues of known *c. elegans* and *Drosophila* proteins, are expressed in the brain and specifically within SGCs in the DRG. Transient expression of the either protein in cultured HEK cells led to binding of neuronal corpses, and overexpression of the proteins in glial cells leads to increased engulfment of dead cells, suggesting a clear role for Jedi-1 and MEGF10 as engulfment receptors. Through their research, Wu et al establish satellite glial cell precursors as members of the 'clean-up' crew in the peripheral nervous system, performing a similar role as microglia in the brain, and importantly implicate two receptors as crucial sensors in the proper clearance of apoptotic cells.

Original Research Article:
HH Wu, E Bellmunt, **JL Scheib**, V Venegas, C Burkert, LF Reichardt, Z Zhou, I Fariñas, BD Carter (2009). Glial precursors clear sensory neuron corpses during development via Jedi-1, an engulfment receptor. *Nat Neurosci.* **12** (12): 1534-1541.

Bad huntingtin, protective effect

Huntington's disease (HD) is a unique neurodegenerative disorder in that it has been mapped to a single locus, the *huntingtin* gene. In the disease, huntingtin protein progressively undergoes an expansion of a polyglutamate region. Onset of symptoms correlates with a specific number of polyglutamate repeats, such that the disease progressively worsens with age. Thus far, research has been unable to explain how mutant huntingtin leads to a remarkably specific loss of medium spiny neurons in the striatum, a primary cause of the motor deficits seen in patients. One hypothesis is that striatal neurons are situated in an environment that may uniquely impact their vulnerability to mutant HTT; a

recent paper from the Bowman lab investigates this gene-environment hypothesis in the context of HD, with specific focus on the impact of metal neurotoxicology.

Williams *et al*. performed a disease-toxicant interaction screen in order to test a correlation between physiological properties shared between metal exposure and Huntington's disease. These include oxidative stress, cell stress, protein aggregation, and alterations in calcium signaling and energy metabolism. To test the correlations, they utilized several HD models in metal exposure paradigms. They first demonstrated that a mouse striatal cell line model of HD demonstrates variable cell survival responses to given metals. They note that mutant Huntingtin cells survive at similar rates over a range of concentrations in the vast majority of metals tested. However, mutant cells were less viable when exposed to cadmium and actually displayed a neuroprotective effect in the presence of manganese, without affecting the level of huntingtin protein.

The lab next capitalized on previous research to investigate the impact of manganese on established physiological processes. First, they demonstrated that HD mutant striatal cells have diminished phosphorylation of Akt, a cell stress signaling pathway associated with previous HD studies. They further show that these mutant cells have impaired accumulation of manganese, which may prevent its toxic intracellular effects. Finally they note that in an *in vivo* mouse model of HD, YAC128Q mice have a striatum-specific reduction of manganese uptake as compared to wild type mice. The neuroprotective effect of mutant HD on manganese exposure is quite surprising, and future experiments will target manganese uptake, export, and storage under the broad hypothesis that HD pathology is regulated in the context of both genetics and environment.

Original Research Article:
BB Williams, D Li, M Wegrzynowicz, BK Vadodaria, JG Anderson, **GF Kwakye**, M Aschner, KM Erikson, AB Bowman(2010). Disease-toxicant screen reveals a neuroprotective interaction between Huntington's disease and manganese exposure. *J. Neurochem*. **112** (1): 227-237.

Worth the 'EEfRT'? Role of motivation in rewarding tasks

Anhedonia is a subtype of the putative psychopathological enophenotypes in major depressive disorder (MDD), which characteristically represents an individual's aberrant motivation and reward responsivity. In order to objectively tap into the measures of reward motivation and test trait anhedonia, the authors in this study developed the Effort-Expenditure for Rewards Task (EEfRT or "effort"), a novel behavioral paradigm to explore effort based decision making in humans. The EEfRT task is a multi-trial game in which participants are given the opportunity on each trial to choose between two different task difficulty levels (hard or easy). They are required to complete the task with a specific number of button responses within a constrained period of time in order to obtain a monetary reward. Along with the EEfRT task, study participants self-reported measures of mood and trait anhedonia. Across multiple analyses, Treadway and colleagues found a significant inverse relationship between the anhedonia trait and a willingness to expend effort for rewards. From these results, the authors postulate that anhedonia is specifically associated with decreased motivation for reward. Additionally, the findings in this study enabled the authors to provide initial validation for the EEfRT behavioral paradigm as a laboratory based measure of reward motivation and effort based decision making in humans.

Original Research Article:
Treadway MT, **Buckholtz JW**, Schwartzman AN, Lambert WE, Zald DH (2009). Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS One*. 4(8):e6598.

IN BRIEF…

Exploring CAMKII associations with calcium L-type channels to regulate physiological changes in tissues

SA Abiria, RJ Colbran (2010). CaMKII associates with CaV1.2 L-type calcium channels via selected beta subunits to enhance regulatory phosphorylation. *J Neurochem*. **112** (1): 150-61.

Voltage activated L-type calcium channels (LTCCs) are known to generate Ca^{2+} signals important for numerous physiological process such as muscle contraction, neurotransmitters, neuronal plasticity and others. In particular the calcium/calmodulin dependant kinase II (CaMKII) protein has been shown to augment the Ca^{2+} signals in response to growth factors or hormones. Excessive $Ca_v1.2$LTCC activity can produce a variety of pathological symptoms including cardiac arrhythmias, multi-organ human genetic disorder and cases of parkinsons disease in animal models. While CAMKII facilitates LTCC activity physiologically, the molecular basis of CAMKII interactions and its modulation of LTCCs is yet to be understood. The authors of this paper transfected HEK293 cells from the forebrain of 7-8week old rats to explore the role of β subunits in targeting CAMKII to LTCC α1 subunits. They found that CAMKII co-immunoprecipitates with forebrain LTCCs that contain $Ca_v1.2$α1 and β1 or β2 subunits, but not LTCC complexes containing b4 subunits. These targeted mechanistic interactions between CAMKII and LTCCs will be crucial in providing insights into our understanding of physiological and pathological process in different tissues.

The Vanderbilt Neuroscience Outreach Initiative

Brandon A. Carver, Ms.E.
Conte Supplement Advisory Board Member

Providing exceptional science, technology, engineering, and mathematics (STEM) education has been at the top of America's education al agenda for nearly a decade. Despite a consensus on the importance of STEM education in developing thoughtful, innovative, and talented students that will help ensure the stren gth of America's intellectual, scientific, and economic base, relatively little guidance exists on how to integrate outreach programs to improve learning outcomes or public awareness of relevant researc h findings. Recently, the Vanderbilt Silvio O. Conte Center for Neuroscience Research and the Vanderbilt Brain Institute (VBI) embarked on a mission to increase public awareness of STEM topics, and specifically neuroscience, in the greater Nashville area. The first and perhaps most critical component of this effort was an application for NIH funds to create a state-of-the-art neuroscience exhibit at a local children's science museum, the Adventure Science Center (ASC). This application was written as a supplement to the already very large Conte Center headed by Randy Blakely, Ph.D. The grant, which was co-written by VBI Director Mark Wallace, Ph.D., was enthusiastically funded by the NIMH for a period of one year. In that time, (July 2010 to July 2011), Vanderbilt neuroscience has the opportunity to make a large and lasting impression on the youth of Greater Nashville—both through this endeavor with the ASC and in its collaboration with the Vanderbilt Center for Science Outreach (VCSO) to bring neuroscience and neuroscientists into Nashville classrooms. A critical component of this effort is, obviously, a measure of its success. Despite th e lack of literature on evaluation studies from the field, we plan to proceed by incorporating standard evaluation principles such as program design and goal setting, establishment of benchmarks and indicators, and fidelity to our objectives. Herein, we will describe our initial plan to report these indicators.

Program design

In creating a collaborative and intricate outreach program such as this, it is important that all colla borators have a clear understanding of the goals, objectives, and intents of the program and that these understandings are mutually agreed upon and shared by all. This consensus step is crucial for programmatic coherence. Construction of a "logic map" or "roadmap" is a valuable exercise that should be conducted to ensure outreach goals are established and actionable. Program benefits should be designed so that they are meaningful to each segment of the target population (students, teachers, parents, young children, adults). For example, in collaboration with the Middle Tennessee Chapter Society for Neuroscience (MTNCSfN) and its President (Vivian Casagrande, Ph.D.), the VBI and the VCSO plan to create brain-based literature for distribution to each child at area schools, but the age, cultural background and comprehension-level must all be considered before conducting an expensive and resource-draining campaign. School curricula and parental involvement must also be considered as serious elements of such a large effort. Because many science outreach initiatives are open to the general public, planners must be cognizant of the diverse nature of their target audience and highlight its benefits accordingly.

All participants should understand whether the program is a partnership (whose design can range from the input of one stakeholder to the input of many stakeholders with a range of expertise) or outreach (which tends to be conducted by experts for the benefit of a targeted group). Lastly, it is also important to note that cultural optics—how different cultural groups within the target audience perceive the program—matter and can easily be overlooked, particularly if being designed by experts with little outreach experience and minimal community input. The incorporation of multicultural community-based knowledge with various methods of learning are critical for the success of any educational outreach effort, and must be cognizant of and engaging to different cultural backgrounds without pandering or being offensive. We hope to achieve these cultural proficiencies through the expertise of our collaborators, and further test curricula and print material before mass public distribution.

Benchmarks and Indicators

Whenever possible, we hope to gather data to ensure that adequate progress is being made to accomplish program goals. Although the impacts of outreach programs are mostly qualitative in nature, relevant data can and will

be collected throughout the implementation process. Attendance logs, procurement records, press releases and print materials are useful indicators of a program's success.

Program Fidelity

Finally, we end with the beginning. Program fidelity returns us to the question of intent. Was our mission accomplished? Was the target population reached? Were the desired observable outcomes achieved? Measures of program fidelity can take years—did the second graders we talked to in 2010 end up with a degree in some STEM discipline in 2024? In the time between our interaction with that child and the completion of her/his degree, was a passion for science nurtured by his/her family/teachers/community. It goes without saying that this endeavor should not be a one-year commitment, but rather a yearly contribution of passionate science from the Vanderbilt Brain Institute and its collaborators.

Conclusion

More formative evaluation of STEM outreach should be conducted to see how to best increase public engagement and awareness of vital health and science issues. Evaluation of this sort is necessary because the literature documenting science outreach is thin and current suggestions for "best practices" and/ or strategies to integrate within high school curricula are even sparser. The aim of our future evaluations will be to inform the field by enriching the "best practices" literature on science education outreach programming so that concrete strategies can be developed to best support the implementation of STEM educational outreach programs in the future…

Program Update

The Neuroscience Graduate Program at Vanderbilt University is entering its twelfth year of existence under the young directorship of Prof. Mark Wallace. For the upcoming 2010-11 academic year, the program will have 71 trainees doing coursework and research in their mentors' laboratories, pursuing their doctoral degrees in Neuroscience. These trainees come from 23 states and 9 foreign counties. We have 94 training faculty committed to preparing our students for careers in teaching and research. Our students receive strong academic and research training from our outstanding training faculty.

Roz Johnson, B.B.A.
Interdisciplinary Program Coordinator

A note from the Director

"Wow!" That was the universal comment I received whenever I showed anyone the inaugural issue of *VRN*. The feedback on last year's issue has been nothing short of phenomenal, and this year's edition looks to be equally exceptional! I carry a copy of *VRN* with me whenever I travel – whether to conferences, to talk at other universities, to study section, etc. When at these venues, I make it a point to show my colleagues the issue, and to watch their faces drop as they leaf through the pages. In today's increasingly electronic age, I've been surprised and delighted that something so tangible as a copy of *VRN* can be such an effective advertisement for the quality of the research and training endeavors within the Vanderbilt Neuroscience Program. We should all be proud to be a part of this remarkable endeavor. A special thanks goes out to Caleb Doll and Mariam Eapen, who have worked tirelessly to begin to assume the reins of editorial leadership from the founding father – Chris Ciarleglio.

Yours in science,

Mark T. Wallace, Ph.D.

Mechanisms for the Interaction of Dopamine and Norepinephrine in the Prefrontal Cortex: Implications for the Treatment of Cognitive Symptoms of Schizophrenia

Peter Vollbrecht

Reductions in prefrontal cortical dopamine (DA) levels have been associated with the cognitive symptoms of schizophrenia. When removal of the dopamine innervation to the prefrontal cortex (PFC) was tested in animal models, researchers reported a loss of dendritic spines. Anatomical arrangements in the PFC suggest that dopamine may play a role in the regulation of dendritic architecture. Atypical antipsychotics, but not typical antipsychotics, reverse the loss of dendritic spines seen upon DA denervation. Atypical antipsychotic drugs have also been reported to reduce cognitive symptoms of schizophrenia. Taken together with their ability to reverse spine loss, these data suggest that spine loss may be a pathological correlate to cognitive deficits associated with the prefrontal cortex. The mechanism by which these drugs act to restore DA tone in the PFC remains unclear. Recent data has suggested that norepinephrine (NE) terminals are capable of releasing the NE "precursor" DA. Atypical antipsychotic drugs have a wide target profile, including antagonism of NE autoreceptors. These data suggest that interactions between the DA and NE systems may play a role in treatment for schizophrenia. Although DA and NE have been implicated in disorders involving the prefrontal cortex such as schizophrenia, affective disorders, and attention-deficit hyperactivity disorder (ADHD), the mechanism for interactions between DA and NE has not been widely investigated. Understanding how these systems interact should have a major impact on therapeutic possibilities for disorders arising from disruption of PFC function.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: peter.j.vollbrecht@vanderbilt.edu.

Dopamine (DA) and norepinephrine (NE) have consistently been shown to play a crucial role in cognitive processes. DA and NE share a common synthetic pathway, and have both been implicated in psychiatric disorders such as attention-deficit hyperactivity disorder (ADHD)[1,2], affective disorders[3], and schizophrenia[4-6]. Both transmitter systems send projections to the prefrontal cortex (PFC), where they have been shown to be involved in processes such as attention, working memory, executive function, and behavioral inhibition[1, 2, 4, 7-14]. Disturbances in PFC function are linked to the cognitive symptoms of schizophrenia, and are thought to be a result of a hypodopaminergic state in the PFC[15]. First generation antipsychotics, such as haloperidol, primarily target the D_2 dopamine receptor[16]. These drugs tend to improve the positive symptoms of the disorder, such as hallucinations and delusions, yet have little effect on cognitive and negative symptoms[17]. Interestingly, second generation antipsychotics, such as clozapine, have a larger target profile and are more effective in treating the cognitive and negative symptoms of schizophrenia, such as deficits in working memory and a flattened affect[18, 19]. Among those receptors targeted by second-generation drugs are NE receptors, including α_{2C}-receptors, increasing interest in the possible interactions of these parallel pathways. Despite commonalities in synthesis, localization, and drug interactions, interactions between DA and NE systems within the prefrontal cortex remain poorly understood. Here we will review evidence for the role of DA and NE in aspects of cognition, then delve into recent studies that investigate possible interactions of these systems at the level of receptors, transporters, and possible co-release, and finally the implications that DA/NE interactions have for our understanding of neuropsychiatric disorders.

DOPAMINE AND NOREPINEPHRINE NEURONS PROJECT TO THE PFC

Early studies of both dopamine and norepinephrine focused on the localization of these transmitters in the brain. Using fluorescent histochemistry, as well as electron microscopy, these studies showed that both DA and NE are present in the prefrontal cortex[20-22].

Prefrontal cortical DA projections originate from neurons in the ventral tegmental area (VTA)[23], while NE projections originate from the locus coeruleous (LC)[24]. Dopamine has an abundance of axon terminals in deep layers of cortex in the rodent[20] and primate[25], primarily in layer V. This coincides well with the distribution of dopamine receptors of which D_1 is the dominant form in the PFC[26]. This selective area of activation implies a selective function for the DA pathway in the PFC, which is further strengthened by the presence of specific synaptic contacts being made onto the shafts and spines of layer V pyramidal cells[25]. Several studies suggest that NE terminals are more evenly distributed than DA terminals throughout the prefrontal cortex[21, 27]. The diffuse nature of NE terminals across both rodent and primate PFC lamina suggests a more general role of this transmitter in the PFC. Norepinephrine terminals lack the synaptic contacts made by DA terminals. However, functional specificity of NE may be determined by NE receptors' laminar distribution. Norepinephrine and DA distribution in the PFC suggests these transmitters' involvement in PFC function.

DOPAMINE AND NOREPINEPHRINE RECEPTOR LOCALIZATION

Following these careful characterizations of dopamine and norepinephrine distribution, investigation began to shift from neurotransmitters to their receptors. Autoradiography was used in early studies, using tridiated ligands that showed specificity for the various dopamine and norepinephrine receptors[28-30].

Currently, five dopamine receptors have been identified, which are classified as adenylate cyclase activating "D_1-like", or inhibiting "D_2-like" receptors, with D1 and D_5 being grouped together and D_{2-4} grouped together[31]. Dopamine receptor identification in early studies made no distinction between the various subtypes, and suggested that DA receptor localization in the PFC was focused in the deep layers V and VI[29]. D_2 receptors are localized to the PFC, yet the relative amount of this receptor is significantly lower than its counterpart[26]. Early studies indicated that D_1 was most abundant in superficial layers I, II, and III, with slightly lower levels in layers V and VI in primates, while showing specificity to deeper layers in a rat model[28, 30]. D_2 receptors show laminar localization primarily to layer V. Findings by Richfield et al. suggest a uniform distribution of D_1 receptors across all lamina in cats and monkeys, but rats had increased D_1 receptor binding in deep layers V and VI[26]. An mRNA expression study of all five receptor subtypes in the PFC of primates found that expression for all five subtypes was highest in layer V[32]. This was in agreement with studies of mRNA levels performed in the human PFC[33], suggesting that layer V has a particularly important role in catecholamine activity in the prefrontal cortex.

Norepinephrine acts on two classes of receptors, both α- and β-adrenergic receptors. These two classes are further broken into α_1 and α_2 as well as β_1, β_2, and β_3 subtypes. The α subtypes are each further divided into three subclasses, A, B and C[31]. The β-receptors activate adenylate cyclase[34], while α_2-receptors act to inhibit this enzyme[35, 36]. The α_1-receptors are linked to PKC and the release of intracellular calcium through G_q coupling[35, 37]. The β receptors appear less abundant in the PFC than the α receptors and show an inverse laminar distribution[28]. The α_1-receptors are more abundant than β-receptors, yet remain less prominent in the PFC than α_2-receptors. Prazosin, a selective ligand for α_1-receptors, exhibits strong binding in deep layers V and VI. The most abundant NE receptor in the PFC is the α_2-receptor. Clonidine binding (α_2) shows a decreasing gradient from superficial to deep layers[28]. Both α_{2A} and α_{2C}-receptors are found both presynaptically and postsynaptically in the PFC[38]. Presynaptic autoreceptors provide feedback inhibition to the NE terminal[38, 39].

It is at the level of receptor binding that it first becomes apparent that interactions between DA and NE systems are likely to occur. It has been shown that DA is capable of acting as an agonist at adrenergic receptors[40, 41]. Likewise, D_1 and D_2 radioligands have been shown to be displaced by both DA and NE, implying NE binding to DA receptors[42]. Cornil et al showed that DA has affinity for the α_{2c}-adrenoceptor in rat brain[43]. It is widely recognized that NE transporters have a higher affinity for DA than for NE, allowing possible interactions through transmitter reuptake. Finally, 2nd generation antipsychotics such as clozapine and olanzapine have affinities for both DA and NE receptors[44-46]. Due to the possibilities for interactions between these systems, an investigation of the mechanisms of these interactions appears critical to our understanding of the effects of either pathway in PFC function.

DOPAMINE AND NOREPINEPHRINE IN THE PREFRONTAL CORTEX

The importance of interactions between NE and DA is underlined by the roles of these transmitters during PFC function. The prefrontal cortex is thought to control such executive cognitive functions as working memory and attention. Given the innervation patterns of both dopamine and norepinephrine, it is not surprising that both have individually been shown to have links to these functions. Through the use of lesion studies within the prefrontal cortex, as well as studies of structures projecting to the PFC, transmitter

loss-of-function has been explored.

Lesion of DA in the PFC can be performed directly by injection of 6-hydroxydopamine (6-OHDA) into the PFC[47], or indirectly by injection of 6-OHDA into the VTA which supplies DA to the PFC[48] along with a NET blocker such as desipramine to spare NE terminals. Studies using both methods have suggested the importance of DA in working memory, and attention[49]. Interestingly, further research has shown that excess DA in the PFC can have detrimental effects on cognitive tasks as well[5].

Through injection of the NE terminal specific toxin DBH-saporin, similar PFC specific lesions of NE can be performed[50]. Before the development of this toxin, DNAB lesions using 6-OHDA were used[1]. Studies using both of these methods have shown cognitive deficits similar to those seen in the DA system[1, 14, 50]. Once again, excess levels of NE can have detrimental effects[37, 51]. These studies suggest that there is an optimal range for DA and NE within the PFC necessary for higher order functioning. Given the involvement of the prefrontal cortex in cognitive functions such as working memory, and attention, as well as the role of DA and NE in these processes, understanding the interactions between these transmitters within the PFC could lead to major changes in the treatment of neurological disorders, such as attention-deficit hyperactivity disorder (ADHD) and schizophrenia.

PREVIOUS HYPOTHESES

Past research often cites potential DA/NE interactions as having an effect on their studies' results[3, 52, 53]. Previous work in this area has focused on drugs that interact with both systems, rather than these systems' interactions with each other, and the body of literature working directly to determine the mechanisms of these interactions is small. It has been well documented that changes in dopamine and norepinephrine in the prefrontal cortex are well-correlated, changing together in disorders such as schizophrenia[53], and as a response to physiological changes, such as stress[51]. (The link to stress may prove to be of further interest, as stress often induces schizophrenia symptoms. However, this link will not be discussed in this review). The correlation between DA and NE levels in the PFC is particularly evident in response to antipsychotic drugs[54, 55]. However, the mechanism of this DA/NE interaction is still being debated. Hypotheses that have been put forth include: a direct effect of NE on DA release[56, 57], an effect of NE on DA reuptake[58, 59], and co-release of DA and NE from NE terminals[60]. Few researchers have actively attempted to validate these hypotheses by studying the mechanisms by which these two transmitters are interacting.

STUDIES ADDRESSING THE DIRECT EFFECT OF NE ON DA RELEASE

Pozzi et al. used lesion studies, along with selective DA and NE reuptake inhibitors, to further the hypothesis that increases in NE directly increase DA levels[57]. This study showed that increasing extracellular NE was correlated to increases in extracellular DA. Similarly, Gresch et al. suggested two possible explanations for their findings, 1) NE regulation of DA through receptors regulating DA release, or 2) transport of DA into noradrenergic terminals[56].

STUDIES ADDRESSING DA REUPTAKE THROUGH NE TERMINALS

The idea of NE affecting the uptake of DA has been suggested given the relatively low abundance of DA transporter (DAT)[61] in the PFC, and the broad coverage of NE transporter (NET) in this area[62]. Moron et al. were able to show that DAT knockout mice had normal rates of DA uptake in the frontal cortex, while NET knockout mice exhibited greater than 50% loss of DA uptake[59]. This indicates that DA uptake in the PFC occurs largely through NET activity. If NE release increases, the probability of DA being taken up by these transporters decreases, thereby increasing the extracellular levels of DA in the region. In this indirect way, NE may increase extracellular DA. Studies using the α_2-receptor antagonist mainserin, along with two NET inhibitors, reboxetine and desipramine, suggest that NET uptake of DA is significantly higher in the PFC than in the parietal cortex or occipital cortex[58] due to a lower NE/DA ratio than in the latter two areas. It is important to note that mainserin was administered via i.p. injection, so effects were global and not PFC specific. Treatment caused an increase in DA levels in all three regions, and the authors attributed this to the effect of the drug in the VTA causing DA neuron firing, rather than action in the PFC. NET's high affinity for DA could cause more rapid clearance of DA than NE, and could cause the increases in DA when extracellular NE is increased. Later research in which mainserin was administered locally suggests that α_2-receptors have a significant effect locally on DA release in the PFC[63].

STUDIES ADDRESSING DOPAMINE AND NOREPINEPHRINE CO-RELEASE

Ahn and Klinman reported on the rate limiting steps of norepinephrine synthesis over 20 years ago[64]. They report that dopamine beta monooxygenase (dopamine beta hydroxylase, DBH), and not tyrosine hydroxylase, may be the rate-limiting step in NE synthesis. DBH is the final enzyme that converts DA into NE within the vesicles of NE terminals. If DBH

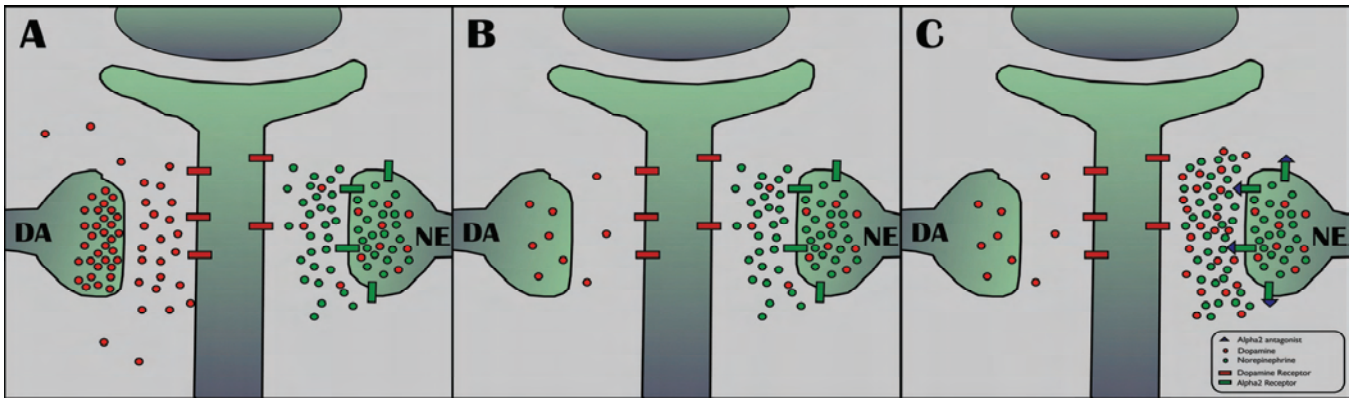


Figure 1 | **A possible mechanism for the effects of olanzapine and clozapine on DA tone** A) Proper DA and NE signaling. B) DA tone disturbance while NE signaling remains intact. C) The effects of a α_2-receptor antagonist as it re-establishes DA tone through the NE terminal. DA (red circles) and receptors (red boxes), NE (green circles) and α_2-receptors (green boxes), α_2-receptor antagonist (blue triangle).

is rate limiting, at times the firing rate of NE neurons could be intensified, causing release of DA from these terminals along with NE. This interaction, coined the "co-release" hypothesis, proposes that DA and NE are released together from the NE terminal[60]. Microdialysis allowed investigation of the possible co-release of DA and NE in the PFC[60]. Looking at DA innervated regions (PFC) and non-, or minutely DA innervated regions (occipital cortex, primary motor cortex) using dialysis, Devoto et al. showed that the extracellular levels of DA were similar in both DA innervated and non-innervated regions. This implies another source of DA in these areas. Using selective α_2-receptor antagonist infused through the probe, investigators saw increases in DA in all three areas, suggesting that DA was being released through NE terminals, and furthermore that this release was, in part, regulated by α_2-receptors[65]. The α_2-receptor agonist, clonidine, reduced extracellular DA levels along with NE levels, while the antagonist, idazoxan, increased DA and NE levels. A further study in 2003 by the same group performed a similar study looking at the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC)[66]. This study further verified that DA is likely being released from both DA and NE terminals in the PFC, but only by DA terminals in subcortical regions. Using clozapine, the first atypical antipsychotic, Devoto et al. showed the effects of this drug on PFC DA and NE levels. In this study, treatment with clozapine elevated both NE and DA levels in the PFC and occipital cortex, as did treatment with a α_2 antagonist. Interestingly, treatment with clonidine, an α_2-receptor agonist, reversed these effects, while treatment with a D_2 agonist, which has been shown to decrease DA release in the striatum, had no effect[66]. This evidence again suggests that DA is being released through the NE terminal, and that the atypical antipsychotic drug clozapine is acting through a α_2-receptor mechanism to restore PFC DA levels. In later experiments, Devoto et al showed that activation of the LC was sufficient to increase extracellular dopamine in the PFC[67]. Considered with the results discussed above, it is likely that this increase is not solely due to the LC acting on the VTA but also through NE terminal firing in the PFC. Finally, Devoto et al. demonstrated that lesioning the VTA and removing DA innervation to the PFC has no affect on extracellular DA levels[68]. Tissue content of DA was significantly reduced, however extracellular levels remained unchanged. These data provide very strong evidence suggesting that NE terminals do, in fact, release DA, providing an alternative explanation to the hypotheses that NE is affecting DA levels through direct interaction with DA terminals or through DA reuptake.

The release of DA from NE terminals may help to explain data derived in our own lab. We have shown that a loss of DA innervation from the VTA causes a loss of dendritic spines on PFC layer V pyramidal cells[48]. This loss of dendritic spines could be related to the loss of cortical volume seen in schizophrenia patients[69], providing a possible pathological correlate to behavioral data suggesting impaired cognitive function in animals with a loss of PFC DA signaling. Interestingly, the loss of spines in these cells could be reversed through treatment with olanzapine, but not haloperidol. Given the ability of atypical, but not typical antipsychotic drugs to help in the relief of cognitive symptoms of schizophrenia, this lends credibility to the importance of dendritic spines in PFC function. Dendritic structure is maintained through DA tone in the striatum[70]. If the same is true for the PFC, it can be hypothesized that following DA depletion of the PFC, atypical drug treatment acts to restore DA tone through an alternative DA source. Under normal conditions, it appears that extracellular DA comes both from the DA and NE terminals, with DA terminals shouldering the majority of this load (**Figure 1a**). However, in certain states such as schizophrenia, these DA levels are reduced, possibly

through reduced transmission through the DA terminals (**Figure 1b**). Through treatments capable of antagonizing the α_{2c}-receptor, DA tone can be restored through release of DA through the NE terminal (**Figure 1c**). Clozapine, the original atypical antipsychotic, as well as olanzapine, has a high affinity for α_2-receptors[44] making these drugs candidates to act at the NE terminal.

CONCLUSIONS

Atypical antipsychotic drugs appear to have effects on cognitive deficits not seen with typical antipsychotic treatments[18]. These drugs also have a restorative effect on DA denervated pyramidal cell morphology in the PFC[48]. Linking these two functions of atypical antipsychotics could provide strong evidence that the ability of atypical antipsychotic drugs to treat the cognitive deficits and negative symptoms of schizophrenia is a result of their ability to affect non-DA receptors, including the α_2-receptor. Data from Devoto et al. have suggested that atypical antipsychotic drugs are capable of causing release of DA from NE terminals[66]. Our own work suggests that this may be a factor in restoring dendritic spines in the PFC. Further research is critical to linking the interactions of the DA and NE systems to the restorative effects of atypical antipsychotic treatment. In the future, understanding the mechanism of interaction of DA and NE should lead to improved treatments of disorders of the prefrontal cortex, ranging from affective disorders to schizophrenia.

REFERENCES

1. Tait DS, Brown VJ, Farovik A, Theobald DE, Dalley JW and Robbins TW (2007). Lesions of the dorsal noradrenergic bundle impair attentional set-shifting in the rat. *Eur J Neurosci*. **25** (12): 3719-3724.
2. Arnsten AF, Scahill L and Findling RL (2007). alpha2-Adrenergic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: emerging concepts from new data. *J Child Adolesc Psychopharmacol*. **17** (4): 393-406.
3. Antelman SM and Caggiula AR (1977). Norepinephrine-dopamine interactions and behavior. *Science*. **195** (4279): 646-653.
4. Bubser M and Koch M (1994). Prepulse inhibition of the acoustic startle response of rats is reduced by 6-hydroxydopamine lesions of the medial prefrontal cortex. *Psychopharmacology (Berl)*. **113** (3-4): 487-492.
5. Murphy BL, Arnsten AF, Goldman-Rakic PS and Roth RH (1996). Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc Natl Acad Sci U S A*. **93** (3): 1325-1329.
6. van Kammen DP, Agren H, Yao JK, O'Connor DT, Gurklis J and Peters JL (1994). Noradrenergic activity and prediction of psychotic relapse following haloperidol withdrawal in schizophrenia. *Am J Psychiatry*. **151** (3): 379-384.
7. Yamamuro Y, Hori K, Iwano H and Nomura M (1994). The relationship between learning performance and dopamine in the prefrontal cortex of the rat. *Neurosci Lett*. **177** (1-2): 83-86.
8. Clinton SM, Sucharski IL and Finlay JM (2006). Desipramine attenuates working memory impairments induced by partial loss of catecholamines in the rat medial prefrontal cortex. *Psychopharmacology (Berl)*. **183** (4): 404-412.
9. Brozoski TJ, Brown RM, Rosvold HE and Goldman PS (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science*. **205** (4409): 929-932.
10. Carter CJ and Pycock CJ (1980). Behavioural and biochemical effects of dopamine and noradrenaline depletion within the medial prefrontal cortex of the rat. *Brain Res*. **192** (1): 163-176.
11. Cooper BR, Howard JL, Grant LD, Smith RD and Breese GR (1974). Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. *Pharmacol Biochem Behav*. **2** (5): 639-649.
12. Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ and Robbins TW (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci*. **20** (3): 1208-1215.
13. Kalsbeek A, de Bruin JP, Matthijssen MA and Uylings HB (1989). Ontogeny of open field activity in rats after neonatal lesioning of the mesocortical dopaminergic projection. *Behav Brain Res*. **32** (2): 115-127.
14. Milstein JA, Lehmann O, Theobald DE, Dalley JW and Robbins TW (2007). Selective depletion of cortical noradrenaline by anti-dopamine beta-hydroxylase-saporin impairs attentional function and enhances the effects of guanfacine in the rat. *Psychopharmacology (Berl)*. **190** (1): 51-63.
15. Abi-Dargham A (2004). Do we still believe in the dopamine hypothesis? New data bring new evidence. *Int J Neuropsychopharmacol*. **7 Suppl 1**: S1-5.
16. Howes OD and Kapur S (2009). The dopamine hypothesis of schizophrenia: version III--the final common pathway. *Schizophr Bull*. **35** (3): 549-562.
17. Kasper S and Resinger E (2003). Cognitive effects and antipsychotic treatment. *Psychoneuroendocrinology*. **28 Suppl 1**: 27-38.
18. Meltzer HY and McGurk SR (1999). The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. *Schizophr Bull*. **25** (2): 233-255.
19. Kane J, Honigfeld G, Singer J and Meltzer H (1988). Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry*. **45** (9): 789-796.
20. Van Eden CG, Hoorneman EM, Buijs RM, Matthijssen MA, Geffard M and Uylings HB (1987). Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. *Neuroscience*. **22** (3): 849-862.
21. Seguela P, Watkins KC, Geffard M and Descarries L (1990). Noradrenaline axon terminals in adult rat neocortex: an immunocytochemical analysis in serial thin sections. *Neuroscience*. **35** (2): 249-264.
22. Levitt P and Moore RY (1978). Noradrenaline neuron innervation of the neocortex in the rat. *Brain Res*. **139** (2): 219-231.
23. Emson PC and Koob GF (1978). The origin and distribution of dopamine-containing afferents to the rat frontal cortex. *Brain Res*. **142** (2): 249-267.

24. Ungerstedt U (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol Scand Suppl*. **367**: 1-48.
25. Goldman-Rakic PS, Leranth C, Williams SM, Mons N and Geffard M (1989). Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proc Natl Acad Sci U S A*. **86** (22): 9015-9019.
Description of synaptic triad arrangement in the prefrontal cortex suggesting an DA plays an important role in regulating excitatory inputs to PFC neurons.
26. Richfield EK, Young AB and Penney JB (1989). Comparative distributions of dopamine D-1 and D-2 receptors in the cerebral cortex of rats, cats, and monkeys. *J Comp Neurol*. **286** (4): 409-426.
27. Geffard M, Patel S, Dulluc J and Rock AM (1986). Specific detection of noradrenaline in the rat brain by using antibodies. *Brain Res*. **363** (2): 395-400.
28. Goldman-Rakic PS, Lidow MS and Gallager DW (1990). Overlap of dopaminergic, adrenergic, and serotoninergic receptors and complementarity of their subtypes in primate prefrontal cortex. *J Neurosci*. **10** (7): 2125-2138.
29. Murrin LC and Kuhar MJ (1979). Dompamine receptors in the rat frontal cortex: an autoradiographic study. *Brain Res*. **177** (2): 279-285.
30. Savasta M, Dubois A and Scatton B (1986). Autoradiographic localization of D1 dopamine receptors in the rat brain with [3H]SCH 23390. *Brain Res*. **375** (2): 291-301.
31. Squire LR (2003). Fundamental neuroscience, 2nd Edition. San Diego, CA: Academic.
32. Lidow MS, Wang F, Cao Y and Goldman-Rakic PS (1998). Layer V neurons bear the majority of mRNAs encoding the five distinct dopamine receptor subtypes in the primate prefrontal cortex. *Synapse*. **28** (1): 10-20.
33. Meador-Woodruff JH, Damask SP, Wang J, Haroutunian V, Davis KL and Watson SJ (1996). Dopamine receptor mRNA expression in human striatum and neocortex. *Neuropsychopharmacology*. **15** (1): 17-29.
34. Ordway GA, O'Donnell JM and Frazer A (1987). Effects of clenbuterol on central beta-1 and beta-2 adrenergic receptors of the rat. *J Pharmacol Exp Ther*. **241** (1): 187-195.
35. Ramos BP and Arnsten AF (2007). Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacol Ther*. **113** (3): 523-536.
36. Ramos BP, Stark D, Verduzco L, van Dyck CH and Arnsten AF (2006). Alpha2A-adrenoceptor stimulation improves prefrontal cortical regulation of behavior through inhibition of cAMP signaling in aging animals. *Learn Mem*. **13** (6): 770-776.
37. Birnbaum SG, Yuan PX, Wang M, Vijayraghavan S, Bloom AK, Davis DJ, Gobeske KT, Sweatt JD, Manji HK and Arnsten AF (2004). Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science*. **306** (5697): 882-884.
38. MacDonald E, Kobilka BK and Scheinin M (1997). Gene targeting--homing in on alpha 2-adrenoceptor-subtype function. *Trends Pharmacol Sci*. **18** (6): 211-219.
39. Ihalainen JA and Tanila H (2002). In vivo regulation of dopamine and noradrenaline release by alpha2A-adrenoceptors in the mouse prefrontal cortex. *Eur J Neurosci*. **15** (11): 1789-1794.
40. Nimit Y, Cantacuzene D, Kirk KL, Creveling CR and Daly JW (1980). The binding of fluorocatecholamines to adrenergic and dopaminergic receptors in rat brain membranes. *Life Sci*. **27** (17): 1577-1585.
41. Weitl N and Seifert R (2008). Distinct interactions of human beta1- and beta2-adrenoceptors with isoproterenol, epinephrine, norepinephrine, and dopamine. *J Pharmacol Exp Ther*. **327** (3): 760-769.
42. Titeler M, Weinreich P, Sinclair D and Seeman P (1978). Multiple receptors for brain dopamine. *Proc Natl Acad Sci U S A*. **75** (3): 1153-1156.
43. Cornil CA and Ball GF (2008). Interplay among catecholamine systems: dopamine binds to alpha2-adrenergic receptors in birds and mammals. *J Comp Neurol*. **511** (5): 610-627.
44. Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, Seeman P and Wong DT (1996). Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology*. **14** (2): 87-96.
45. Gioanni Y, Thierry AM, Glowinski J and Tassin JP (1998). Alpha1-adrenergic, D1, and D2 receptors interactions in the prefrontal cortex: implications for the modality of action of different types of neuroleptics. *Synapse*. **30** (4): 362-370.
46. Briand LA, Gritton H, Howe WM, Young DA and Sarter M (2007). Modulators in concert for cognition: modulator interactions in the prefrontal cortex. *Prog Neurobiol*. **83** (2): 69-91.
47. Venator DK, Lewis DA and Finlay JM (1999). Effects of partial dopamine loss in the medial prefrontal cortex on local baseline and stress-evoked extracellular dopamine concentrations. *Neuroscience*. **93** (2): 497-505.
48. Wang HD and Deutch AY (2008). Dopamine depletion of the prefrontal cortex induces dendritic spine loss: reversal by atypical antipsychotic drug treatment. *Neuropsychopharmacology*. **33** (6): 1276-1286.
First description of spine changes in the PFC and alterations through antipsychotic treatment.
49. Sokolowski JD, McCullough LD and Salamone JD (1994). Effects of dopamine depletions in the medial prefrontal cortex on active avoidance and escape in the rat. *Brain Res*. **651** (1-2): 293-299.
50. Newman LA, Darling J and McGaughy J (2008). Atomoxetine reverses attentional deficits produced by noradrenergic deafferentation of medial prefrontal cortex. *Psychopharmacology (Berl)*. **200** (1): 39-50.
51. Arnsten AF (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci*. **10** (6): 410-422.
52. Tassin JP (1992). NE/DA interactions in prefrontal cortex and their possible roles as neuromodulators in schizophrenia. *J Neural Transm Suppl*. **36**: 135-162.
53. Lieberman JA and Koreen AR (1993). Neurochemistry and neuroendocrinology of schizophrenia: a selective review. *Schizophr Bull*. **19** (2): 371-429.
54. Westerink BH, de Boer P, de Vries JB, Kruse CG and Long SK (1998). Antipsychotic drugs induce similar effects on the release of dopamine and noradrenaline in the medial prefrontal cortex of the rat brain. *Eur J Pharmacol*. **361** (1): 27-33.
55. Li XM, Perry KW, Wong DT and Bymaster FP (1998). Olanzapine increases in vivo dopamine and norepinephrine release in rat prefrontal cortex, nucleus accumbens and striatum. *Psychopharmacology (Berl)*. **136** (2): 153-161.

Describes the correlation of DA and NE levels in response to antipsychotic treatment.

56. Gresch PJ, Sved AF, Zigmond MJ and Finlay JM (1995). Local influence of endogenous norepinephrine on extracellular dopamine in rat medial prefrontal cortex. *J Neurochem*. **65** (1): 111-116.
57. Pozzi L, Invernizzi R, Cervo L, Vallebuona F and Samanin R (1994). Evidence that extracellular concentrations of dopamine are regulated by noradrenergic neurons in the frontal cortex of rats. *J Neurochem*. **63** (1): 195-200.
58. Valentini V, Frau R and Di Chiara G (2004). Noradrenaline transporter blockers raise extracellular dopamine in medial prefrontal but not parietal and occipital cortex: differences with mianserin and clozapine. *J Neurochem*. **88** (4): 917-927.
59. 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. *J Neurosci*. **22** (2): 389-395.
60. Devoto P, Flore G, Pani L and Gessa GL (2001). Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol Psychiatry*. **6** (6): 657-664.
61. Freed C, Revay R, Vaughan RA, Kriek E, Grant S, Uhl GR and Kuhar MJ (1995). Dopamine transporter immunoreactivity in rat brain. *J Comp Neurol*. **359** (2): 340-349.
62. Miner LH, Jedema HP, Moore FW, Blakely RD, Grace AA and Sesack SR (2006). Chronic stress increases the plasmalemmal distribution of the norepinephrine transporter and the coexpression of tyrosine hydroxylase in norepinephrine axons in the prefrontal cortex. *J Neurosci*. **26** (5): 1571-1578.
63. Devoto P, Flore G, Pira L, Longu G and Gessa GL (2004). Alpha2-adrenoceptor mediated co-release of dopamine and noradrenaline from noradrenergic neurons in the cerebral cortex. *J Neurochem*. **88** (4): 1003-1009.
64. Ahn NG and Klinman JP (1989). Nature of rate-limiting steps in a compartmentalized enzyme system. Quantitation of dopamine transport and hydroxylation rates in resealed chromaffin granule ghosts. *J Biol Chem*. **264** (21): 12259-12265.
65. Devoto P, Flore G, Longu G, Pira L and Gessa GL (2003). Origin of extracellular dopamine from dopamine and noradrenaline neurons in the medial prefrontal and occipital cortex. *Synapse*. **50** (3): 200-205.
66. Devoto P, Flore G, Vacca G, Pira L, Arca A, Casu MA, Pani L and Gessa GL (2003). Co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex induced by clozapine, the prototype atypical antipsychotic. *Psychopharmacology (Berl)*. **167** (1): 79-84.
67. Devoto P, Flore G, Saba P, Fa M and Gessa GL (2005). Co-release of noradrenaline and dopamine in the cerebral cortex elicited by single train and repeated train stimulation of the locus coeruleus. *BMC Neurosci*. **6**: 31.
68. Devoto P, Flore G, Saba P, Castelli MP, Piras AP, Luesu W, Viaggi MC, Ennas MG and Gessa GL (2008). 6-Hydroxydopamine lesion in the ventral tegmental area fails to reduce extracellular dopamine in the cerebral cortex. *J Neurosci Res*. **86** (7): 1647-1658.

Shows through lesion studies that co-release of DA is occurring through NE terminals.

69. Broadbelt K, Byne W and Jones LB (2002). Evidence for a decrease in basilar dendrites of pyramidal cells in schizophrenic medial prefrontal cortex. *Schizophr Res*. **58** (1): 75-81.
70. Ingham CA, Hood SH, van Maldegem B, Weenink A and Arbuthnott GW (1993). Morphological changes in the rat neostriatum after unilateral 6-hydroxydopamine injections into the nigrostriatal pathway. *Exp Brain Res*. **93** (1): 17-27.

FURTHER INFORMATION

Ariel Deutch's Lab:
http://www.mc.vanderbilt.edu/root/vumc.php?site=deutchlab

Role of the $5HT_{2c}$ receptor in regulation of metabolism and mesolimbic dopamine

Richard O'Neil

Serotonin (5HT) in the CNS plays an important role in regulation of behavioral and motivational states, and has an implied role in a number of behavioral pathologies including depression, schizophrenia, and drug abuse. 5HT acts in the CNS by binding receptors on the surface of neurons which then facilitate modulation of cellular processes and neuronal activity. The $5HT_{2c}$ receptor is a G-protein coupled receptor (GPCR) primarily expressed in the central nervous system. The $5HT_{2c}$ receptor is the only known GPCR subject to a form of post transcriptional modification known as RNA editing, a process in which specific adenosine residues are converted to inosine resulting in functional recoding of the mRNA. Editing of $5HT_{2c}$ receptor transcripts alters the functional signaling characteristics of the receptor, thus modulating the role the receptor plays in various neural processes. This review discusses evidence for the role of the $5HT_{2c}$ receptor in regulating feeding behavior, and mesoaccumbal dopamine signaling. Furthermore, it addresses the possible implications of dynamic RNA editing in these processes and discusses approaches to better understand the relationship *between* RNA editing and receptor function in vivo.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: richard.t.oneil@vanderbilt.edu.

A fundamental requirement for the evolution of complex nervous systems is the capacity for plasticity at the molecular level. Enzymes, known as Adensosine Deaminases which Act on RNA (ADARs), have coevolved with nervous systems in many species to catalyze hydrolytic deamination of specific adenosine residues on RNA sequences, resulting in functional alteration of RNA transcripts[1]. In mammals, two genes have been shown to encode catalytically active ADARs (ADAR 1 and ADAR 2), and both are expressed in most tissues but are highly enriched in the CNS[2,3]. Through their ability to convert adenosines to inosines at specific residues on protein coding RNAs, ADARs have been shown to alter amino acid codons with profound functional implications in a number of genes. This recoding occurs because inosine is processed by the translational machinery as guanosine. This results in a functional coding alteration of A-to-G at edited residues[1]. Most of the characterized substrate RNAs modified by ADARs encode proteins associated with neuronal signaling. These include voltage gated and ligand gated ion channels[4,5], fast synaptic release machinery[6], and at least one G-protein coupled neurotransmitter receptor; the $5HT_{2c}$ receptor[7]. The necessity of ADAR activity for viability and nervous system function has been clearly demonstrated by a number of genetically modified mouse models in which activity of the respective ADARs or their editing activity on specific substrates has been disrupted[8,9,10].

RNA editing is a conserved mechanism allowing for precise and dynamic modulation of protein functions within the nervous system. This unique process provides insight into the critical aspects of protein function and makes it a powerful beacon to guide further scientific inquiry in molecular neuroscience. For example, the transcript encoding the serotonin 2c receptor ($5HT_{2c}$) can be edited at 5 adenosines in exon 5 of the mature mRNA. These sites are denoted A, B, E, C, and D sites respectively 5' to 3' (**Figure 1c**). Considerable differences in signaling properties have been observed *in vitro* for a number of the unique protein isoforms generated by different combinations of editing at these 5 sites. The functional differences result from modulation of three amino acid codons which genomically encode isoleucine-asperigine-isoleucine respectively at residues 156-158-160 located within the second intracellular loop of the mature receptor. Editing at these amino acid codons allows for the production of 24 unique protein isoforms which can differ by as few as one or as many as three amino acids[7] (**Figure 1**). The second intercellular loop of the receptor is known to be important for g-protein coupling and efficient activation of the $G_{q/11}$- phospholipase C beta (PLCβ) signaling cascade[11-13]. *In vitro* evidence suggests that protein isoforms generated by different combinations of editing at these three amino acid codons have profoundly altered properties with respect to trafficking and signaling efficacy [7,14,15,18]. Among the differences between edited isoforms is variability in ligand independent signaling capacity, termed constitutive activity. Constitutive activation of the

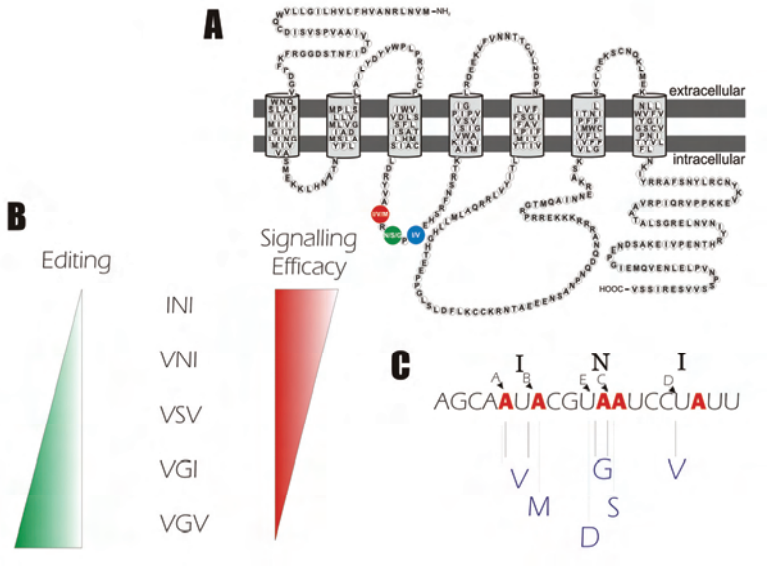


Figure 1 | **$5HT_{2c}$ isoforms. a |** Proposed 7TM domain structure and amino acid sequence of the Serotonin 2c receptor amino acids subject to aleration by editing shown in colored spheres. **b |** Schematic representation summarizing the relationship between editing and signaling efficacy of respective receptor isoforms. **c |** Pre-mRNA sequence of edited region showing editing sites and assigned names designated for each site. Single letter codes for amino acids encoded by non-edited transcript shown in black; amino acids encoded after editing at each position are indicated in blue.

$5HT_{2c}$ receptor has been observed in multiple cell lines transfected with transcripts encoding several different isoforms of the receptor [14,16,18]. Importantly, this constitutive activity is not detectable in cells expressing only the fully edited receptor isoform encoding valine-glycine-valine (VGV) and the activity is significantly reduced in isoforms encoded by more edited transcripts VNV and VSV[14]. With respect to ligand-dependant receptor activation, $5HT_{2c}$ receptor agonists have reduced signaling efficacy and affinity for isoforms encoded by more edited transcripts (**Figure 1b**). This reduction in agonist efficacy has been attributed to the existence of high and low affinity state receptors. According to the modified ternery complex model; receptors with constitutive activity fluctuate between inactive (R) and active (R*-G) conformations and the level of constitutive activity is a reflection of the relative time spent in R*-G state. Agonist binding promotes formation of R*-G complex and the affinity of an agonist is higher for the R*-G complex than for R. The more edited isoforms are less likely to achieve the R*-G complex in absence of agonist and therefore have reduced constitutive activity and exist predominately in a low affinity state[16]. The existence of multiple affinity states is supported by experiments in which non-hydrolysable GTP analogs are used to fully uncouple g-proteins (G) from the R-G complex, resulting in only low affinity state receptors[14,16]. The fact that all receptor isoforms tested can achieve similar maximal activation of PI hydrolysis suggests that the ability of $5HT_{2c}$ receptors to couple with g-proteins is not affected by editing and that signaling efficacy is primarily altered by ligand affinity and constitutive receptor activation[16]. While a great deal of progress has been made in characterizing the role of editing at this receptor for signaling in vitro, the role of distinct isoforms *in vivo* is not understood.

The $5HT_{2c}$ receptor has a broad expression profile in the CNS [17] and has been repeatedly implicated in the regulation of feeding behavior[20] and reward pathways[19]. Based on the *in vitro* data, the expression of distinct $5HT_{2c}$ receptor protein isoforms by RNA editing has the potential to profoundly affect the role of the receptor in these systems. This review will focus on recent work illuminating the role of the $5HT_{2c}$ receptor in the regulation of mesoaccumbal dopamine, as well as this receptor's role in regulating feeding behavior. Furthermore, it will discuss approaches to better characterize the distribution of edited isoforms in various cell types and brain regions. Finally, it will outline efforts to test the hypothesis that dynamic regulation of editing allows for precise modulation of behavior.

$5HT_{2C}$ RECEPTORS REGULATE METABOLISM AND FEEDING BEHAVIOR IN MICE

Regulating metabolism and feeding behavior is one of the most ubiquitous and fundamental functions facilitated by the mammalian nervous system. The brain maintains metabolic homeostasis through regulation of autonomic tone, glucose homeostasis, and by providing the motivational drive to consume food. The notion that 5HT plays an important role in the regulation of feeding became evident in the early 1970's when the drug fenfluramine came on the market for weight loss. The anorexigenic effects of fenfluramine have been attributed to its ability to increase extracellular 5HT levels[20]. Recent studies by Vickers et al, in which knockout mice lacking $5HT_{2c}$ receptors are treated with fenflouramine, provided strong evidence that the $5HT_{2c}$ receptor is primarily responsible for mediating the inhibition of feeding observed with fenfluramine treatment. It was observed that the anorexigenic efficacy of fenflouramine was greatly attenuated in these $5HT_{2c}$ null animals[21]. Interestingly, phenotypic characterization of these mice lacking the $5HT_{2c}$ receptor revealed that they are hyperphagic and develop adult onset obesity[22]. More recently, electrophysiological and molecular techniques have allowed for a more precise characterization of the $5HT_{2c}$ receptor's role in feeding. Heisler et al provide strong evidence that pro-opiomelanocortin (POMC) neurons located within the arcuate nucleus of the

hypothalamus express the $5HT_{2c}$ receptor and that receptor activation promotes excitability and α-MSH release[23]. Accumulating evidence implicating the $5HT_{2c}$ receptor as a key component in the regulation of feeding behavior has made the receptor an attractive target for development of anti-obesity drugs[20].

To understand the $5HT_{2c}$ receptor's role in physiology and attempt to develop informed pharmacologic interventions it is imperative to consider the variable signaling properties observed among different receptor isoforms. RNA editing of transcripts encoding $5HT_{2c}$ receptor results in production of receptor proteins with profound differences in constitutive activity and 5HT mediated signaling efficacy. Several labs have generated mice genetically modified to express only protein isoforms resulting from fully edited transcripts (VGV) or non-edited transcripts (INI) to study the effects of these isoforms respectively *in vivo*. Significant metabolic alterations in the animals which only express the VGV receptor isoform suggest that the role of this receptor in regulating metabolism is more complicated that previously appreciated. Specifically, these mice display increased basal metabolic rate which was found to be independent of the melanocortin- 4 receptor. Consistent with the hypothesis that efficient excitatory drive onto POMC neurons by the $5HT_{2c}$ receptor is required for normal inhibition of feeding, these mice display adult onset hyperphagia similar to that observed in the $5HT_{2c}$ null animals[24]. Currently, it is not clear if this hyperphagia is directly related deficient activation POMC neurons in the Arc, or an indirect feedback mechanism related the enhanced basal metabolic rate. Importantly, these results demonstrate a previously unappreciated fundamental role for the $5HT_{2c}$ receptor in regulating metabolic function.

Throughout evolution, animals have had to cope with tremendous variations in food availability, across seasons and through generations, requiring the evolution of highly dynamic metabolic regulation. Serotonin plays an important role in maintaining many aspects of homeostasis, but the specific role that RNA editing plays in these processes is not known. Interestingly, it has been observed that prenatal dietary deficiencies can lead to altered brain serotonin homeostasis[25], metabolic disfunction, and reduced sensitivity to d-fenflouramine[26]. Specifically, Lopez de Souza et al observed significant reductions in d-fenfluramine-induced suppression of feeding and arcuate c-fos activation in rats exposed to perinatal protein deficiency. The levels of $5HT_{2c}$ receptor transcripts are unchanged in these animals suggesting that a mechanism downstream of receptor expression is responsible for this lack of sensitivity[26]. The RNA-editing profile for the $5HT_{2c}$ receptor has not been characterized in these rats, but alterations in editing have the potential to contribute to the findings observed in these studies. Characterization of RNA-editing dynamics in POMC neurons during normal development and in response to prenatal malnutrition may elucidate the relationship between metabolic disfunction and $5HT_{2c}$ receptor function.

$5HT_{2C}$ RECEPTORS REGULATE MESOLIMBIC DOPAMINE SIGNALING

Animals possess the unique ability to physically interact with their environment in order to promote their own survival and reproduction. Goal directed behavior and adaptive learning have evolved in higher vertebrates to facilitate this need to efficiently respond to the nearly infinite possible circumstances an animal may find itself in. Mechanistically, goal directed behavior and adaptive learning requires; goal identification, perception of relevant sensory stimuli, generation of possible motor sequences, and anticipation of the relative utility of particular motor sequences in attaining the goal. Furthermore, the ability to encode reward upon the execution of effective motor sequences allows animals to modify and perfect behavioral sequences to efficiently achieve goals. The mesolimbic system, which includes; the Nucleus Accumbens (NAc), olfactory tubercle, ventral pallidum (VP), mediodorsal thalamus, ventral tegmental area, lateral hypothalamus, limbic cortex, and amygdala provides the neural substrate for reward associated learning and motivated behavior[27]. Dopamine (DA) release in the NAc and olfactory tubercle is thought to be a key component in goal directed selection of motor programs and underlie the neural coding of reward[28]. Dopamine is supplied to the ventral striatum primarily by groups of neurons located in the midbrain known collectively as the ventral tegmental area (VTA), and its release in the medial shell of the NAc and medial olfactory tubercle directly correlates with the rewarding effects of achieving goals as well as the reinforcing effects of drugs of abuse[28].

5HT is supplied to the structures of the mesolimbic system by neurons in the dorsal raphe and plays a significant role in the regulation of NAc DA release[33] and $5HT_{2c}$ receptor transcripts can be detected by in situ hybridization the VTA and throughout the NAc[17]. Studies in which selective $5HT_{2c}$ receptor agonists, antagonists, and inverse agonists are administered while monitoring DA levels in NAc via microdialysis suggest that activation of $5HT_{2c}$ receptors have a net inhibitory effect on both baseline and drug induced DA release in the ventral striatum. Navailles et al showed that intra-accumbal infusion of inverse agonist SB 206553, but not antagonist SB 242084, increased basal DA efflux in the NAc. They also showed that the acute decrease in

basal accumbal DA levels elicited by systemic infusion of agonist Ro 60-0175 was attenuated by intra VTA infusion of antagonist SB 242084[39]. Taken together, these results suggest that phasic activation of receptors in the VTA attenuates DA release and constitutive activity of receptors in the NAc provide tonic suppression of DA release. Further supporting an inhibitory role for $5HT_{2c}$ receptors with respect to DA release, Gobert et al showed that systemic administration of inverse agonist and agonist dose dependently increased and decreased respectively, the firing rate of DAergic neurons in the VTA[33]. Activation of $5HT_{2c}$ receptors has been shown to excite cells via activation of PLC and subsequent modulation of ion channel activity[29,30]. These observations have lead to the hypothesis that $5HT_{2c}$ receptors in the NAc and VTA negatively regulate DA release by DAergic neurons by activating inhibitory GABAergic interneurons which, in turn, directly inhibit DAergic neuron activity. In agreement with this hypothesis, immunoreactivity has been observed primarily in GABAergic cell bodies in the VTA and NAc as implied by co-staining of cells with antibodies against glutamate decarboxylase the enzyme that synthesizes GABA from glutamate[31, 32].

Recently, several studies have attempted to evaluate the effects of various serotonin receptor ligands on the acquisition of stimulant addiction and on reinstatement of drug seeking behavior after extinction. The inhibitory role of $5HT_{2c}$ receptor agonists on DA release has lead to the hypothesis that such drugs could be effective in treating addiction. Indeed, several studies have indicated that systemic administration of agonists decreases the acute hyperlocamotive response to stimulants and reduces self-administration and reinstatement of cocaine seeking bahavior[34]. As noted above, investigation into the regulation of DA by $5HT_{2c}$ receptors has indicated that several distinct populations of receptors exist each with different functional roles in regulation of DA[19, 34]. All of the subcortical $5HT_{2c}$ receptor populations analyzed to date seem to provide a net inhibitory drive on NAc DA release, but the differential responses to inverse agonists, antagonists, and agonists suggests that the receptors play distinct roles in responding to 5HT. Functional differences of 5HT populations merit further investigation; it is possible that select cell types express different edited isoforms with variable levels of constitutive activity and provide tonic or phasic control of DA release respectively.

CONCLUSIONS

A number of studies have attempted to profile the distribution of $5HT_{2c}$ receptor edited isofoms in the brains regions of humans who had suffered from neuro-pathologies, particularly depression and schizophrenia[35,36]. One study by Gurevich et al reported that changes in editing observed in pre-frontal cortex of suicide victims was the opposite (at each respective editing site) of the changes that could be induced by treating mice with the SSRI fluoxetine[36]. The authors suggested not only that altered editing may contribute to pathology in humans, but also that editing could be manipulated by pharmacologic intervention affecting 5HT tone. Following these findings, effort was spent attempting to elucidate how sustained alterations in 5HT tone via pharmacologic intervention might affect editing of $5HT_{2c}$ receptor transcripts. Englander and Gurevich subsequently provided evidence for dynamic modulation of RNA editing by a number of factors including stress[37], 5HT depletion[38], and SSRI treatment[36,37]. The possibility that RNA editing can be dynamically modulated in a substrate specific manner carries compelling implications with respect to normal and pathologic brain function. While the relationship between RNA editing dynamics, drug treatment, and behavioral pathology is not understood, it is an important phenomenon to consider when studying CNS function.

Accumulating evidence implies that the $5HT_{2c}$ receptor is a key component in the transduction of 5HT's signal in the CNS. This review discussed receptor function in two key systems which regulate fundamental aspects of behavior; hypothalamic regulation of metabolism, and DA regulation in the ventral striatum. In addition to being medically relevant behavioral systems, these circuits are relatively well characterized, have readily observable outputs, and several well established experimental paradigms. With the recent development of tools such as knock-in mice expressing only specific edited isoforms of the receptor, and selective ligands, it will be possible to begin understanding the role of this neurotransmitter receptor's unique and complex molecular biology. Serotonin related drug intervention is at the forefront of the struggle to treat a wide array of psychiatric disorders. To develop informed therapies based on modulation of serotonin signaling it is imperative to understand the role played by this prominent monoamine receptor.

REFERENCES:

1. Sansam, C.L. and R.B. Emeson (2005). mRNA Editing. In: Encyclopedia of Life Sciences. John Wiley & Sons.
2. Melcher T, Maas S, Herb A, Sprengel R, Seeburg PH, Higuchi M. (1996) A mammalian RNA editing enzyme. *Nature.* **379**: 460–464.
3. Chen CX, Cho DS, Wang Q, Lai F, Carter KC, Nishikura K. *(2000)* A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *RNA.* **6**: 755–767.

4. Sommer B, Kohler M, Sprengel R, Seeburg PH. (1991). RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell*. **67**: 11–19.
5. Bhalla T, Rosenthal JJ, Holmgren M, Reenan R. (2004). Control of human potassium channel inactivation by editing of a small mRNA hairpin. *Nat. Struct. Mol. Biol*. **11**: 950–956
6. Reenan RA. (2005). Molecular determinants and guided evolution of species specific RNA editing. *Nature*. **434**: 409–413.
7. Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson R.B. (1997). Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature.* **387**: 303–308.
8. Brusa R, Zimmermann F, Koh DS, Feldmeyer D, Gass P, Seeburg PH, Sprengel R. (1995) Early-onset epilepsy and postnatal lethality associated with an editing-deficient GluR-B allele in mice. *Science.* **270**: 1677–1680
9. Hartner JC, Schmittwolf C, Kispert A, Muller AM, Higuchi M, Seeburg PH (2004). Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. *J. Biol. Chem.* **279:** 4894–4902.
10. Higuchi M, Maas S, Single FN, Hartner J, Rozov A, Burnashev N, Feldmeyer D, Sprengel R, Seeburg PH (2000). Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature.* **406**: 78–81.
11. Chang M, Zhang LS, Tap JP, Sanders-Bush E (2000). Disecting G protein coupled receptor signaling pathways with membrane-permeable blocking peptides – endogenous $5HT_{2c}$ receptors in choroid plexus epithelial cells. *J. Biol. Chem.* **275**: 7021-7029.
12. Moro O, Lameh J, Hogger P, Sadee W (1993). Hydrophobic amino acid in the i2 loop plays a key role in receptor-G protein coupling. *J. Biol. Chem.* **268**: 22273-22276
13. Burstein ES, Spalding TA, Brann MR (1998). The second intracellular loop of the m5 muscarinic receptor is the switch which enables G-protein coupling. *J. Biol. Chem.* **273**: 24322-24327.
14. Herrick-Davis K, Grinde E, Niswender CM (1999). $5HT_{2c}$ Receptor RNA Editing Alters Receptor Basal Activity: Implications for Serotonergic Signal Transduction. *Journal of Neurochemistry*. **73** (4): 1711-1717.
15. Marion S, Weiner DM, Caron MG (2004). RNA Editing Induces Variation in Desensitization and Trafficking of $5HT_{2c}$ Receptor Isoforms. *J. Biol. Chem.* **279** (4): 2945-2954.
16. Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E (1999). RNA editing of the Human $5HT_{2c}$ Receptor silences constitutive activity. *J. Biol. Chem.* **274** (14): 9472-9478.
This paper shows the profound changes in the capacity for ligand independent signaling that can result from editing.
17. Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L (1995). Comparative Localization of Serotonin 1a, 1c, and 2 Receptor Subtype mRNAs in Rat Brain. *Journal of Comparative Neurology.* **351**: 357-373
18. Wang Q, O'Brian PJ, Chen CX, Cho DC, Murray JM, Nishikura K (2000). Altered G-Protein coupling functions of RNA Editing Isoform and Splicing Variant Serotonin 2c Receptors. *Journal of Neurochemistry.* **74** (3): 1290-1300.
19. Alex KD, Pehek EA (2007). Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacology and Thereputics*. **113**: 296-320.
20. Miller KJ, Azzara AV (2008). An Old Dog Learns a New Trick: Regulation of Peripheral Glucose Homeostasis by the Serotonin(5-HT)2C Receptor. *Molecular Interventions.* **8** (2): 73-77.
21. Vickers SP, Clifton PG, Dourish CT, Tecott LH (1999). Reduced satiating effect of d-fenfluramine in serotonin 5-HT(2C) receptor mutant mice. *Psychopharmacology(Berl).* **143** (3): 309-314.
22. Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D (1995). Eating disorder and epilepsy in mice lacking $5HT_{2c}$ receptors. *Nature*. **374**: 542-546.
23. Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD, Elmquist JK (2002). Activation of central melanocortin pathways by fenflouramine. *Science.* **297**: 609-611.
This study showed that serotonin 2c receptors on POMC neurons in the arcuate nucleus play an important role in the hypothalamic regulation of feeding behavior.
24. Kawahara Y, Grimberg A, Teegardern S, Mombereau C, Liu S, Bale TL, Blendy JA, Nishikura K (2008). Dysregulated Editing of Serotonin 2C Receptor mRNAs Results in Energy Dissipation and Loss of Fat Mass. *J. Neuroscience.* **28** (48): 12834-12844.
25. Manjarrez GG, Chagoya GG, Hernandez J (1994). Early nutritional changes modify the kinetics and phosphorylation capacity of tryptophan-5-hydroxylase. *Int. J. Dev. Neurosci*. **12**: 695–702.
26. Souza SL, Orozco-Solis R, Grit I, Castro RM, Bolanos-Jimanez F (2008). Perinatal protein restriction reduces the inhibitory action of serotonin on food intake. *Eur. J. Neuroscience.* **27**: 1400-1408
27. Swanson LW (2000). Cerebral hemisphere regulation of motivated behavior. *Brain Research.* **886**:113-164.
28. Ikemoto (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Reviews.* **56** (1): 27-78.
29. Stanford IM, Kantarina MA, Chahal HS, Loucif KC, Wilson CL (2005). 5-Hydroxytryptamine induced excitation and inhibition in the STN: action at 5HT(2c), 5HT(4), and 5HT(1a) receptors. *Neuropharmacology.* **49**: 1228-1234.
30. Qiu J, Xue C, Bosch MA, Murphy JG, Fan W, Rønnekleiv OK, Kelly MJ (2007). Serotonin 2c receptor signaling in hypothalamic POMC neurons: role for energy homeostasis in females. Molecular Pharmacology. **72** (4):885-896
31. Bubar MJ, Cunningham KA (2007). Distribution of serotonin 5-HT2C receptors in the ventral tegmental area. Neuroscience. **146** (1):286-97
32. Liu S, Bubar MJ, Lanfranco MF, Hillman GR, Cunningham KA (2007). Serotonin2C receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. *Neuroscience*. **146** (4):1677-88
33. Gobert A, Rivet JM, LeJeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP, Cistarelli L, Melon

C, Millan MJ (2000). Serotonin2c receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: A combined dialysis and electrophysiological analysis in the rat. *Synapse*. **36** (3):205-21

34. Dhonnchadha BA, Cunningham KA (2008). Serotonergic mechanisms in addiction related memories. *Behavioral Brain Research*. **195** (1):39-53.
35. Niswender CM, Herrick-Davis K, Dilley GE, Meltzer HY, Overholser JC, Stockmeier CA, Emeson RB, Sanders-Bush E (2001). RNA editing of the human serotonin 5-HT2C receptor. alterations in suicide and implications for serotonergic pharmacotherapy. *Neuropsychopharmacology.* **24** (5):478-91
36. Gurevich I, Hadassah T, Arango V, Dwork A, Mann JJ, Schmauss C (2002). Altered Editing of Serotonin 2C Receptor Pre-mRNA in the Prefrontal Cortex of Depressed Suicide Victims. *Neuron.* **34**: 349-356.
    This paper provides evidence that altered editing contributes to suicidal depression and that SSRI treatment can result in reversal of these alterations.
37. Englander MT, Dulawa SC, Bhansali P, Schmauss C (2005). How stress and fluoxetine modulate serotonin 2c receptor pre-mRNA editing. *J. Neuroscience.* **25** (3): 648-651.
38. Gurevich I, Englander MT, Adlersberg M, Seigal NB, Schmauss C (2002). Modulation of serotonin 2c receptor editing by sustained changes in serotonergic neurotransmission. *J. Neuroscience*. **22** (24): 10529-10532.
39. Navailles S, Moison D, Ryczko D, Spampinato U (2006). Region-dependant regulation of mesoaccumbens dopamine neurons *in vivo* by the constitutive activity of central serotonin 2c receptors. *Journal of Neurochemistry*. **99**: 1311-1319.
    This study provides evidence for the roles of different populations of receptors in distinct brain regions regulating NAc dopamine release.

FURTHER INFORMATION

Ron Emeson's Lab:
http://kc.vanderbilt.edu/site/people/1450/emeson-ron.aspx

Assembly and Heterogeneity of $GABA_A$ Receptors

Katharine N. Gurba

$GABA_A$ receptors ($GABA_A$Rs) are pentameric, ligand-gated chloride channels that mediate the majority of fast inhibitory synaptic neurotransmission in the brain. The receptors are assembled from a repertoire of 19 subunits (α1-6, β1-3, γ1-3, δ, ε, π, and ρ1-3), providing the possibility for vast isoform heterogeneity. Because the subunit subtypes included in a receptor determine its physiological and pharmacological properties, identification of receptor isoforms has clear clinical relevance. A large body of literature indicates that $GABA_A$Rs do not assemble randomly; rather, incorporation of specific subunits into a receptor is regulated at many levels. Each subunit has a characteristic temporal and spatial expression pattern; however, most neurons express many $GABA_A$R subunits at once. Consequently, certain "rules" of assembly must exist to limit receptor heterogeneity. In this review, we discuss the regulation of $GABA_A$R biogenesis, including limitation of heterogeneity, as well as the specific receptor isoforms that have been identified *in vivo.*

Phasic inhibition
Inhibition resulting from transient activation of synaptic $GABA_A$ receptors by presynaptically-released GABA; gives rise to inhibitory postsynaptic currents (IPSCs).

Tonic inhibition
Inhibition resulting from persistent activation of peri- or extrasynaptic $GABA_A$ receptors by ambient GABA.

Benzodiazepines
Compounds that potentiate the response of certain $GABA_A$ receptors; used clinically for their anticonvulsant, anxiolytic, sedative, and amnestic effects.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: kate.gurba@vanderbilt.edu.

The vast majority of inhibitory neurotransmission in the brain is mediated by γ-aminobutyric acid (GABA). It has been detected in approximately 30% of all synapses[1] and acts via ionotropic $GABA_A$ receptors, which mediate fast inhibitory neurotransmission[2], and metabotropic $GABA_B$ receptors, which mediate slower inhibitory effects[3]. $GABA_A$ receptors ($GABA_A$Rs) are chloride channels belonging to the Cys-loop receptor superfamily of ligand-gated ion channels (LGIC), which also includes nicotinic acetylcholine receptors (nAChR), 5-hydroxytryptamine type 3 receptors (5-HT3), and glycine receptors (GlyR)[4]. Like most members of this superfamily, $GABA_A$Rs are pentamers that are assembled from an array of homologous subunits. All subunits share a common structure: each contains a large, extracellular N-terminal domain, which contains the ligand-binding site and the eponymous Cys-loop; four α-helical transmembrane domains (M1-4); a large intracellular loop between the third and fourth transmembrane helices (M3-M4 loop); and a very short, extracellular C-terminal domain[5] (**Figure 1a**).

Nineteen subunits, grouped by sequence homology into eight subunit families, have been identified for the $GABA_A$ receptor: α1-6, β1-3, γ1-3, δ, ε, π, and ρ1-3[6]. Several of these subunit subtypes also undergo alternative splicing and/or RNA editing, further increasing the potential diversity of $GABA_A$ receptor isoforms. Each subunit exhibits a characteristic expression pattern in the brain; however, these patterns overlap extensively. Indeed, a single neuron can express many subunits simultaneously. Consequently, many but not all of the mathematically-possible $GABA_A$R isoforms could exist somewhere in the brain. The most common isoforms, however, are thought to comprise two α subunits, two β subunits, and one γ or δ subunit[7-9] (**Figure 1b**), though this remains a subject of vigorous debate.

The large variety of $GABA_A$R isoforms exhibit a concomitant variety of physiological properties[2]. For instance, most receptors containing a γ subunit are located in the synapse, where they mediate phasic inhibition in response to presynaptically-released GABA[10]. These receptors have a relatively low affinity for GABA, activate quickly, desensitize extensively, and deactivate slowly. Conversely, receptors containing a δ subunit are located outside the synapse, where they mediate tonic inhibition in response to low concentrations of ambient GABA. Unsurprisingly, δ-subunit-containing receptors also differ physiologically; they have a relatively high affinity for GABA, activate slowly, desensitize minimally, and deactivate rapidly[11].

Additionally, $GABA_A$Rs have been linked to many diseases and disorders, including epilepsy[12-14], insomnia[15], anxiety[16], depression[16], schizophrenia[17], alcoholism[18], and autism[19]. Predictably, then, $GABA_A$Rs are targeted by numerous drugs, particularly sedatives, anxiolytics, and anticonvulsants; examples include benzodiazepines, zolpidem, etomidate, and propofol[20, 21]. Both the pathology and the pharmacology of $GABA_A$Rs depend highly upon receptor subunit composition – for instance, epilepsy-associated mutations have been identified only in the α1, β3, γ2, and δ subunits, and benzodiazepines act only at receptor isoforms containing both a γ subunit and certain α subunits.

Given the prevalence of $GABA_A$R expression, the pathology resulting from receptor malfunction, and the pharmacological dependence upon isoform

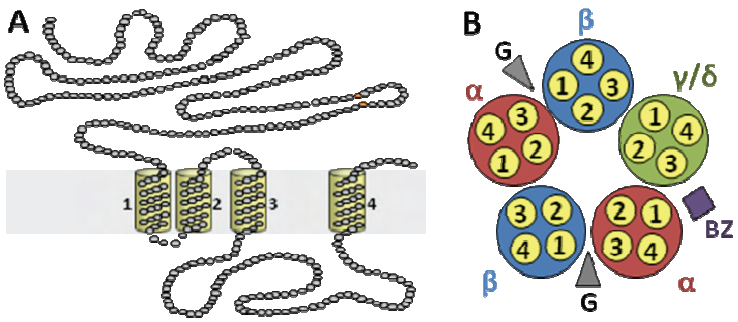


Figure 1 | **$GABA_A$ receptor morphology. a |** Structure of a $GABA_AR$ subunit. Cys-loop cysteines marked in orange; transmembrane domains enclosed in cylinders and numbered 1-4. **b |** Schematic view of most common $GABA_AR$ isoform (putative) from the synaptic cleft . G = GABA binding site; BZ = benzodiazepine binding site.

Zolpidem
Compound with structure and physiological effects similar to those of benzodiazepines; used clinically to treat insomnia.

Etomidate and propofol
Intravenous general anesthetics that potentiate the response of certain $GABA_A$ receptors.

N-linked glycosylation
The transfer, by the ER-resident enzyme oligosaccharyl transferase, of a 14-sugar "core" oligosaccharide to asparagines on newly-synthesized polypeptides. Asparagines contained in the sequence Asn-Xaa-Ser/Thr (where Xaa is any amino acid other than proline) are candidates for glycosylation.

Glycan trimming
The modification of core oligosaccharides by enzymes in the Golgi apparatus.

Palmitoylation
The covalent attachment of palmitate, a 16-carbon saturated fatty acid, to cysteine residues.

identity, it is clearly important to understand the process of receptor assembly. Therefore, in this review, we will examine the generation of $GABA_AR$ diversity. First, we will review the general processes of receptor biogenesis, after which we will discuss the selective oligomerization of $GABA_AR$ subunits. Finally, we will examine the ultimate product of these processes: native $GABA_A$ receptor isoforms.

BIOGENESIS OF $GABA_A$ RECEPTORS

As with other LGICs, $GABA_A$ receptor subunits are inserted co-translationally into the membrane of the endoplasmic reticulum (ER). There, they fold and oligomerize in a process that depends heavily upon ER-resident chaperones. The process of receptor oligomerization is slow and inefficient; studies suggest that approximately 70% of subunits are degraded without being incorporated into a pentameric receptor, and receptors do not appear on the cell surface for several hours following transfection[22]. While in the ER, $GABA_A$ receptor subunits also undergo typical protein modifications, including the early stages of N-linked glycosylation. Interestingly, however, N-linked glycosylation is not required for subsequent forward trafficking, although multiple glycosylation sites have been identified on all subunits[23] and glycosylation is necessary for proper assembly and trafficking of other Cys-loop receptors[24, 25]. Properly folded and assembled subunits proceed to the Golgi apparatus, where they undergo further modification such as palmitoylation and glycan trimming[26]. With the assistance of multiple $GABA_AR$-associated proteins, receptors are then trafficked to the neuronal surface. They may be inserted directly into their final subcellular location (*i.e.* post-, peri-, or extrasynaptic), or they may diffuse into that location after membrane insertion[27]. Finally, $GABA_ARs$ undergo constitutive and activity-dependent endocytosis (both clathrin-dependent and clathrin-independent)[28], after which they are recycled to the cell surface or targeted for lysosomal degradation. Every step of $GABA_A$ receptor assembly and trafficking is regulated by signals within the subunits[29] as well as by various associated proteins[30].

SELECTIVE OLIGOMERIZATION OF $GABA_A$ RECEPTOR SUBUNITS

After temporal and spatial regulation of subunit expression, the first (and, arguably, the most important) opportunity for a neuron to control what $GABA_A$ receptor isoforms it will produce is the process of selective subunit oligomerization. Presumably, a neuron expressing many $GABA_AR$ subunit subtypes would have a hierarchical yet flexible assembly mechanism that favors association between certain subunits and, ultimately, directs the incorporation of assembly intermediates (e.g. dimers, trimers) into full receptors. Indeed, several studies have indicated that, though all subunit combinations can form oligomers, only a subset can form pentamers[23]. This is a key distinction because pentamers are trafficked to the cell surface, but oligomers of lower molecular weight are retained in the ER and subsequently degraded[23, 31]. Importantly, some disease-causing mutations appeared to reduce surface expression and function by disrupting the process of oligomerization[14].

Expression of recombinant subunits in heterologous cells has provided insight into the "rules" governing assembly of the most prevalent subunit subtypes. When expressed individually, α1, β2, and γ2 subunits formed primarily monomers and dimers, as did combinations of γ2 with either α1 or β2/3. Conversely, co-expression of α1 and β2/3 subunits, with or without γ2 subunits, predominantly yielded pentamers, indicating that the combination of α and β subunits is necessary and sufficient for complete receptor assembly[31, 32]. Interestingly, however, receptors including a third (non-α/β) subunit appear to assemble more efficiently. When α, β, and a third subunit (either γ, δ, ε, or π) were co-expressed in heterologous systems, the kinetic signature of αβ receptors could not be detected[33-35]; furthermore, that signature has been detected in very few neurons[36, 37]. Clearly, both neurons and heterologous cells are capable of selective oligomerization, suggesting the existence of assembly signals within the subunits themselves.

Several studies have, in fact, isolated amino acid sequences and individual residues that are important for specific subunit interactions[29, 38]. These sequences have been identified in the α1[39-43], α6[39], β3[42-45], γ2[42, 46], and γ3[47] subunits, primarily in the large N-terminal domain, though there were some reports of assembly sequences in the M3-M4 loop[48, 49]. Although homology modeling based on the

nAChR[50] and AChBP[51] has provided some insight into the structural basis of these interactions, it is important to note that these sequences might not directly contact adjacent subunits; rather, they might simply facilitate oligomerization by encouraging proper protein folding.

HETEROGENEITY *IN VIVO*: NATIVE $GABA_A$ RECEPTOR ISOFORMS

Most studies mentioned thus far have been conducted in heterologous expression systems or in cultured neurons. Because of the great potential for $GABA_AR$ heterogeneity, it is necessary to use such systems to investigate properties of specific subunits (*i.e.* assembly sequences) and isoforms (*i.e.* kinetic and pharmacological properties). Unfortunately, these studies cannot answer a crucial question: what $GABA_A$ receptor isoforms actually exist in the brain? In an attempt to construct a standardized response to that question, the International Union of Pharmacology recently established a list of potential native $GABA_AR$ oligomers[6]. These receptor isoforms were divided into three categories ("identified", "existence with high probability", and "tentative") based on multiple types of evidence. The authors also specified a logical strategy, summarized below, for determining whether or not a receptor isoform exists *in vivo*. First, the long list of potential isoforms can be narrowed based on subunit co-expression patterns, which can be ascertained by *in situ* hybridization and immunoreactivity. If subunits are indeed co-expressed in a specific cell type, evidence for association of those subunits should then be sought, primarily through co-immunoprecipitation. Subunits that associate should be co-expressed in heterologous systems, where electrophysiology can be performed and characteristic kinetics and pharmacology can be assessed. These characteristic properties can then be sought in neurons. Finally, knockout animals can be created and studied for the absence of characteristic physiology and pharmacology associated with isoforms containing the deleted subunit. The list of "identified" and "high probability" isoforms, along with their localization (regional and subcellular) and basic forms of inhibition (phasic or tonic), is presented in **Table 1**.

Isoforms that have been unequivocally identified

Given the widespread distribution of the α1β2γ2 $GABA_AR$ isoform, it is perhaps unsurprising that this isoform is thought to account for up to 60% of all $GABA_A$ receptors in the brain[20]. Mice lacking either the α1 or β2 subunit have been generated; in both lines, total $GABA_AR$ expression in the brain was reduced by more than 50%[52]. A γ2 knockout mouse was found to lack 94% of all benzodiazepine binding sites[53] (recall that the BZ binding site is located at the interface of an α and a γ subunit; consequently, this result indicates that receptors including the γ1 or γ3 subunit might make up only 6% of all αβγ receptors). As indicated in **Table 1**, the other five α subunits can likewise co-assemble with β and γ2 subunits. Strong evidence for the existence of these αxβxγ2 receptors is provided by isoform-specific pharmacology from benzodiazepine (BZ) site ligands. Such ligands include classic benzodiazepines (*i.e.* diazepam); imidazobenzodiazepines (*i.e.* flumazenil and Ro15-4513); and the so-called "Z-drugs" (*i.e.* zolpidem and zaleplon).

Classic benzodiazepines cannot bind receptors containing α4 or α6 subunits, and they have much lower affinity for receptors containing γ1 or γ3 subunits than for receptors containing γ2 subunits. Furthermore, through the use of transgenic mice, the various actions of benzodiazepines have been attributed to specific α subunit subtypes. Point mutations conferring diazepam insensitivity were introduced into the genes of individual α subunits and the resulting mice were subjected to behavioral tests with and without administration of diazepam[54, 74, 75]. Results indicated that the α1 subunit mediated the sedative, anterograde amnestic, and some of the anticonvulsant effects of diazepam[74, 76]; the α2 and α3 subunits mediated the anxiolytic and muscle-relaxant effects[54, 75] and the α5 subunit was involved in amnestic effects as well as other aspects of learning and memory. Imidazobenzodiazepines, however, bind without regard to α subunit subtype. Therefore, receptors that are benzodiazepine-insensitive but imidazobenzodiazepine-sensitive can be identified as α4βγ2 or α6βγ2 isoforms. Conversely, Z-drugs act with differing potency at BZ-sensitive isoforms containing α1,2,3, or 5; specifically, they display high potency at α1βγ2 isoforms, lower potency at α2βγ2 and α3βγ2 isoforms, and no action at α5βγ2[77]. Taken together, these pharmacological properties allow positive identification of α1βγ2 and α5βγ2 receptors, as well as tentative identification of α(2,3)βγ2 and α(4,6)βγ2 receptors; however, expression patterns can differentiate these latter two pairs of isoforms. Consequently, all αβγ2 isoforms are considered to have been identified *in vivo*.

The aforementioned evidence accounts for six of the 11 identified native isoforms. Four of the remaining five isoforms contain the δ subunit, which possesses many unusual properties that help to identify δ-subunit-containing isoforms *in vivo*. First, the δ subunit has been found exclusively in extrasynaptic membranes, where it is incorporated into receptors that have a high affinity for GABA and mediate a constant, "tonic" current with low amplitude and little desensitization[11, 78]. The pharmacology of δ-subunit-containing receptors is

Table 1 | **$GABA_AR$ isoforms likely to exist *in vivo*.**

	Areas of high expression	Subcellular localization	Type of inhibition	Refs
Identified				
α1β2γ2	cerebral cortex (all layers) hippocampus (interneurons, principal cells) thalamus (relay nuclei) cerebellum (Purkinje and granule cells)	synaptic, extrasynaptic	phasic, tonic	52
α2βγ2	cerebral cortex (layers I-IV) hippocampus (pyramidal cells) striatum hypothalamus motor neurons	synaptic (most), extrasynaptic	phasic, tonic	54
α3βγ2	cerebral cortex (layers V-VI) hippocampus thalamus (nRT) cerebellum	synaptic (most), extrasynaptic	phasic, tonic	54
α4βγ2	hippocampus (granule cells) thalamus (relay nuclei)	synaptic, extrasynaptic	phasic, tonic	55
α4β2δ	thalamus (relay nuclei)	extrasynaptic	tonic	55, 56
α4β3δ	dentate gyrus (granule cells); thalamus	extrasynaptic	tonic	55
α5βγ2	hippocampus (pyramidal cells)	extrasynaptic – clustered (minor synaptic population)	tonic	57
α6βγ2	cerebellum (granule cells)	extrasynaptic	phasic	58, 59
α6β2δ	cerebellum (granule cells)	extrasynaptic	tonic	58-60
α6β3δ	cerebellum (granule cells)	extrasynaptic	tonic	58-60
ρ	retina (bipolar cells)	synaptic, extrasynaptic?	tonic?	61-63
Existence with high probability				
α1β3γ2	cortex? hippocampus?	synaptic?	phasic?	6, 64
α1βδ	hippocampus (interneurons)	extrasynaptic	tonic	65
α5β3γ2	hippocampus (pyramidal cells, granule cells)	extrasynaptic	tonic	66
αβ1γ/ αβ1δ	cerebral cortex	?	?	67-69
αβ	hippocampus (pyramidal cells)	extrasynaptic	tonic	36, 37
α1α6βγ/ α1α6βδ	cerebellum (granule cells)	synaptic/extrasynaptic	phasic	58, 60

List of isoforms from reference 6, which also identifies "tentative" isoforms that assembled in heterologous systems (ρ1-3, αβγ1, αβγ3, αβε, αβθ, αβπ, and αxαγβγ2). Also see the following general references: *in situ* hybridization[70]; immunohistochemistry[71, 72]; reviews[20, 73] .

also very different from that of γ-subunit-containing receptors. Though GABA binds to δ-containing isoforms with high affinity, its efficacy is relatively low. Conversely, ethanol[79] and neuroactive steroids[80] act strongly at δ-subunit-containing receptors. Demonstration of these properties *in vivo*[56], combined with co-localization, co-immunoprecipitation, and gene deletion studies[81], have allowed identification of the δ-subunit-containing receptors listed in **Table 1**[55].

The last isoform that has been identified unequivocally *in vivo* comprises ρ subunits alone. These receptors, previously classified as $GABA_C$ receptors due to their unique pharmacology, are expressed predominantly in retinal bipolar cells[63]; however, low levels of ρ subunit transcripts have also been detected in hippocampus[82], cerebellum[83], amygdala[84], and certain brain areas important for visual signal processing (superior colliculus, lateral geniculate nucleus, and visual cortex)[62, 83] . Evidence for both homomeric and heteromeric ρ isoforms has been reported[85, 86]; consequently, the subunit subtypes present in these receptors remain undefined.

Isoforms that exist with high probability

Finally, we will briefly discuss the evidence supporting the "existence with high probability" of certain key $GABA_AR$ isoforms listed in **Table 1**. Each of these isoforms assembles efficiently and has been studied extensively in heterologous systems[11, 31, 33, 35, 80, 87-89]; moreover, the subunits are co-expressed *in vivo*[70-72]. Indeed, most were not classified as "identified" simply because few animal studies have been conducted. First, although α1 and γ2 subunits seem to partner most frequently with the β2 subunit, expression patterns indicate that this cannot always be the case, because certain areas expressing the α1 and γ2 subunits do not express the β2 subunit[71]. In these areas, it is quite likely that α1β3γ2 receptors are formed, as indicated by various pharmacological properties[64]. The evidence supporting the existence of α5β3γ2 is also extensive; the only reason that it is not considered to be unequivocally identified is that, to date, α5 and β3 have not been co-immunoprecipitated[6]. However, these three subunits have been co-localized[71], α5 and β3 subunits were co-depleted in knockout mice[6], α5-selective etomidate effects have been identified[90], and electrophysiology indicates that this isoform mediates tonic inhibition in the hippocampus[66]. Another widely-accepted isoform, α1βδ, clearly assembled in heterologous systems and responded to known modulators of δ-subunit-containing receptors. Furthermore, one recent report identified this isoform in molecular layer interneurons of the hippocampus[65]. Finally, as previously mentioned, two different αβ isoforms have been identified in rat brain via sequential co-immunoprecipitation[37] and electrophysiology[36].

CONCLUDING REMARKS

$GABA_A$ receptors in the brain are ubiquitous, implicated in many diseases, and highly heterogeneous. Each receptor isoform exhibits unique physiological and pharmacological properties and a characteristic expression pattern. Consequently, a thorough understanding of $GABA_AR$ assembly, trafficking, and function could yield significant therapeutic advantages, such as isoform-specific drugs that minimize unwanted side effects. Currently, only 11 $GABA_AR$ isoforms have been conclusively identified *in vivo*, and the existence of another six is considered to be highly probable. Further study of the assembly, trafficking, and function of these receptors may improve clinical practice, as will attempts to identify other $GABA_AR$ isoforms that occur in the brain.

REFERENCES

1. Bloom FE and Iversen LL (1971). Localizing 3H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. *Nature*. **229** (5287): 628-630.
2. Farrant M and Nusser Z (2005). Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. **6** (3): 215-229.
3. Couve A, Moss SJ and Pangalos MN (2000). GABAB receptors: a new paradigm in G protein signaling. *Mol Cell Neurosci*. **16** (4): 296-312.
4. Connolly CN and Wafford KA (2004). The Cys-loop superfamily of ligand-gated ion channels: the impact of receptor structure on function. *Biochem Soc Trans*. **32** (Pt3): 529-534.
5. Campagna-Slater V and Weaver DF (2007). Molecular modelling of the GABAA ion channel protein. *J Mol Graph Model*. **25** (5): 721-730.
6. Olsen RW and Sieghart W (2008). International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev*. **60** (3): 243-260.
7. Tretter V, Ehya N, Fuchs K and Sieghart W (1997). Stoichiometry and assembly of a recombinant GABAA receptor subtype. *J Neurosci*. **17** (8): 2728-2737.
8. Baumann SW, Baur R and Sigel E (2001). Subunit arrangement of gamma-aminobutyric acid type A receptors. *J Biol Chem*. **276** (39): 36275-36280.
9. Baumann SW, Baur R and Sigel E (2002). Forced subunit assembly in alpha1beta2gamma2 GABAA receptors. Insight into the absolute arrangement. *J Biol Chem*. **277** (48): 46020-46025.
10. Mozrzymas JW (2004). Dynamism of GABA(A) receptor activation shapes the "personality" of inhibitory synapses. *Neuropharmacology*. **47** (7): 945-960.
11. Haas KF and Macdonald RL (1999). GABAA receptor subunit gamma2 and delta subtypes confer unique kinetic properties on recombinant GABAA receptor currents in mouse fibroblasts. *J Physiol*. **514 (Pt 1)**: 27-45.
12. Macdonald RL and Kang JQ (2009). Molecular Pathology of Genetic Epilepsies Associated with

GABA(A) Receptor Subunit Mutations. *Epilepsy Curr*. **9** (1): 18-23.

13. Gallagher MJ, Shen W, Song L and Macdonald RL (2005). Endoplasmic reticulum retention and associated degradation of a GABAA receptor epilepsy mutation that inserts an aspartate in the M3 transmembrane segment of the alpha1 subunit. *J Biol Chem*. 280 (45): 37995-38004.
14. Frugier G, Coussen F, Giraud MF, Odessa MF, Emerit MB, Boue-Grabot E and Garret M (2007). A gamma 2(R43Q) mutation, linked to epilepsy in humans, alters GABAA receptor assembly and modifies subunit composition on the cell surface. *J Biol Chem*. 282 (6): 3819-3828.
    The preceding two papers elegantly demonstrate that altered $GABA_A$ receptor biogenesis and subunit composition may lead to epilepsy.
15. Buhr A, Bianchi MT, Baur R, Courtet P, Pignay V, Boulenger JP, Gallati S, Hinkle DJ, Macdonald RL and Sigel E (2002). Functional characterization of the new human GABA(A) receptor mutation beta3(R192H). *Hum Genet*. **111** (2): 154-160.
16. Kalueff AV and Nutt DJ (2007). Role of GABA in anxiety and depression. *Depress Anxiety*. **24** (7): 495-517.
17. Blum BP and Mann JJ (2002). The GABAergic system in schizophrenia. *Int J Neuropsychopharmacol*. **5** (2): 159-179.
18. Enoch MA (2008). The role of GABA(A) receptors in the development of alcoholism. *Pharmacol Biochem Behav*. **90** (1): 95-104.
19. Fatemi SH, Reutiman TJ, Folsom TD and Thuras PD (2009). GABA(A) receptor downregulation in brains of subjects with autism. *J Autism Dev Disord*. **39** (2): 223-230.
20. Mohler H (2006). GABA(A) receptor diversity and pharmacology. *Cell Tissue Res*. **326** (2): 505-516.
21. Rudolph U and Antkowiak B (2004). Molecular and neuronal substrates for general anaesthetics. *Nat Rev Neurosci*. **5** (9): 709-720.
22. Green WN and Millar NS (1995). Ion-channel assembly. *Trends Neurosci*. 18 (6): 280-287.
23. Connolly CN, Krishek BJ, McDonald BJ, Smart TG and Moss SJ (1996). Assembly and cell surface expression of heteromeric and homomeric gamma-aminobutyric acid type A receptors. *J Biol Chem*. 271 **(1): 89-96.**
    This study presents one of the first methodical analyses of selective subunit oligomerization and its effects on forward trafficking.
24. Blount P and Merlie JP (1990). Mutational analysis of muscle nicotinic acetylcholine receptor subunit assembly. *J Cell Biol*. **111** (6 Pt 1): 2613-2622.
25. Ramanathan VK and Hall ZW (1999). Altered glycosylation sites of the delta subunit of the acetylcholine receptor (AChR) reduce alpha delta association and receptor assembly. *J Biol Chem*. **274** (29): 20513-20520.
26. Keller CA, Yuan X, Panzanelli P, Martin ML, Alldred M, Sassoe-Pognetto M and Luscher B (2004). The gamma2 subunit of GABA(A) receptors is a substrate for palmitoylation by GODZ. *J Neurosci*. **24** (26): 5881-5891.
27. Bogdanov Y, Michels G, Armstrong-Gold C, Haydon PG, Lindstrom J, Pangalos M and Moss SJ (2006). Synaptic GABAA receptors are directly recruited from their extrasynaptic counterparts. *EMBO J*. **25** (18): 4381-4389.
28. Kanematsu T, Fujii M, Mizokami A, Kittler JT, Nabekura J, Moss SJ and Hirata M (2007). Phospholipase C-related inactive protein is implicated in the constitutive internalization of GABAA receptors mediated by clathrin and AP2 adaptor complex. *J Neurochem*. **101** (4): 898-905.
29. Sarto-Jackson I and Sieghart W (2008). Assembly of GABA(A) receptors (Review). *Mol Membr Biol*. **25** (4): 302-310.
30. Jacob TC, Moss SJ and Jurd R (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci*. **9** (5): 331-343.
31. Gorrie GH, Vallis Y, Stephenson A, Whitfield J, Browning B, Smart TG and Moss SJ (1997). Assembly of GABAA receptors composed of alpha1 and beta2 subunits in both cultured neurons and fibroblasts. *J Neurosci*. **17** (17): 6587-6596.
32. Klausberger T, Ehya N, Fuchs K, Fuchs T, Ebert V, Sarto I and Sieghart W (2001). Detection and binding properties of GABA(A) receptor assembly intermediates. *J Biol Chem*. **276** (19): 16024-16032.
33. Angelotti TP and Macdonald RL (1993). Assembly of GABAA receptor subunits: alpha 1 beta 1 and alpha 1 beta 1 gamma 2S subunits produce unique ion channels with dissimilar single-channel properties. *J Neurosci*. **13** (4): 1429-1440.
34. Fisher JL and Macdonald RL (1997). Single channel properties of recombinant GABAA receptors containing gamma 2 or delta subtypes expressed with alpha 1 and beta 3 subtypes in mouse L929 cells. *J Physiol*. **505 (Pt 2)**: 283-297.
35. Saxena NC and Macdonald RL (1994). Assembly of GABAA receptor subunits: role of the delta subunit. *J Neurosci*. **14** (11 Pt 2): 7077-7086.
36. Mortensen M and Smart TG (2006). Extrasynaptic alphabeta subunit GABAA receptors on rat hippocampal pyramidal neurons. *J Physiol*. **577** (Pt 3): 841-856.
37. Bencsits E, Ebert V, Tretter V and Sieghart W (1999). A significant part of native gamma-aminobutyric AcidA receptors containing alpha4 subunits do not contain gamma or delta subunits. *J Biol Chem*. **274** (28): 19613-19616.
38. Bollan K, Robertson LA, Tang H and Connolly CN (2003). Multiple assembly signals in gamma-aminobutyric acid (type A) receptor subunits combine to drive receptor construction and composition. *Biochem Soc Trans*. **31** (Pt 4): 875-879.
39. Srinivasan S, Nichols CJ, Lawless GM, Olsen RW and Tobin AJ (1999). Two invariant tryptophans on the alpha1 subunit define domains necessary for GABA(A) receptor assembly. *J Biol Chem*. **274** (38): 26633-26638.
40. Taylor PM, Connolly CN, Kittler JT, Gorrie GH, Hosie A, Smart TG and Moss SJ (2000). Identification of residues within GABA(A) receptor alpha subunits that mediate specific assembly with receptor beta subunits. *J Neurosci*. **20** (4): 1297-1306.
41. Klausberger T, Sarto I, Ehya N, Fuchs K, Furtmuller R, Mayer B, Huck S and Sieghart W (2001). Alternate use of distinct intersubunit contacts controls GABAA receptor assembly and stoichiometry. *J Neurosci*. **21** (23): 9124-9133.
42. Sarto I, Wabnegger L, Dogl E and Sieghart W (2002). Homologous sites of GABA(A) receptor alpha(1), beta(3) and gamma(2) subunits are important for assembly. *Neuropharmacology*. **43** (4): 482-491.

43. Bollan K, King D, Robertson LA, Brown K, Taylor PM, Moss SJ and Connolly CN (2003). GABA(A) receptor composition is determined by distinct assembly signals within alpha and beta subunits. *J Biol Chem*. **278** (7): 4747-4755.
44. Taylor PM, Thomas P, Gorrie GH, Connolly CN, Smart TG and Moss SJ (1999). Identification of amino acid residues within GABA(A) receptor beta subunits that mediate both homomeric and heteromeric receptor expression. *J Neurosci*. 19 (15): 6360-6371.
This study identifies specific residues mediating the unusual assembly patterns of the β3 subunit, which may promote significant heterogeneity of recombinant receptors.
45. Ehya N, Sarto I, Wabnegger L and Sieghart W (2003). Identification of an amino acid sequence within GABA(A) receptor beta3 subunits that is important for receptor assembly. *J Neurochem*. **84** (1): 127-135.
46. Klausberger T, Fuchs K, Mayer B, Ehya N and Sieghart W (2000). GABA(A) receptor assembly. Identification and structure of gamma(2) sequences forming the intersubunit contacts with alpha(1) and beta(3) subunits. *J Biol Chem*. **275** (12): 8921-8928.
47. Sarto I, Klausberger T, Ehya N, Mayer B, Fuchs K and Sieghart W (2002). A novel site on gamma 3 subunits important for assembly of GABA(A) receptors. *J Biol Chem*. **277** (34): 30656-30664.
48. Nymann-Andersen J, Sawyer GW and Olsen RW (2002). Interaction between GABAA receptor subunit intracellular loops: implications for higher order complex formation. *J Neurochem*. **83** (5): 1164-1171.
49. Lo WY, Botzolakis EJ, Tang X and Macdonald RL (2008). A conserved Cys-loop receptor aspartate residue in the M3-M4 cytoplasmic loop is required for GABAA receptor assembly. *J Biol Chem*. **283** (44): 29740-29752.
50. Unwin N (2005). Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol*. **346** (4): 967-989.
51. Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB and Sixma TK (2001). Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature*. **411** (6835): 269-276.
52. Sur C, Wafford KA, Reynolds DS, Hadingham KL, Bromidge F, Macaulay A, Collinson N, O'Meara G, Howell O, Newman R, Myers J, Atack JR, Dawson GR, McKernan RM, Whiting PJ and Rosahl TW (2001). Loss of the major GABA(A) receptor subtype in the brain is not lethal in mice. *J Neurosci*. **21** (10): 3409-3418.
53. Gunther U, Benson J, Benke D, Fritschy JM, Reyes G, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y and et al. (1995). Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci U S A*. **92** (17): 7749-7753.
54. Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H and Rudolph U (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. *Science*. **290** (5489): 131-134.
55. Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR and McKernan RM (1999). Preferential coassembly of alpha4 and delta subunits of the gamma-aminobutyric acidA receptor in rat thalamus. *Mol Pharmacol*. **56** (1): 110-115.
56. Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL and Goldstein PA (2005). An extrasynaptic GABAA receptor mediates tonic inhibition in thalamic VB neurons. *J Neurophysiol*. **94** (6): 4491-4501.
57. Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF and Orser BA (2004). Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci U S A*. **101** (10): 3662-3667.
58. Jechlinger M, Pelz R, Tretter V, Klausberger T and Sieghart W (1998). Subunit composition and quantitative importance of hetero-oligomeric receptors: GABAA receptors containing alpha6 subunits. *J Neurosci*. **18** (7): 2449-2457.
59. Nusser Z, Sieghart W and Somogyi P (1998). Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J Neurosci*. **18** (5): 1693-1703.
60. Poltl A, Hauer B, Fuchs K, Tretter V and Sieghart W (2003). Subunit composition and quantitative importance of GABA(A) receptor subtypes in the cerebellum of mouse and rat. *J Neurochem*. **87** (6): 1444-1455.
61. Qian H and Dowling JE (1995). GABAA and GABAC receptors on hybrid bass retinal bipolar cells. *J Neurophysiol*. **74** (5): 1920-1928.
62. Zhang D, Pan ZH, Awobuluyi M and Lipton SA (2001). Structure and function of GABA(C) receptors: a comparison of native versus recombinant receptors. *Trends Pharmacol Sci*. **22** (3): 121-132.
63. Enz R, Brandstatter JH, Wassle H and Bormann J (1996). Immunocytochemical localization of the GABAc receptor rho subunits in the mammalian retina. *J Neurosci*. **16** (14): 4479-4490.
64. Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B and Rudolph U (2003). General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J*. **17** (2): 250-252.
65. Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR and Mody I (2007). A new naturally occurring GABA(A) receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci*. **10** (1): 40-48.
66. Sur C, Quirk K, Dewar D, Atack J and McKernan R (1998). Rat and human hippocampal alpha5 subunit-containing gamma-aminobutyric AcidA receptors have alpha5 beta3 gamma2 pharmacological characteristics. *Mol Pharmacol*. **54** (5): 928-933.
67. Li M and De Blas AL (1997). Coexistence of two beta subunit isoforms in the same gamma-aminobutyric acid type A receptor. *J Biol Chem*. **272** (26): 16564-16569.
68. Mossier B, Togel M, Fuchs K and Sieghart W (1994). Immunoaffinity purification of gamma-aminobutyric acidA (GABAA) receptors containing gamma 1-subunits. Evidence for the presence of a single type of gamma-subunit in GABAA receptors. *J Biol Chem*. **269** (41): 25777-25782.
69. Mtchedlishvili Z and Kapur J (2006). High-affinity, slowly desensitizing GABAA receptors mediate tonic inhibition in hippocampal dentate granule cells. *Mol Pharmacol*. **69** (2): 564-575.

70. Laurie DJ, Wisden W and Seeburg PH (1992). The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci*. **12** (11): 4151-4172.
71. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W and Sperk G (2000). GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience*. **101** (4): 815-850.
72. Sieghart W and Sperk G (2002). Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr Top Med Chem*. **2** (8): 795-816.
73. Fritschy JM and Brunig I (2003). Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol Ther*. **98** (3): 299-323.
74. Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H and Mohler H (1999). Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature*. **401** (6755): 796-800.
75. Morris HV, Dawson GR, Reynolds DS, Atack JR and Stephens DN (2006). Both alpha2 and alpha3 GABAA receptor subtypes mediate the anxiolytic properties of benzodiazepine site ligands in the conditioned emotional response paradigm. *Eur J Neurosci*. **23** (9): 2495-2504.
76. McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR and Whiting PJ (2000). Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. *Nat Neurosci*. **3** (6): 587-592.
77. Winsky-Sommerer R (2009). Role of GABAA receptors in the physiology and pharmacology of sleep. *Eur J Neurosci*. **29** (9): 1779-1794.
78. Mody I, Glykys J and Wei W (2007). A new meaning for "Gin & Tonic": tonic inhibition as the target for ethanol action in the brain. *Alcohol*. **41** (3): 145-153.
79. Olsen RW, Hanchar HJ, Meera P and Wallner M (2007). GABAA receptor subtypes: the "one glass of wine" receptors. *Alcohol*. **41** (3): 201-209.
80. Wohlfarth KM, Bianchi MT and Macdonald RL (2002). Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci*. **22** (5): 1541-1549.
81. Jia F, Pignataro L and Harrison NL (2007). GABAA receptors in the thalamus: alpha4 subunit expression and alcohol sensitivity. *Alcohol*. **41** (3): 177-185.
82. Didelon F, Sciancalepore M, Savic N, Mladinic M, Bradbury A and Cherubini E (2002). gamma-Aminobutyric acidA rho receptor subunits in the developing rat hippocampus. *J Neurosci Res*. **67** (6): 739-744.
83. Harvey VL, Duguid IC, Krasel C and Stephens GJ (2006). Evidence that GABA rho subunits contribute to functional ionotropic GABA receptors in mouse cerebellar Purkinje cells. *J Physiol*. **577** (Pt 1): 127-139.
84. Fujimura J, Nagano M and Suzuki H (2005). Differential expression of GABA(A) receptor subunits in the distinct nuclei of the rat amygdala. *Brain Res Mol Brain Res*. **138** (1): 17-23.
85. Enz R and Cutting GR (1999). GABAC receptor rho subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties. *Eur J Neurosci*. **11** (1): 41-50.
86. Pan Y, Ripps H and Qian H (2006). Random assembly of GABA rho1 and rho2 subunits in the formation of heteromeric GABA(C) receptors. *Cell Mol Neurobiol*. **26** (3): 289-305.
87. Feng HJ and Macdonald RL (2004). Multiple actions of propofol on alphabetagamma and alphabetadelta GABAA receptors. *Mol Pharmacol*. **66** (6): 1517-1524.
88. Fisher JL (2002). A histidine residue in the extracellular N-terminal domain of the GABA(A) receptor alpha5 subunit regulates sensitivity to inhibition by zinc. *Neuropharmacology*. **42** (7): 922-928.
89. Luddens H and Korpi ER (1995). GABA antagonists differentiate between recombinant GABAA/benzodiazepine receptor subtypes. *J Neurosci*. **15** (10): 6957-6962.
90. Cheng VY, Martin LJ, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC, Macdonald JF, Bhambri A, Collinson N, Wafford KA and Orser BA (2006). Alpha5GABAA receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. *J Neurosci*. **26** (14): 3713-3720.

FURTHER INFORMATION

Robert L. Macdonald Lab:
http://www.mc.vanderbilt.edu/neurology/faculty/macdonald.htm

The Role of the Amygdala in Emotion-Attention Interactions

Maureen McHugo

The amygdala is a subcortical medial temporal lobe structure that is widely thought to be involved in processing emotional information[1]. Amygdala dysfunction and abnormal attention to affective events have been commonly observed in anxiety disorders[2]. Characterizing how the amygdala interacts with other brain regions to influence attention in healthy adults could prove useful in developing a better understanding of the mechanisms underlying these illnesses. This paper will primarily review evidence from human neuroimaging studies that have examined a potential role for the amygdala in emotion-attention interactions.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: maureen.mchugo@vanderbilt.edu.

Imagine that while watching the latest episode of your favorite television show, you suddenly hear glass breaking at your kitchen door. Although you're in the middle of an important scene, you freeze and strain your ears to try and listen for other sounds. Is someone trying to break in or has your cat knocked over a glass? Attention allows us to process important stimuli, like the sound of a possible intruder, at the expense of other items present in our environment (e.g. the television)[3]. As noted by the authors of a recent model of the neural systems of attention, reorienting attention toward "novel, potentially threatening" stimuli is of great importance[4, 5]. Fear conditioning studies in rodents and humans have shown that the amygdala is important for the acquisition and expression of conditioned fear and mediates a variety of behavioral and autonomic responses to threat-related cues[6-8]. Using positron emission tomography (PET) and functional magnetic resonance imaging (FMRI), investigators have found that the amygdala also responds preferentially to emotional faces[9, 10] and scenes[11]. For scenes, this response may depend on the arousal level of a stimulus rather than its valence[12-14]. Arousal refers to the energy or intensity level of a stimulus and can range from calm to excited, whereas valence indicates how pleasant or unpleasant a stimulus might be[15]. Early lesion studies in animals suggested that the amygdala might play a role in orienting to novel events[16] and low level amygdala stimulation can lead to "attention"-like orienting responses[17] and increased cortical arousal[18]. Behavioral data show that emotionally salient stimuli can be better identified than neutral items[19-21], may lead to facilitated detection of subsequent stimuli[22, 23] and can impair detection of other important events[24-27], possibly by "capturing" attention. The anatomical projections of the amygdala may enable it to influence attention via modulation of sensory areas[28], cortical regions implicated in attentional orienting and control[29], and subcortical structures involved in modulating arousal and attention[30]. This review will focus on evidence for the amygdala's role in modulating attention based on these three patterns of connectivity.

SENSORY MODULATION

According to the biased competition model of attention[31], stimuli in the environment compete for processing resources based on a combination of sensory salience and relevance to current goals. Top-down attention that biases sensory processing based on behavioral relevance is thought to be allocated by a frontoparietal network that includes the frontal eye fields (FEF) and areas along the intraparietal sulcus (IPS)[4]. These regions modulate activity in sensory areas in response to cues in order to direct covert attention[32-35] by increasing gain at attended locations[36] or by altering feature tuning[37]. Anatomical tracing studies in non-human primates have demonstrated that the amygdala sends topographically organized feedback projections to higher order visual areas (e.g. areas TE and TEO in the macaque) and sparser projections to earlier levels of the visual pathway including primary and secondary visual cortices[28, 38, 39]. These feedback connections from the amygdala to visual cortex may act in parallel to top-down attention by transiently boosting perceptual processing of emotional stimuli, allowing them to "out-compete" non-emotional items for available resources[28, 40].

Increased activity in primary and secondary visual cortices and the fusiform gyrus has been observed while participants view emotional relative to neutral faces[41-43] and scenes,[12, 44, 45] with a greater response to scenes than faces[11, 46]. The level of activity in the face responsive region of the fusiform gyrus varies as a

function of the amygdala response to fearful compared to neutral faces[42] even when the faces appear outside the current focus of attention,[43] as long as attentional or perceptual resources are available[47, 48]. Vuilleumier and colleagues[49] performed a novel FMRI study using patients with damage to the amygdala and/or hippocampus due to medial temporal lobe epilepsy and healthy adult controls in order to examine the necessity of the amygdala for affective perceptual enhancement. Healthy adults and patients with damage limited to the hippocampus showed the expected increase in fusiform activity in response to fearful compared to neutral faces. Critically, this differential fusiform response to emotion was attenuated in patients who had amygdala damage and the level of right or left fusiform activity decreased as the level of ipsilateral amygdala sclerosis increased.

If emotional stimuli processed by the amygdala are more strongly represented in sensory areas, two potential predictions follow: when these stimuli appear at task relevant locations, they should be more readily detected and when they are task-irrelevant (*i.e.* distractors) they should interfere with detection of concurrent stimuli. Anderson and Phelps have proposed that the amygdala enhances sensory processing to facilitate attention for emotional stimuli based on a manipulation of the attentional blink (AB) paradigm[50]. In the AB task, detection of a target during a rapid serial visual presentation display temporarily impairs processing of a subsequent target[51, 52]. If an arousing, aversive word appears as a target at a short interval following the first target, it is more accurately identified than a neutral word even when emotion is irrelevant for the task[20]. Unlike healthy adults, patients with left or bilateral amygdala damage do not exhibit increased identification of aversive second targets[50]. However, there is no direct evidence that this results from sensory enhancement. Several studies have looked at how fearful faces impact performance on visual search tasks. One behavioral study found that task-irrelevant fearful faces may facilitate subsequent search for neutral items appearing in the same spatial location[23]. Greater amygdala activation in response to masked fearful faces correlates with faster detection of positive or negative schematic faces during a subsequent behavioral task[53]. In contrast to these findings, Williams and colleagues[54] observed that participants were worse at detecting fearful compared to happy faces in the presence of neutral face distractors even though the amygdala was more active when fearful faces were present in a search display. However, perceptual differences between the face stimuli may have led to the results of this study.

Lavie's model of selective attention under load indicates that processing of distractors decreases if the perceptual load of a task is high[55]. This suggests that emotional distractors present outside the current focus of attention may not always be processed by the amygdala and would therefore be incapable of influencing behavior. Consistent with this model, Hsu and Pessoa found that activity for fearful and neutral face distractors in the amygdala and fusiform gyrus decreased when the number of distinct items in a search display increased (reflecting greater perceptual load). When the sensory salience of the search display was degraded to reach the same level of difficulty as the perceptual load condition, face related activity increased relative to a baseline condition[56]. Reaction times on the search task were slower when faces were present, as compared to absent, during the salience condition, supporting the idea of increased distractor interference. Although activity in the amygdala and fusiform gyrus was greater for fearful faces relative to neutral faces during this condition, reaction times did not differ by expression. In summary, although much data strongly suggests that the amygdala can modulate sensory areas, there is little evidence that this modulation has a behavioral correlate.

ATTENTIONAL MODULATION

The effect of emotion on spatial attention has been extensively studied using a modified spatial cueing paradigm called the dot probe task[57, 58]. In this task, subjects typically view a pair of words, faces, or scenes in which one item is threat-related and the other neutral. A brief target stimulus is then presented in the same or opposite location as the emotional item. The affective cue is thought to attract attention in a stimulus-driven manner because it is not predictive of the upcoming target location. Several neuroimaging studies have used this task to examine the possibility that the amygdala facilitates spatial attention by interacting with regions involved in attentional allocation rather than by sensory enhancement alone. Armony and Dolan[59] used a version of this task combined with differential classical conditioning in an FMRI experiment to examine whether aversively conditioned cues could direct spatial attention. During trials in which an angry face that had previously been paired with an unpleasant noise (CS+) was presented with a different angry face unpaired with noise (CS-), participants were faster to detect a target when it appeared in the same location as the CS+ (cued trials) and slower when the target followed the CS- (uncued trials). The amygdala and fusiform gyrus were more active during presentations of the CS+ than the CS-. Crucially, the putative FEF, IPS, and lateral orbitofrontal cortex were more active during cued and uncued trials compared to when only the CS+ or CS- was presented on both sides of fixation. The behavioral and imaging data thus support the hypothesis that attention was modulated by the conditioned stimulus. Pourtois and

colleagues found that following the appearance of a fearful face cue, the response in IPS contralateral to the fearful face was decreased for uncued targets, an effect that was not observed during cue-only trials[60]. The authors interpreted this to mean that allocating attention to emotional stimuli may produce a "processing cost" when subjects must subsequently reorient attention. However, the consequence of a potential processing cost in IPS is unclear, since there was no behavioral difference associated with fearful face cues and no amygdala activation was observed during this experiment. In typical spatial cueing studies, differences in reaction time between cued and uncued trials are not always found when the cue-target stimulus onset asynchrony (SOA) is approximately 200-500 msec[61], the interval used in the Pourtois study. The two experiments using a short cue-target SOA (<150 msec) in the dot probe task have found amygdala activation to fearful or angry face cues as well as cue validity effects, consistent with a role for the amygdala in orienting attention to potential threat[59, 62, 63].

Novel, unexpected environmental stimuli attract attention and elicit an orienting response that habituates rapidly in the absence of a significant associated outcome[64, 65]. The human amygdala responds to unfamiliar neutral faces[66, 67] and unusual scenes[12], but this response decreases quickly. Lesion studies in rats have shown that the amygdala is necessary for the acquisition of conditioned orientation to visual or auditory cues that cue food delivery, but does not participate in unconditioned orienting[65, 68, 69]. Several investigators have proposed that the amygdala acts as a detector for emotionally or biologically salient information[67, 70] and may provide an interrupt signal to reorient attention to highly important events[7, 63]. In contrast, an influential model of attention suggests that a ventral frontoparietal network, including the temporoparietal junction (TPJ), anterior insula and regions of the middle and inferior frontal gyri, is responsible for reorienting attention to behaviorally relevant events[4, 5]. A recent study found that the inferior frontal component of this network was specifically engaged during infrequent, presumably unexpected attentional shifts[71]. The inferior frontal gyrus makes up a significant portion of the ventrolateral prefrontal cortex[72] (VLPFC). Although the amygdala has few connections to lateral prefrontal and posterior parietal cortices, it has moderate reciprocal connections to the ventral-most portion of the inferior frontal gyrus, corresponding to area 47/12 of the VLPFC[29, 39, 73]. The potential functional similarities and anatomical connections of the VLPFC and amygdala suggest a possible mechanism by which the amygdala could influence cortical attentional networks in response to emotionally salient events, particularly if they are unanticipated. Brain regions involved in detecting unexpected or infrequent environmental changes have often been studied using oddball paradigms in which subjects detect a rare discrepant target among a series of standard stimuli[4]. The amygdala and VLPFC respond more to rare targets in auditory oddball tasks[74, 75] when they elicit an arousal response[76] and to aversive words presented among neutral words, but not to neutral oddballs differing in semantic or perceptual features[77]. Fichtenholtz and colleagues presented two groups of subjects with infrequent squares and aversive or neutral scenes among standard circle stimuli to examine whether attentional networks responded differently depending on whether the emotional items were relevant to current goals[78, 79]. The VLPFC was engaged by infrequently presented scenes regardless of task relevance but the response was greater for aversive than for neutral items. Reaction times were slowest for aversive scenes regardless of target status and fastest for square targets. These data support the idea that the VLPFC is involved in redirecting attention to novel events and suggest that it can be modulated by stimulus valence and/or arousal possibly due to input from the amygdala[78, 79]. In contrast, the IPS and TPJ did not appear to respond to infrequent aversive stimuli unless they were targets.[79] However, several complications arise when interpreting these results because the emotional stimuli used were negative and arousing. The right VLPFC has been linked to emotion regulation[80, 81] and response inhibition[82], and it is possible that the response to aversive items reflects greater cognitive control rather than attentional capture. Additionally, several studies have suggested that arousal itself may be particularly important for engaging the VLPFC[76, 83], and different parts of the inferior frontal gyrus may be sensitive to valence and arousal[84]. Two recent studies suggest that the amygdala and VLPFC may interact to evaluate emotional stimuli[85, 86]. Future studies could vary stimulus valence and arousal and more rigorously manipulate task-relevance and attentional focus to investigate the specific conditions under which the VLPFC and amygdala are employed.

When an affectively salient stimulus is irrelevant to ongoing goal-directed behavior and is not sufficiently important, attention is not fully redirected and is instead maintained on the current task. For example, distracting emotional information can cause subjects to respond more slowly[78], but performance failure occurs only when processing capacity is nearly exhausted[25]. The rostral, pregenual cingulate region corresponding to areas 24 and caudal 32 (rACC) is thought to have a role in detecting or resolving emotional distraction[87, 88] and has direct reciprocal projections with the amygdala[29]. The rACC is more active when participants must ignore negative

compared to neutral word content during emotional Stroop tasks[87, 89], when fearful faces appear at unattended locations[43] and when aversive scene or fearful face distractors appear unexpectedly[78, 90]. Although these studies suggest a role for the rACC in detecting or resolving emotional distraction, most failed to show a behavioral correlate reflecting interference. In a modified attentional blink paradigm, task-irrelevant emotional items presented shortly before a target decrease target detection accuracy compared to neutral distractors[25]. Interestingly, this effect occurs for aversive, erotic, and conditioned complex scenes[25-27] but not fearful faces[91], possibly indicating the importance of arousal in capturing attention. Using FMRI, Most and colleagues[92] found that aversive scene distractors interfered with target detection and were associated with increased amygdala activation when compared to neutral scenes. Greater rACC activation in this study appeared to be driven by individual differences in subjects' ability to ignore emotional scenes, as evidenced by decreased amygdala activity. Conversely, a recent FMRI study found that more accurate detection of fearful relative to neutral face second targets in the AB task was related to greater rACC response in the absence of amygdala activation[21]. In this case, the fearful expression was unimportant for reporting facial identity and greater rACC activity could have reflected increased attention when participants were aware of the emotional stimulus. From these studies, it is unclear whether the rACC detects and/or resolves affective interference. Control of attention over distracting information is typically studied using Stroop-type paradigms in which conflict must be monitored or resolved[93] and none of these studies examined rACC function in conflict situations *per se*[94]. Etkin and colleagues[94, 95] developed a task in which participants had to report whether faces were fearful or happy while ignoring a congruent or incongruent word label, thereby providing response conflict. Conflict resolution was defined based on trials in which an incongruent trial followed a previous incongruent trial whereas conflict detection was thought to occur when an incongruent trial followed a congruent trial. In support of this dichotomy, amygdala activation increased during the conflict detection condition and subjects were slower to respond compared to the conflict resolution condition, which was associated with increased rACC activity and a concurrent decrease in amygdala response[94].

LINKS TO NEUROMODULATORY SYSTEMS

The amygdala may also influence sensory processing and attention through its connections with subcortical neuromodulatory systems[39]. Numerous studies have examined the importance of the amygdala and locus coeruleus noradrenergic system for emotional memory[96], but little work has been done to determine whether norepinephrine modulates attention to affective stimuli. A behavioral study in humans showed that increasing the availability of norepinephrine through a reuptake inhibitor improved the ability of subjects to detect emotional compared to neutral targets in an attentional blink task[97]. Tentative support for amygdala involvement in this process comes from a recent FMRI experiment showing increased amygdala activity to fearful faces in participants who had taken the same reuptake inhibitor[98]. The amygdala is also reciprocally connected with the nucleus basalis[39], which provides cholinergic input throughout cortex and is thought to be important for a variety of attentional functions such as normal attentional shifting to unattended targets[99-101]. Classical conditioning studies in animals have shown that amygdala-mediated release of acetylcholine is important to return auditory cortex neuron receptive fields to prefer a conditioned stimulus[102]. In humans, frequency specific changes in auditory cortex during differential classical conditioning are correlated with increased amygdala and basal forebrain[103] activity, which appears to be dependent on acetylcholine[104]. The amygdala also contributes to acetylcholine-dependent EEG desynchronization, which is thought to reflect increased cortical arousal or attention[105, 106]. When the relationship between a cue and its conditioned outcome changes unexpectedly, the amygdala interacts with a network that includes the nucleus basalis, substantia nigra and posterior parietal cortex to increase attention to the cue[65, 107-109]. Participants who had taken a cholinesterase inhibitor initially showed impaired performance during a house-matching task in the presence of unattended fearful faces compared to those who received a placebo, suggesting greater attentional capture by the fearful faces with increased acetylcholine levels[110]. The subjects who had taken the drug also showed greater activity to unattended fearful faces in the dorsal anterior cingulate, intraparietal sulcus and a region of the lateral orbitofrontal cortex similar to that observed during attentional shifts to conditioned stimuli[59]. The amygdala-mediated release of acetylcholine may therefore facilitate attention to emotional stimuli[99].

CONCLUSION

Current data suggest that the amygdala modulates sensory cortices to bias activity for emotional stimuli such that they compete more effectively than non-emotional items for attention. Enhanced attention to affective events may be bolstered by amygdala-VLPFC or neuromodulatory interactions and weakened by rACC influence. Future studies should more rigorously manipulate attentional demands and

address the relative importance of arousal versus valence, specific emotions and possible differences resulting from stimulus type (e.g. faces versus scenes) in these processes.

REFERENCES

1. Zald DH (2003). The human amygdala and the emotional evaluation of sensory stimuli. *Brain Res Rev*. **41** (1): 88-123.
2. Bishop SJ (2007). Neurocognitive mechanisms of anxiety: an integrative account. *Trends Cogn Sci (Regul Ed)*. **11** (7): 307-316.
3. Egeth HE and Yantis S (1997). Visual attention: control, representation, and time course. *Annu Rev Psychol*. **48**: 269-297.
4. Corbetta M and Shulman GL (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nat Rev Neurosci*. **3** (3): 201-215.
5. Corbetta M, Patel G and Shulman GL (2008). The reorienting system of the human brain: from environment to theory of mind. *Neuron*. **58** (3): 306-324.
6. Paré D, Quirk GJ and Ledoux JE (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol*. **92** (1): 1-9.
7. LeDoux JE (2000). Emotion circuits in the brain. *Annu Rev Neurosci*. **23**: 155-184.
8. Davis M and Whalen PJ (2001). The amygdala: vigilance and emotion. *Mol Psychiatry*. **6** (1): 13-34.
9. Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ and Dolan RJ (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature*. **383** (6603): 812-815.
10. Hoffman KL, Gothard KM, Schmid MC and Logothetis NK (2007). Facial-expression and gaze-selective responses in the monkey amygdala. *Curr Biol*. **17** (9): 766-772.
11. Hariri AR, Tessitore A, Mattay VS, Fera F and Weinberger DR (2002). The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage*. **17** (1): 317-323.
12. Hamann SB, Ely TD, Hoffman JM and Kilts CD (2002). Ecstasy and agony: activation of the human amygdala in positive and negative emotion. *Psychol sci*. **13** (2): 135-141.
13. Kensinger EA and Schacter DL (2006). Processing emotional pictures and words: effects of valence and arousal. *Cogn Affect Behav Ne*. **6** (2): 110-126.
14. Garavan H, Pendergrass JC, Ross TJ, Stein EA and Risinger RC (2001). Amygdala response to both positively and negatively valenced stimuli. *Neuroreport*. **12** (12): 2779-2783.
15. Lang PJ, Bradley MM and Cuthbert BN (1990). Emotion, attention, and the startle reflex. *Psychol rev*. **97** (3): 377-395.
16. Bagshaw MH, Mackworth NH and Pribram KH (1972). The effect of resections of the inferotemporal cortex or the amygdala on visual orienting and habituation. *Neuropsychologia*. **10** (2): 153-162.
17. Ursin H and Kaada BR (1960). Subcortical structures mediating the attention response induced by amygdala stimulation. *Exp Neurol*. **2**: 109-122.
18. Kapp BS, Supple WF and Whalen PJ (1994). Effects of electrical stimulation of the amygdaloid central nucleus on neocortical arousal in the rabbit. *Behav Neurosci*. **108** (1): 81-93.
19. Ohman A, Flykt A and Esteves F (2001). Emotion drives attention: detecting the snake in the grass. *J Exp Psychol Gen*. **130** (3): 466-478.
20. Anderson AK (2005). Affective influences on the attentional dynamics supporting awareness. *J Exp Psychol Gen*. **134** (2): 258-281.
21. De Martino B, Kalisch R, Rees G and Dolan RJ (2009). Enhanced processing of threat stimuli under limited attentional resources. *Cereb Cortex*. **19** (1): 127-133.
22. Phelps EA, Ling S and Carrasco M (2006). Emotion facilitates perception and potentiates the perceptual benefits of attention. *Psychol Sci*. **17** (4): 292-299.
23. Becker MW (2009). Panic search: fear produces efficient visual search for nonthreatening objects. *Psychol Sci*. **20** (4): 435-437.
24. Yiend J and Mathews A (2001). Anxiety and attention to threatening pictures. *Q J Exp Psychol-A*. **54** (3): 665-681.
25. Most SB, Chun MM, Widders DM and Zald DH (2005). Attentional rubbernecking: cognitive control and personality in emotion-induced blindness. *Psychon B Rev*. **12** (4): 654-661.
26. Most S, Smith S, Cooter A, Levy B and Zald D (2007). The naked truth: Positive, arousing distractors impair rapid target perception. *Cognition Emotion*. **21** (5): 964-981.
27. Smith SD, Most SB, Newsome LA and Zald DH (2006). An emotion-induced attentional blink elicited by aversively conditioned stimuli. *Emotion*. **6** (3): 523-527.
28. Amaral DG, Behniea H and Kelly JL (2003). Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. *Neuroscience*. **118** (4): 1099-1120.
29. Ghashghaei HT, Hilgetag CC and Barbas H (2007). Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage*. **34** (3): 905-923.
30. Price JL and Amaral DG (1981). An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *J Neurosci*. **1** (11): 1242-1259.
31. Desimone R and Duncan J (1995). Neural mechanisms of selective visual attention. *Annu Rev Neurosci*. **18**: 193-222.
32. Kastner S, Pinsk MA, De Weerd P, Desimone R and Ungerleider LG (1999). Increased activity in human visual cortex during directed attention in the absence of visual stimulation. *Neuron*. **22** (4): 751-761.
33. Corbetta M, Kincade JM, Ollinger JM, McAvoy MP and Shulman GL (2000). Voluntary orienting is dissociated from target detection in human posterior parietal cortex. *Nat Neurosci*. **3** (3): 292-297.
34. Hopfinger JB, Buonocore MH and Mangun GR (2000). The neural mechanisms of top-down attentional control. *Nat Neurosci*. **3** (3): 284-291.
35. Kincade JM, Abrams RA, Astafiev SV, Shulman GL and Corbetta M (2005). An event-related functional magnetic resonance imaging study of voluntary and stimulus-driven orienting of attention. *J Neurosci*. **25** (18): 4593-4604.
36. Reynolds JH and Chelazzi L (2004). Attentional modulation of visual processing. *Annu Rev Neurosci*. **27**: 611-647.
37. Martinez-Trujillo JC and Treue S (2004). Feature-based attention increases the selectivity of population responses in primate visual cortex. *Curr Biol*. **14** (9): 744-751.

38. Iwai E and Yukie M (1987). Amygdalofugal and amygdalopetal connections with modality-specific visual cortical areas in macaques (Macaca fuscata, M. mulatta, and M. fascicularis). *J Comp Neurol*. **261** (3): 362-387.
39. Amaral DG, Price JL, Pitkänen A and Carmichael ST (1992). Anatomical organization of the primate amygdaloid complex. In: The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction (Aggleton JP, ed), pp 1-66. New York: Wiley-Liss.
40. Pessoa L, Kastner S and Ungerleider LG (2002). Attentional control of the processing of neutral and emotional stimuli. *Cognitive brain res*. **15** (1): 31-45.
41. Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, Strauss MM, Hyman SE and Rosen BR (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron*. **17** (5): 875-887.
42. Morris JS, Friston KJ, Büchel C, Frith CD, Young AW, Calder AJ and Dolan RJ (1998). A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain*. **121 (Pt 1)**: 47-57.
43. Vuilleumier P, Armony JL, Driver J and Dolan RJ (2001). Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. *Neuron*. **30** (3): 829-841.
44. Lang PJ, Bradley MM, Fitzsimmons JR, Cuthbert BN, Scott JD, Moulder B and Nangia V (1998). Emotional arousal and activation of the visual cortex: an fMRI analysis. *Psychophysiology*. **35** (2): 199-210.
45. Sabatinelli D, Bradley MM, Fitzsimmons JR and Lang PJ (2005). Parallel amygdala and inferotemporal activation reflect emotional intensity and fear relevance. *Neuroimage*. **24** (4): 1265-1270.
46. Britton JC, Taylor SF, Sudheimer KD and Liberzon I (2006). Facial expressions and complex IAPS pictures: common and differential networks. *Neuroimage*. **31** (2): 906-919.
47. Pessoa L, McKenna M, Gutierrez E and Ungerleider LG (2002). Neural processing of emotional faces requires attention. *Proc Natl Acad Sci USA*. **99** (17): 11458-11463.
48. Pessoa L, Padmala S and Morland T (2005). Fate of unattended fearful faces in the amygdala is determined by both attentional resources and cognitive modulation. *Neuroimage*. **28** (1): 249-255.
49. Vuilleumier P, Richardson MP, Armony JL, Driver J and Dolan RJ (2004). Distant influences of amygdala lesion on visual cortical activation during emotional face processing. *Nat Neurosci*. 7 (11): 1271-1278.
    This paper demonstrates the necessity of the amygdala for enhanced fusiform cortex activity in response to emotional faces.
50. Anderson AK and Phelps EA (2001). Lesions of the human amygdala impair enhanced perception of emotionally salient events. *Nature*. 411 (6835): 305-309.
    This paper shows that the amygdala is necessary for emotional facilitation of attention.
51. Raymond JE, Shapiro KL and Arnell KM (1992). Temporary suppression of visual processing in an RSVP task: an attentional blink? *J Exp Psychol Hum Percept Perform*. **18** (3): 849-860.
52. Chun MM and Potter MC (1995). A two-stage model for multiple target detection in rapid serial visual presentation. *J Exp Psychol Hum Percept Perform*. **21** (1): 109-127.
53. Ohrmann P, Rauch A, Bauer J, Kugel H, Arolt V, Heindel W and Suslow T (2007). Threat sensitivity as assessed by automatic amygdala response to fearful faces predicts speed of visual search for facial expression. *Exp Brain Res*. **183** (1): 51-59.
54. Williams MA, McGlone F, Abbott DF and Mattingley JB (2008). Stimulus-driven and strategic neural responses to fearful and happy facial expressions in humans. *Eur J Neurosci*. **27** (11): 3074-3082.
55. Lavie N (2005). Distracted and confused?: selective attention under load. *Trends Cogn Sci (Regul Ed)*. **9** (2): 75-82.
56. Hsu SM and Pessoa L (2007). Dissociable effects of bottom-up and top-down factors on the processing of unattended fearful faces. *Neuropsychologia*. **45** (13): 3075-3086.
57. Bar-Haim Y, Lamy D, Pergamin L, Bakermans-Kranenburg MJ and van IJzendoorn MH (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychol Bull*. **133** (1): 1-24.
58. Frewen PA, Dozois DJ, Joanisse MF and Neufeld RW (2008). Selective attention to threat versus reward: meta-analysis and neural-network modeling of the dot-probe task. *Clin Psychol Rev*. **28** (2): 307-337.
59. Armony JL and Dolan RJ (2002). Modulation of spatial attention by fear-conditioned stimuli: an event-related fMRI study. *Neuropsychologia*. 40 (7): 817-826.
    This paper characterizes the involvement of the amygdala and other brain regions involved in facilitating spatial attention in response to emotional stimuli.
60. Pourtois G, Schwartz S, Seghier ML, Lazeyras F and Vuilleumier P (2006). Neural systems for orienting attention to the location of threat signals: an event-related fMRI study. *Neuroimage*. **31** (2): 920-933.
61. Posner M and Cohen Y (1984). Components of visual orienting. In: Attention and performance X (Bouma H and Bouwhuis DG, eds), pp 531–556. Hillsdale: Lawrence Erlbaum Associates.
62. Carlson JM, Reinke KS and Habib R (2009). A left amygdala mediated network for rapid orienting to masked fearful faces. *Neuropsychologia*. **47** (5): 1386-1389.
63. Armony J, Servan-Schreiber D, Cohen J and LeDoux J (1997). Computational modeling of emotion: Explorations through the anatomy and physiology of fear conditioning. *Trends Cogn Sci (Regul Ed)*. **1** (1): 28-34.
64. Sokolov EN (1963). Higher nervous functions; the orienting reflex. *Annu Rev Physiol*. **25**: 545-580.
65. Holland PC and Gallagher M (1999). Amygdala circuitry in attentional and representational processes. *Trends Cogn Sci (Regul Ed)*. **3** (2): 65-73.
66. Schwartz CE, Wright CI, Shin LM, Kagan J, Whalen PJ, McMullin KG and Rauch SL (2003). Differential amygdalar response to novel versus newly familiar neutral faces: a functional MRI probe developed for studying inhibited temperament. *Biol Psychiatry*. **53** (10): 854-862.
67. Wright CI, Martis B, Schwartz CE, Shin LM, Fischer H H, McMullin K and Rauch SL (2003). Novelty responses and differential effects of order in the

amygdala, substantia innominata, and inferior temporal cortex. *Neuroimage*. **18** (3): 660-669.
68. Gallagher M, Graham PW and Holland PC (1990). The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. *J Neurosci*. **10** (6): 1906-1911.
69. McDannald M, Kerfoot E, Gallagher M and Holland PC (2004). Amygdala central nucleus function is necessary for learning but not expression of conditioned visual orienting. *Eur J Neurosci*. **20** (1): 240-248.
70. Sander D, Grafman J and Zalla T (2003). The human amygdala: an evolved system for relevance detection. *Rev Neuroscience*. **14** (4): 303-316.
71. Shulman GL, Astafiev SV, Franke D, Pope DL, Snyder AZ, McAvoy MP and Corbetta M (2009). Interaction of stimulus-driven reorienting and expectation in ventral and dorsal frontoparietal and basal ganglia-cortical networks. *J Neurosci*. **29** (14): 4392-4407.
72. Petrides M (2005). Lateral prefrontal cortex: architectonic and functional organization. *Philos Trans R Soc Lond, B, Biol Sci*. **360** (1456): 781-795.
73. Petrides M and Pandya DN (2002). Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. *Eur J Neurosci*. **16** (2): 291-310.
74. Kiehl KA, Laurens KR, Duty TL, Forster BB and Liddle PF (2001). Neural sources involved in auditory target detection and novelty processing: an event-related fMRI study. *Psychophysiology*. **38** (1): 133-142.
75. Kiehl KA, Stevens MC, Laurens KR, Pearlson G, Calhoun VD and Liddle PF (2005). An adaptive reflexive processing model of neurocognitive function: supporting evidence from a large scale (n = 100) fMRI study of an auditory oddball task. *Neuroimage*. **25** (3): 899-915.
76. Williams LM, Felmingham K, Kemp AH, Rennie C, Brown KJ, Bryant RA and Gordon E (2007). Mapping frontal-limbic correlates of orienting to change detection. *Neuroreport*. **18** (3): 197-202.
77. Strange BA, Henson RN, Friston KJ and Dolan RJ (2000). Brain mechanisms for detecting perceptual, semantic, and emotional deviance. *Neuroimage*. **12** (4): 425-433.
78. Yamasaki H, LaBar KS and McCarthy G (2002). Dissociable prefrontal brain systems for attention and emotion. *Proc Natl Acad Sci USA*. 99 (17): 11447-11451.
Shows that distracting, highly arousing emotional stimuli are processed in the amygdala, inferior frontal gyrus and rostral anterior cingulate.
79. Fichtenholtz HM, Dean HL, Dillon DG, Yamasaki H, McCarthy G and LaBar KS (2004). Emotion-attention network interactions during a visual oddball task. *Cog Brain Res*. **20** (1): 67-80.
80. Dolcos F, Kragel P, Wang L and McCarthy G (2006). Role of the inferior frontal cortex in coping with distracting emotions. *Neuroreport*. **17** (15): 1591-1594.
81. Lieberman MD, Eisenberger NI, Crockett MJ, Tom SM, Pfeifer JH and Way BM (2007). Putting feelings into words: affect labeling disrupts amygdala activity in response to affective stimuli. *Psychol Sci*. **18** (5): 421-428.
82. Aron AR, Robbins TW and Poldrack RA (2004). Inhibition and the right inferior frontal cortex. *Trends Cogn Sci (Regul Ed)*. **8** (4): 170-177.
83. Schmidt L, Cléry-Melin ML, Lafargue G, Valabrègue R, Fossati P, Dubois B and Pessiglione M (2009). Get aroused and be stronger: emotional facilitation of physical effort in the human brain. *J Neurosci*. **29** (30): 9450-9457.
84. Dolcos F, LaBar KS and Cabeza R (2004). Dissociable effects of arousal and valence on prefrontal activity indexing emotional evaluation and subsequent memory: an event-related fMRI study. *Neuroimage*. **23** (1): 64-74.
85. Wager TD, Davidson ML, Hughes BL, Lindquist MA and Ochsner KN (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron*. **59** (6): 1037-1050.
86. Lee KH and Siegle GJ (2009). Common and distinct brain networks underlying explicit emotional evaluation: a meta-analytic study. *Soc Cogn Affect Neur*.
87. Whalen PJ, Bush G, McNally RJ, Wilhelm S, McInerney SC, Jenike MA and Rauch SL (1998). The emotional counting Stroop paradigm: a functional magnetic resonance imaging probe of the anterior cingulate affective division. *Biol Psychiatry*. **44** (12): 1219-1228.
88. Bush G, Luu P and Posner MI (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci (Regul Ed)*. **4** (6): 215-222.
89. Mohanty A, Engels AS, Herrington JD, Heller W, Ho MH, Banich MT, Webb AG, Warren SL and Miller GA (2007). Differential engagement of anterior cingulate cortex subdivisions for cognitive and emotional function. *Psychophysiology*. **44** (3): 343-351.
90. Bishop S, Duncan J, Brett M and Lawrence AD (2004). Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nat Neurosci*. **7** (2): 184-188.
91. Stein T, Zwickel J, Ritter J, Kitzmantel M and Schneider WX (2009). The effect of fearful faces on the attentional blink is task dependent. *Psychon B Rev*. **16** (1): 104-109.
92. Most SB, Chun MM, Johnson MR and Kiehl KA (2006). Attentional modulation of the amygdala varies with personality. *Neuroimage*. 31 (2): 934-944.
This paper demonstrates the involvement of the amygdala in generating emotional interference and how this effect is modulated by attention and the rostral anterior cingulate.
93. Raz A and Buhle J (2006). Typologies of attentional networks. *Nat Rev Neurosci*. **7** (5): 367-379.
94. Etkin A, Egner T, Peraza DM, Kandel ER and Hirsch J (2006). Resolving emotional conflict: a role for the rostral anterior cingulate cortex in modulating activity in the amygdala. *Neuron*. **51** (6): 871-882.
95. Egner T, Etkin A, Gale S and Hirsch J (2008). Dissociable neural systems resolve conflict from emotional versus nonemotional distracters. *Cereb Cortex*. **18** (6): 1475-1484.
96. McGaugh JL (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci*. **27**: 1-28.
97. De Martino B, Strange BA and Dolan RJ (2008). Noradrenergic neuromodulation of human attention for emotional and neutral stimuli. *Psychopharmacology (Berl)*. **197** (1): 127-136.

98. Onur OA, Walter H, Schlaepfer TE, Rehme AK, Schmidt C, Keysers C, Maier W and Hurlemann R (2009). Noradrenergic enhancement of amygdala responses to fear. *Soc Cogn Affect Neur*. **4** (2): 119-126.
99. Sarter M, Hasselmo ME, Bruno JP and Givens B (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Brain Res Rev*. **48** (1): 98-111.
100. Davidson MC and Marrocco RT (2000). Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *J Neurophysiol*. **83** (3): 1536-1549.
101. Chiba AA, Bushnell PJ, Oshiro WM and Gallagher M (1999). Selective removal of cholinergic neurons in the basal forebrain alters cued target detection. *Neuroreport*. **10** (14): 3119-3123.
102. Weinberger NM (2004). Specific long-term memory traces in primary auditory cortex. *Nat Rev Neurosci*. **5** (4): 279-290.
103. Morris JS, Friston KJ and Dolan RJ (1998). Experience-dependent modulation of tonotopic neural responses in human auditory cortex. *Proc Biol Sci*. **265** (1397): 649-657.
104. Thiel CM, Friston KJ and Dolan RJ (2002). Cholinergic modulation of experience-dependent plasticity in human auditory cortex. *Neuron*. **35** (3): 567-574.
105. Kapp BS, Whalen PJ, Supple WF and Pascoe JP (1992). Amygdaloid contributions to conditioned arousal and sensory information processing. In: The amygdala: neurobiological aspects of emotion, memory, and mental dysfunction (Aggleton JP, ed), pp 229-254. New York: Wiley-Liss.
106. Whalen PJ, Kapp BS and Pascoe JP (1994). Neuronal activity within the nucleus basalis and conditioned neocortical electroencephalographic activation. *J Neurosci*. **14** (3 Pt 2): 1623-1633.
107. Holland PC and Gallagher M (1993). Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behav Neurosci*. **107** (2): 246-253.
108. Holland PC and Gallagher M (2006). Different roles for amygdala central nucleus and substantia innominata in the surprise-induced enhancement of learning. *J Neurosci*. **26** (14): 3791-3797.
109. Lee HJ, Youn JM, Gallagher M and Holland PC (2008). Temporally limited role of substantia nigra-central amygdala connections in surprise-induced enhancement of learning. *Eur J Neurosci*. **27** (11): 3043-3049.
110. Bentley P, Vuilleumier P, Thiel CM, Driver J and Dolan RJ (2003). Cholinergic enhancement modulates neural correlates of selective attention and emotional processing. *Neuroimage*. **20** (1): 58-70.
111. Indovina I and Macaluso E (2007). Dissociation of stimulus relevance and saliency factors during shifts of visuospatial attention. *Cereb Cortex*. **17** (7): 1701-1711.

FURTHER INFORMATION

David Zald's Lab:
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At the Edge: Neuroethological Approaches to Reptilian Mechanoreception

Duncan B. Leitch

The neural circuitry directing behavior is one of the fundamental questions of neurobiology. Historically, studies of ethology have been descriptive in nature; however, by adopting a comparative biological perspective, patterns of animal behavior and corresponding neural substrates can be examined systematically. Although they are morphologically similar to early tetrapod ancestors, modern crocodilians have adapted distinct sensory abilities that have made them impressive predators in both terrestrial and aquatic environments. Characterization of the activity of peripheral nervous system adaptations, central nervous system organization, and accompanying behaviors remain open questions suited to comparative neuroethological strategies.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: duncan.b.leitch@vanderbilt.edu.

THE NEUROETHOLOGICAL PERSPECTIVE

Insights into the organization and function of the nervous system have arisen through assimilation of a variety of experimental approaches ranging from molecular and cellular techniques to behavioral analyses. Similarly, neuroethology represents a field at the crossroads, fostering interdisciplinary research with methods familiar to zoology, physiology, evolutionary biology, and neurobiology[1]. These efforts are unified in their foundations in understanding animal behavior and its underlying neural processes. However, given the complexity of nervous systems within highly developed animals, broader questions of the neural basis of behavior are explored through a comparative approach. Fundamental to these ideas is an appreciation for the "ethology" – that is, the behaviors observable both in animals' natural habitats – balanced with the rigorous control of stimuli to elicit these patterns[2]. Pioneering work from animal behaviorists Lorenz[3], von Frisch[4], and Tinbergen[5] in the past century have provided a framework for investigation, as outlined in Tinbergen's classification of four explanations for behavior[6], which are equally relevant in approaching neuroethology. Rather than broadly grouping proximate and ultimate explanations for behavior, Tinbergen argued that physiological and mechanistic bases, development or ontogeny, functional contributions to survival and reproduction, and evolutionary history or phylogeny provide a structure for addressing questions of ethology[6,7].

Within the realm of sensory biology, comparative neuroethological approaches have proven effective. For example, the circuits underlying electroreception and the "jam avoidance response" in afferents from gymnotiform fish *Eigenmannia* have been identified in detail[8-10]. This weakly electric fish discharges its electric organ in order to "electrolocate" or sense perturbations in the electric field of the surrounding environment. In order to accommodate the presence of other actively electrolocating fish which could potentially mask the reception of subtle electric field variations from prey, *Eigenmannia* and other species of gymnotiforms can alter the frequency of the discharge of the electric generating organ, thereby increasing the frequency difference relative to the neighboring fish. As electroreception is widespread among vertebrates with examples in all classes of fish, some amphibians[11,12], and potentially mammals[13,14], this "exotic" sensory modality has evolved multiple times through non-homologous receptor and neural circuits over the course of vertebrate evolution[15].

Neuroethology and comparative studies of nervous systems integrate information from two different levels. Even distantly related organisms can show similarities in motor patterns and sensory processing (indeed these functional and structural homologies contribute to the utility of commonly used animal models in studying human neurobiology). Comparative analysis using organisms of varying phylogenetic relationships provides a framework for understanding nervous system organization that takes historical forces and evolutionary pressures into account in the shaping of the final architecture of neural circuits. Through a comparative approach, information on the uniqueness of a particular behavior or sensory processing mechanism is revealed. One example of the benefit of this combined perspective can be seen in studies of somatosensory processing among members of the mammalian order *Insectivora*[16-18]. Included in this group are shrews and moles - animals that have been used in examinations of mammalian nervous system evolution as they have retained morphological traits

similar to those found in fossils of small-bodied eutherian ancestors. Through comparisons of nervous system organization among these related animals, as well as their behaviors, the uniqueness of certain species becomes apparent. The star-nosed mole (*Condylura cristata*) has a specialized symmetrical star array of 22 appendages, covered in more than 25,000 highly-innervated Eimer's organ mechanoreceptors[19,20] and a somatosensory cortical organization dominated by the star's representation with the most densely-innervated appendages occupying the most cortical space[21]. Using its star appendages to minimize prey searching and handling latencies, the star-nosed mole is the fastest mammalian forager[22]. The unusual anatomical and neural circuits underlying distinct sensory processing abilities is revealed through integration of behavioral and comparative perspectives.

This review discusses unique mechanosensory structures found among some of the most phylogenetically ancient tetrapods: extant members of the order *Crocodilia.* Respecting Tinbergen's ethological considerations, as well as integrating a comparative neuroanatomical perspective, we describe the evolution and ecological pressures faced by these species. Lastly, we present the current understanding of receptor physiology and corresponding neural circuitry.

THE EVOLUTION OF TETRAPODS AND CROCODILIANS

About 370 million years ago, a large lobe-finned fish emerged from the water, having evolved into a giant-salamander-like "labyrinthodont" amphibian[23]. The evolution of this amphibian in the Upper Devonian period of the Paleozoic era marked a transition among vertebrates from an aquatic fish-like lifestyle to terrestrial life, and consequently, heralded unique physiological and morphological changes[24]. Among these adaptations was the development of four paired limbs, replacing four paired fins, giving rise to a new group of vertebrate organisms: the tetrapods, a group with modern amphibian, reptile, bird, and mammal representatives. Approximately 320 million years ago, with the advent of fluid-filled amniotic membranes and yolk sacs to prevent dehydration of eggs and embryos among the synapsids (mammals and "proto-mammals") and sauropsids (dinosaurs, reptiles, and birds), the transition to terrestrial life was complete[25,26]. Some tetrapods became amphibious and continued to occupy the transitional water-to-land habitats. Living in these environments at the water-to-land matrix, these organisms evolved distinct sensory systems that allowed them to face the series of challenges presented in aquatic and terrestrial environments[27]. This semi-aquatic tetrapod lifestyle is seen today in some species of modern mammals, birds, reptiles, and amphibians, including members of the order *Crocodilia.*

Extant members of the order *Crocodilia* (referred to collectively as "crocodilians") are represented in twenty-three species, occupying semi-aquatic habitats throughout North and South America, Africa, Asia, and Australia. Furthermore, crocodilians are generally regarded as a sister group and the closest modern relatives to birds, based on morphological and genomic comparisons[28]. The order is divided into three families – Crocodylidae, Alligatoridae, and Gavialidae. All modern crocodilians are descendants of archosaurian ancestors[29,30]. Furthermore, modern crocodilians occupy a range of aquatic environments and vary in the amount of time spent in the water. The most aquatic species include the gharials (*Gavialis*) which have large, narrow snouts adapted to their primarily piscivorous diets[31]. Many species, including the saltwater crocodile (*Crocodylus porosus*), Nile crocodile (*C. niloticus*), and the American crocodile (*C. acutus*), can accommodate both freshwater and marine environments to differing degrees as they swim from coastal areas into the ocean[32]. Evidence from phylogenetic and physiological studies suggests transoceanic migrations might have occurred among crocodilian lineages.

Modern crocodilian tolerance to a variety of external chemical[33] and environmental conditions, even among areas developed by humans[34], are reflected in their archosaurian ancestors' survival through both the Triassic-Jurassic and Cretaceous-Tertiary mass extinction events[35]. Crocodilian fossils have retained the general skeletal and morphological features of extant crocodilians since the late Triassic/early Jurassic period, 180-200 MYA[36], underscoring the great degree of conservation in the evolution of their body plans. Under periods of prolonged development and a growth rate similar to juvenile extant crocodilians, extinct species such as the so-called *Deinosuchus* "terror-crocodile" of the Late Cretaceous period attained dinosaur-like lengths of 8 to 10 meters and masses between 2,500 and 5,000 kg, growing into broad-snouted crocodilians similar in appearance to modern alligators[37].

Although crocodilian populations have become vulnerable to declines in population even within the recent past[38], crocodilians have shown a remarkable degree of resilience when sufficient habitats are recovered[39]. In fact, no crocodilian species have been driven to extinction during recorded human history despite considerable economic incentives to hunt and kill them[40,41]. These reptiles inspire both fear and fascination as successful large-bodied, long-lived ectotherms thriving among a world of endotherms, having survived extinction events to remain the closest living reptilian relatives to the Dinosauria, comprising one branch of its extant phylogenetic

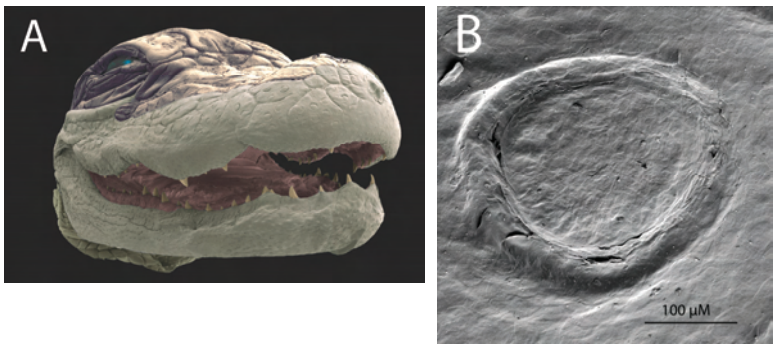

Figure 1 | **Mechanoreception in *A. mississippiensis*. a |** Pseudo-colored scanning electron micrograph of head of juvenile *A. mississippiensis.* Epidermal touch papillae appear over upper and lower jaw regions (paler scales). The head is 4.5 cm in length. **b |** Scanning electron micrograph of single epidermal touch papilla from the upper jaw. These dome-like structures are innervated by many mechanoreceptors. The scale bar is 100 μM.

bracket[42,43]. Beyond their armored osteoderm-plated bodies and their abilities to tolerate and adapt to a variety of environmental conditions, crocodilians have distinct sensory specializations, enabling them to rapidly process stimuli to determine the presence of potential prey, making them apex[44] predators and survivors.

MECHANORECEPTION IN CROCODILIANS

In the evolution of a sensitive mechanosensory system balanced with the development of a protected body surface, all orders of reptiles developed arrays of touch papillae that vary in morphology, function, and distribution among taxa. Initial studies using the American alligator (*Alligator mississippiensis)* identified myelinated afferents for rapidly adapting fibers responsive to transient "on" and "off" stimuli and afferents for slowly adapting fibers[45,46]. Considering their semi-aquatic habitats which include murky bodies of water with poor illumination, it is likely that mechanoreception plays a significant role in prey localization over other sensory modalities[47]. This is not to say that their visual[48,49], chemosensory[50], and auditory[51-53] systems are poorly represented, and in fact, several anatomical[54] and functional[51,55-57] studies, primarily in *A. mississippiensis* and the caiman (*C. crocodilus*), have examined these modalities. However, comprehensive discussion of these sensory systems is beyond the scope of this review.

As first characterized by von Düring, arrays of sensory organs in the form of spot-like touch papillae cover crocodilians[58,59]. These dense arrangements of darkly pigmented pits, also called "follicle pits," "follicle glands," or "integumentary sense organs," have been used in the identification of crocodilian skin[60]. Among species in the alligatorid family (including alligators and caimans), these touch papillae are found on cranial scales surrounding the face of the animal (**Figure 1a**), whereas they are distributed post-cranially on ventral integumentary scales in members of the crocodylid family. Despite speculation on their possible function as secretory pores[61] or their osmoreceptive properties[62-64], these touch papillae are dome-like structures (**Figure 1b**), lacking pore or hair follicles, and externally resemble specialized mechanoreceptors such as the push rod organs on the hairless bills of monotremes[13] and the Eimer's organs on the rhinarium of moles[19,65,66]. Noting the pronounced thinning of the keratin and stratum corneum epidermal layers, the complex organization of discoid receptors through the stratum spinosum, the Merkel cell neurite columns and complexes in the dermis and epidermis, and the presence of encapsulated and unencapsulated lamellated receptors, von Düring proposed that these papillae were "particularly complex" sensory structures, especially when compared to tactile structures identified in other reptiles[59].

Using *A. mississippiensis*, which have these specialized touch papillae on only the cranial scales, Soares demonstrated that partially submerged alligators could orient themselves to water surface disturbances created by a single drop of water in complete darkness[67]. This behavior was abolished when the animals were completely submerged, had their heads completely out of the water, or had their touch papillae covered by a thin plastic elastomer. In recording from the trigeminal ganglion, neurons produced single spikes phase-locked to water surface wave stimuli, with increasing wave amplitude producing increased spike firing probability. Based on these examinations, Soares coined a new term for these sensory organs, calling them "dome pressure receptors;" however, this nomenclature has led to some confusion by grouping together post-cranial receptors and von Düring's "touch papillae," a categorization that has yet to be confirmed functionally or physiologically[62].

NEURAL TARGETS FOR UNIQUE SENSORY ORGANS

One aspect of touch papillae-mediated orientation not yet understood is the neuroanatomical representation of water surface movements. Forming the majority of the reptilian midbrain roof is the optic tectum (the homolog to the mammalian superior colliculus), the single largest visual center in reptiles. Examined in great detail for patterns in cytoarchitecture and afferent and efferent connections[68,69], the optic tectum (or tecta mesencephali) is notable for its concentric laminated structure, consisting of 14 layers, divided into periventricular, central, and superficial zones[70] (Alternatively, different nomenclatures have been proposed which group together some laminae[71]). Of

the tectal organization patterns observed in reptiles, the so-called lacertid pattern shows relatively poorly developed superficial layers and more prominent periventricular layers, as noted in crocodilians, turtles, and several families of lizards[72]. As the layers of the superficial zone, characterized by horizontal cells and vertically-arranged fusiform cells, are the primary target for retinal ganglion axons, this atrophy of lamination in the lacertid pattern suggests a lessened degree of dependence on visual processing, especially compared to the clearly defined laminae of highly visual *Iguanidae* and *Chamaeloeonidae* lizards.

The contralateral retina provides the single largest source of afferents to the optic tectum in most reptiles, and precise retinotopic projections on the tectum have been established for a variety of reptiles[73-76]. With the exception of the *Iguana iguana*, in all examined reptiles the nasotemporal visual axis is oriented along the tectum's rostrocaudal axis, and the dorsoventral visual axis is oriented along the tectum's mediolateral axis. Despite having receptive fields significantly smaller in size, the central 10º of the visual field is expanded in representation and occupies approximately 20% of the tectal surface, thus allowing a degree of magnified foveal representation, similar to that noted in other tetrapods[76,77].

Similar to other vertebrates, the reptilian optic tectum receives non-visual afferents, particularly in deeper layers where overlap of visual, auditory, and tactile modalities occurs, via unimodal and multimodal neurons. Based on HRP and fast blue retrograde experiments, a number of non-visual diencephalic structures were found to project to the optic tectum in reptiles[78,79]. Hartline and colleagues demonstrated that specialized infrared receptors known as "pit organs," found in *Boidae* and *Viperidae* snakes, project to the central layers of the contralateral optic tectum via the lateral descending trigeminal tract, thereby providing the snake with cues in guiding orientation towards warm-blood prey[75,80,81]. As observed in rattlesnakes, the upper and lower regions of the infrared fields are mapped onto the medial and lateral tectal areas, with infrared and visual units responding to stimuli in roughly the same spatial region of the tectum. Through tectobulbar projections, the optic tectum has indirect control of spinal cord activity via the brainstem reticular formation[82,83]. In *Iguana*, somatotopic receptive fields responsive to tactile stimulation of the contralateral body surface have been detected in deeper layers of the tectum[76]. These studies found somatosensory receptive fields for the face to be roughly in register with the iguana's visual fields, with the smallest fields corresponding to stimulation of face regions. For somatosensation, the horizontal body axis (head to tail) is oriented along the tectum's lateromedial axis, and the vertical body axis (dorsal to ventral skin surface) is oriented along the tectum's rostrocaudal axis. In light of Stein and Gaither's somatotopic organization found within the contralateral optic tectum, it is evident that the tectum is receiving input via both spinotectal and dorsal column projections, and the crossing of these pathways has been seen in a variety of reptiles[84-86].

As opportunistic, ambush predators, crocodilians are successful in localizing stimuli and rapidly orienting towards prey both in aquatic and terrestrial environments. With specialized epidermal receptors covering either the facial regions or the entire integument as in alligators and crocodiles respectively, it is likely that mechanoreception of water movements contributes to unique sensory processing and thus, their formidable predatory behaviors. However, it remains to be seen how activity from these receptors is represented and how this might vary between different species of crocodiles. Is there a "computational" map, integrating combinations of action potentials from specific receptors thereby guiding orientation[87-90]? Is distance from the stimuli encoded through mechanoreception? Is this map in spatial registration with receptive fields for other sensory modalities? Although impressive bodies of literature exist delineating tectal laminar patterns, cell morphology, and general patterns of tectal efferents and afferents among vertebrates, the behavioral output of these neural circuits are open question amenable to neuroethological strategies.

SUMMARY

Research in neuroethology seeks to determine the neural mechanisms underlying patterns of animal behavior. In adopting a comparative approach, distinct behavioral and physiological mechanisms that have evolved in response to ecological constraints can be identified. As relatives to early tetrapods and having retained similar morphological traits for more than 180 million years, modern crocodilians present a unique opportunity in the study of nervous system organization and evolution. With body surfaces covered by arrays of specialized touch papillae, crocodilians can detect minute movements on the water surface. Investigations into the neural circuitry of these sensations and their influence on crocodilian behavior can yield insight into the evolution and organization of vertebrate nervous systems.

REFERENCES

1. Ewert J-P (1980). *Neuroethology: An Introduction to the Neurophysiological Fundamentals of Behavior*, First Edition. Berlin: Springer Verlag.
2. Carew TJ (2004). *Behavioral Neurobiology: The Cellular Organization of Natural Behavior*, Second Edition. Sunderland, MA: Sinauer Associates.

3. Lorenz KZ (1937). The companion in the bird's world. *The Auk.* **54**: 245-273.
4. von Frisch K (1974). Decoding the language of the bee. *Science.* **185**: 663-668.
5. Tinbergen N (1959). Comparative studies of the behaviour of gulls (laridae): a progress report. *Behaviour*. **15**: 1-70.
6. Tinbergen N (1963). On aims and methods of ethology. *Animal Biology* **55**: 297-321.
7. Enquist M and Ghirlanda S (2005). *Neural Networks and Animal Behavior,* First Edition. Princeton, NJ: Princeton University Press.
8. Heiligenberg W (1987). Central processing of sensory information in electric fish. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology.* **161**: 621-631.
9. Rose GJ (2004). Insights into neural mechanisms and evolution of behaviour from electric fish. *Nat. Rev. Neurosci* **5**: 943-951.
10. Bullock TH (1982) Electroreception. *Annu. Rev. Neurosci.* **5**: 121-170.
11. Fritzsch B and Wahnschaffe U (1983). The electroreceptive ampullary organs of urodeles. *Cell and Tissue Res.* **229**: 483-503.
12. Bullock TH, Bodznick DA and Northcutt RG (1983). The phylogenetic distribution of electroreception: evidence for convergent evolution of a primitive vertebrate sense modality. *Brain Res.* **287**: 25-46.
13. Andres KH, von Düring M, Iggo A and Proske U (1991). The anatomy and fine structure of the echidna Tachyglossus aculeatus snout with respect to its different trigeminal sensory receptors including the electroreceptors. *Anat. Embryol.* **184**: 371-393.
14. Manger PR and Pettigrew JD (1995). Electroreception and the feeding behaviour of platypus (Ornithorhynchus anatinus: Monotremata: Mammalia). *Philosophical Transactions: Biological Sciences.* **347**: 359-381.
15. Northcutt RG (1986). Electroreception in non-teleosts bony fishes. In: *Electroreception,* (Bullock TH, Hopkins CD, Popper AN and Fay RR). New York: Springer.
16. Catania KC (2000). Cortical organization in insectivora: the parallel evolution of the sensory periphery and the brain. *Brain Behav Evol.* **55**: 311-321.
17. Catania KC, Lyon DC, Mock OB and Kaas JH (1999). Cortical organization in shrews: evidence from five species. *J. Comp. Neurol.* **410**: 55-72.
18. Catania KC (2000). Epidermal sensory organs of moles, shrew moles, and desmans: a Study of the family Talpidae with comments on the function and evolution of Eimer's organ. *Brain Behav Evol.* **56**: 146-174.
19. Catania KC (1996). Ultrastructure of the Eimer's organ of the star-nosed mole. *J. Comp. Neurol.* **365**: 343-354.
20. Marasco PD, Tsuruda PR, Bautista DM, Julius D and Catania, KC (2006). Neuroanatomical evidence for segregation of nerve fibers conveying light touch and pain sensation in Eimer's organ of the mole. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 9339-9344.
This series of experiments outlines immunocytochemical, fluorescent staining, and scanning electron microscopy techniques used to differentiate between sensory afferents conveying nociceptive and mechanoreceptive information through Eimer's organ complexes in two species of mole.
21. Catania KC and Kaas JH (1997). Somatosensory fovea in the star-nosed mole: behavioral use of the star in relation to innervation patterns and cortical representation. *J. Comp. Neurol.* **387**: 215-233.
22. Catania KC and Remple FE (2005). Asymptotic prey profitability drives star-nosed moles to the foraging speed limit. *Nature.* **433**: 519-522.
23. Carroll RL (1992). The primary radiation of terrestrial vertebrates. *Annu. Rev. Earth. Planet. Sci.* **20**: 45-84.
24. Carroll RL (1995). Between fish and amphibian. *Nature.* **373**: 389-390.
25. Reisz RR (1997). The origin and early evolutionary history of amniotes. *Trends in Ecology & Evolution.* **12**: 218-222.
26. Carroll RL (1969). Problems of the origin of reptiles. *Biological Reviews.* **44**: 393-431.
27. Thewissen JGM and Nummela S (2008). *Sensory Evolution on the Threshold,* First Edition. Berkeley: University of California Press.
28. Janke A and Arnason U (1997). The complete mitochondrial genome of Alligator mississippiensis and the separation between recent archosauria (birds and crocodiles). *Mol Biol Evol.* **14**: 1266-1272.
29. Sereno PC (1991). Basal archosaurs: phylogenetic relationships and functional implications. *Memoir (Society of Vertebrate Paleontology).* **2**: 1-53.
30. Tarsitano SF, Frey E and Reiss J (1989). The evolution of the crocodilia: a conflict between morphological and biochemical Data. *Amer. Zool.* **29**: 843-856.
31. Huchzermeyer FW (2003). *Crocodiles: Biology, Husbandry and Diseases*. Cambrdige, MA: CABI.
32. Mazzotti FJ and Dunson WA (1989). Osmoregulation in crocodilians. *Amer. Zool.* **29**: 903-920.
33. Ellis TM (1981). Tolerance of sea water by the American crocodile, Crocodylus acutus. *Journal of Herpetology* **15**: 187-192.
34. Gaby R, McMahon MP, Mazzotti FJ, Gillies WN and Wilcox JR (1985). Ecology of a population of Crocodylus acutus at a power plant site in Florida. *Journal of Herpetology.* **19**: 189-198.
35. Benton MJ (1990). Phylogeny of the major tetrapod groups: morphological data and divergence dates. *Journal of Molecular Evolution.* **30**: 409-424.
36. Carroll RL (1987). *Vertebrate Paleontology and Evolution*. W.H. Freeman & Company.
37. Erickson GM and Brochu CA (1999). How the "terror crocodile" grew so big. *Nature.* **398**: 205-206.
38. Groombridge B and Wright L (1982). *IUCN amphibia-reptilia Red Data Book. Part 1*. IUCN.
39. Webb GJW and Carrillo CE (2000). Risk of extinction and categories of endangerment: perspectives from long-lived reptiles. *Population Ecology.* **42**: 11-17.
40. Santiapillai C and de Silva M (2001). Status, distribution and conservation of crocodiles in Sri Lanka. *Biological Conservation.* **97**: 305-318.
41. Thorbjarnarson J (1999). Crocodile tears and skins: international trade, economic constraints, and limits to the sustainable use of crocodilians. *Conservation Biology.* **13**: 465-470.
42. Dodson P (2003). Allure of El Lagarto - Why do dinosaur paleontologists love alligators, crocodiles, and their kin?. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology.* **274A**: 887-890.
43. Thomason J (1997). *Functional Morphology in Vertebrate Paleontology*. Cambridge, UK:

Cambridge University Press.
44. Sergio F, Caro T, Brown D, Clucas B, Hunter J, Ketchum J, McHugh K and Hiraldo F (2008). Top predators as conservation Tools: ecological rationale, assumptions, and efficacy. *Ecology, Evolution, and Systematics.* **39**: 1-19.
45. Siminoff R and Kruger L (1968). Properties of reptilian cutaneous mechanoreceptors. *Exp. Neurol.* **20**: 403-414.
46. Kenton B, Kruger L and Woo M (1971). Two classes of slowly adapting mechanoreceptor fibres in reptile cutaneous nerve. *J. Physiol. (Lond.).* **212**: 21-44.
47. Fleishman LJ, Howland HC, Howland MJ, Rand AS and Davenport ML (1988). Crocodiles don't focus underwater. *J. Comp. Physiol. A.* **163**: 441-443.
48. Pritz MB and Stritzel ME (1992). Interconnections between the pretectum and visual thalamus in reptiles, Caiman crocodilus. *Neurosci. Lett.* **143**, 205-209.
49. Pritz MB (1997). Some morphological features of a visual thalamic nucleus in a reptile: observations on nucleus rotundus in Caiman crocodilus. *Brain Behav. Evol.* **49**: 237-248.
50. Weldon PJ and Ferguson MW (1993). Chemoreception in crocodilians: anatomy, natural history, and empirical results. *Brain Behav. Evol.* **41**: 239-245.
51. Klinke R and Pause M (1980). Discharge properties of primary auditory fibres in Caiman crocodilus: comparisons and contrasts to the mammalian auditory nerve. *Exp Brain Res.* **38**: 137-150.
52. Pritz MB and Stritzel ME (1990). Thalamic projections from a midbrain somatosensory area in a reptile, Caiman crocodilus. *Brain Behav. Evol.* **36**: 1-13.
53. Pritz MB (1974). Ascending connections of a midbrain auditory area in a crocodile, Caiman crocodilus. *J. Comp. Neurol.* **153**: 179-197.
54. Dooling RJ, Fay RR and Popper AN (2000). *Comparative Hearing*. New York: Springer.
55. Higgs DM, Brittan-Powell EF, Soares D, Souza DJ, Carr CE, Dooling RJ and Popper AN (2002). Amphibious auditory responses of the American alligator (Alligator mississipiensis). *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **188**: 217-223.
56. Carr CE, Soares D, Smolders J and Simon, JZ (2009). Detection of interaural time differences in the Alligator. *J. Neurosci.* **29**: 7978-7990.
57. Wever EG (1971). Hearing in the crocodilia. *Proc. Natl. Acad. Sci. U.S.A.* **68**: 1498-1500.
58. von Düring M (1973). The ultrastructure of lamellated mechanoreceptors in the skin of reptiles. *Z Anat Entwicklungsgesch* **143**: 81-94.
59. von Düring M and Miller M (1979). Sensory nerve endings of the skin and deeper structures. In: *Biology of the Reptilia*, First edition (Gans C, Northcutt RG, and Ulinski PS), 407-441. London: Academic Press.
60. Brazaitis P, Eberdong J, Brazaitis PJ and Watkins-Colwell GJ (2009). Notes on the saltwater crocodile, Crocodylus porosus, in the republic of Palau. *Bulletin of the Peabody Museum of Natural History.* **50**: 27-48.
61. Grigg G and Gans C (1993). Morphology and physiology of the Crocodilia. In: *Fauna of Australia 2A: Amphibia and Reptilia*, 326-336. Canberra: AGPS Press.
62. Jackson K and Brooks DR (2007). Do crocodiles co-opt their sense of "touch" to "taste"? A possible new type of vertebrate sensory organ. *Amphibia-Reptilia.* **28**: 277-285.
63. Jackson K, Butler DG and Brooks DR (1996). Habitat and phylogeny influence salinity discrimination in crocodilians: implications for osmoregulatory physiology and historical biogeography. *Biological Journal of the Linnean Society.* **58**: 371-383.
64. Jackson K, Butler DG and Youson JH (1996). Morphology and ultrastructure of possible integumentary sense organs in the estuarine crocodile, Crocodylus porosus. *J. Morphol.* **229**: 315-324.
65. Eimer T (1871). Die Schnautze des Maulwurfs als Tastwerkzeug. *Archiv für Mikroskopische Anatomie.* **7**: 181-191.
66. Catania KC (1995). A comparison of the Eimer's organs of three North American moles: The hairy-tailed mole (Parascalops breweri), the star-nosed mole (Condylura cristata), and the eastern mole (Scalopus aquaticus). *J. Comp. Neurol.* **354**: 150-160.
67. Soares D (2002). Neurology: an ancient sensory organ in crocodilians. *Nature.* **417**: 241-242.
This article reflects the current state of understanding of the function of "dome pressure receptors" in crocodilians explored through work in *A. mississippiensis* and their orienting behavior towards water disturbances. Soares also examines the foramina in fossilized crocodilian skulls to speculate on the evolution of these receptors.
68. Senn DG and Northcutt RG (1973). The forebrain and midbrain of some squamates and their bearing on the origin of snakes. *J. Morphol.* **140**: 135-151.
69. Northcutt RG (1978). Forebrain and midbrain organization in lizards and its phylogenetic significance. In: *Behavior and Neurology of Lizards,* (Greenberg N and MacLean PD), 11-64. Rockville, MD: NIMH.
70. Northcutt RG (1984). Anatomical organization of the optic tectum in reptiles. In: *Comparative Neurology of the Optic Tectum,* First Edition (Vanegas H), 547-600. New York: Plenum Press.
71. Huber GC and Crosby EC (1933). A phylogenetic consideration of the optic tectum. *Proc. Natl. Acad. Sci. U.S.A.* **19**: 15-22.
72. ten Donkelaar HJ (1998). Reptiles. In: *The Central Nervous System of Vertebrates*, (Niewenhuys R, ten Donkelaar HJ and Nicholson C), 1315-1524. Berlin: Springer.
73. Heric TM and Kruger L (1966). The electrical response evoked in the reptilian optic tectum by afferent stimulation. *Brain Res.* **1**: 187-199.
74. Terashima SI and Goris RC (1975). Tectal organization of pit viper infrared reception. *Brain Res.* **83**: 490-494.
75. Hartline PH, Kass L and Loop MS (1978). Merging of modalities in the optic tectum: infrared and visual integration in rattlesnakes. *Science.* **199**: 1225-1229.
76. Stein BE and Gaither NS (1981). Sensory representation in reptilian optic tectum: some comparisons with mammals. *J. Comp. Neurol.* **202**: 69-87.
The authors map visual and somatotopic receptive fields in *Iguana iguana* in the optic tectum, noting patterns in field size, location, and depth. This article presents some of the only data

reflecting a rough tectal topographic orientation corresponding to tactile stimuli among reptiles.

77. Gaither NS and Stein BE (1979). Reptiles and mammals use similar sensory organizations in the midbrain. *Science.* **205**: 595-597.
78. ten Donkelaar HJ and de Boer-van Huizen R (1981). Basal ganglia projections to the brain stem in the lizard Varanus exanthematicus as demonstrated by retrograde transport of horseradish peroxidase. *J. Neurosci.* **6**: 1567-1590.
79. ten Donkelaar HJ and de Boer-van Huizen R (1987). A possible pain control system in a non-mammalian vertebrate (a lizard, Gekko gecko). *Neurosci. Let.t* **83**: 65-70.
80. Hartline PH (1984). The optic tectum of reptiles: neurophysiological studies. In: *Comparative Neurology of the Optic Tectum,* First Edition (Vanegas H), 601-618. New York: Plenum Press.
81. Molenaar GJ and Fizaan-Oostveen JL (1980). Ascending projections from the lateral descending and common sensory trigeminal nuclei in python. *J. Comp. Neurol.* **189**: 555-572.
82. Dacey DM and Ulinski PS (1986). Optic tectum of the eastern garter snake, Thamnophis sirtalis. V. morphology of brainstem afferents and general discussion. *J. Comp. Neurol.* **245**: 423-453.
83. Dacey DM and Ulinski PS (1986). Optic tectum of the eastern garter snake, Thamnophis sirtalis. IV. morphology of afferents from the retina. *J. Comp. Neurol.* **245**: 301-318.
84. Pritz MB and Northcutt RG (1980). Anatomical evidence for an ascending somatosensory pathway to the telencephalon in crocodiles, Caiman crocodilus. *Exp Brain Res* **40**: 342-345.
85. Ebbesson SOE and Goodman DC (1981). Organization of ascending spinal projections in Caiman crocodilus. *Cell and Tissue Res.* **215**: 383-395.
86. Lohman AH and van Woerden-Verkley I (1978). Ascending connections to the forebrain in the Tegu lizard. *J. Comp. Neurol.* **182**: 555-574.
87. Jeffress LA (1948). A place theory of sound localization. *J. Comp. Physiol Psychol.* **41**: 35-39.
88. Knudsen EI, du Lac S, and Esterly SD (1987). Computational maps in the brain. *Annu. Rev. Neurosci.* **10**: 41-65.
89. Konishi M (2003). Coding of auditory space. *Annu. Rev. Neurosci.* **26**: 31-55.
90. Carr CE and Konishi M (1990). A circuit for detection of interaural time differences in the brain stem of the barn owl. *J. Neurosci.* **10**: 3227-3246.

    This series of experiments utilizes a neuroethological approach in exploring sound localization in the barn owl (*Tyto alba*). Fitting with Jeffress' proposed model, the authors find and functionally characterize neural circuitry corresponding to delay lines and coincidence detectors within brainstem structures.

FURTHER INFORMATION

Kenneth Catania's Lab:
http://www.vanderbilt.edu/exploration/stories/starnosedmole.html

Getting beyond Prozac: A *C. elegans* approach

Leda Ramoz

Since its debut in 1986 the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Prozac™) has taken society and mental illness by storm, becoming one of the most widely prescribed medications in America for the treatment of depression, obsessive-compulsive-disorder, bulimia nervosa, and anxiety[1]. Despite its pervasiveness in society, the exact mechanism of action of these and other antidepressants as well as their effects on endogenous regulation of their target protein, the serotonin transporter[2] are largely unknown. Synaptic serotonergic activity is primarily regulated by recycling of serotonin (5-hydroxytryptamine, 5-HT) from the synaptic cleft through activity of the presynaptic serotonin transporter (SERT, 5-HTT, SLC6A4)[3, 4], a transmembrane protein that is a major target of psychostimulants such as MDMA ("ecstasy") as well as many antidepressants such as fluoxetine[5,2]. The monoamine neurotransmitter 5-HT is an important modulator of vertebrate cardiovascular and cognitive function regulating a wide range of physiological and behavioral processes including gut function, body temperature, sleep, appetite, aggression, and mood[6]. SERT deregulation is linked to a variety of disease states, those listed above as well as alcoholism and autism[1,7-9], yet we are only beginning to understand the mechanisms behind endogenous regulation of SERT.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: leda.ramoz@vanderbilt.edu.

Current investigations of SERT regulation implicate several Ser/Thr kinases in modulation of both activity and localization, possibly in part through presynaptic receptor activity [10-15]. Rodent models demonstrate the impact of a loss in SERT activity and SERT alleles on behavior[16, 17] and are critical for understanding the complex role of 5-HT in human disease states. However, there is a pressing need for identification of endogenous regulators of 5-HT signaling, particularly SERT, and these investigations can profit from tools drawn from the behaviorally straightforward model organism *Caenorhabditis elegans* (*C. elegans)*. Although unsuitable for modeling most human disease states, this model system offers approaches that are impractical with mammalian SERT to provide insight into the mechanism of action of antidepressants, potential drug targets for treatment of 5-HT-linked disorders, and identify genes responsible for behavior. This review describes the power of forward genetics in this model organism to investigate the mechanisms regulating 5-HT transporter activity by examining the role of 5-HT and SERT in *C. elegans* behavior, particularly how these behaviors may serve as the basis for a forward genetic screen.

C. elegans AND FORWARD GENETICS

The nematode *C. elegans* is an excellent model system neurogenetic research: animals are transparent and therefore ideal for fluorescent reporter imaging, there are many viable neuronal knockouts available where the cognate disruption in mammals is inviable, the core synaptic machinery is well conserved from invertebrates to man (**Figure 1**). In addition, there are a plethora of well-developed techniques for studying this organism including genetics, biochemistry, primary cell cultures, and RNAi technology. *C. elegans* are easily cultivated in the laboratory, withstand cryopreservation, and in sub-optimal environmental conditions (such as prolonged starvation) maintain a metabolically inactive state known as dauer arrest for months. Each individual *C. elegans* contains a numerically and morphologically invariant 959 cells, including 302 neurons, enabling lineage mapping for each cell[27, 28] and reconstruction of the entire animal by serial electron micrograph[29]. These provide an intimate knowledge of the structure and connectivity of the nematode nervous system. In particular, the easily monitored behaviors (egg-laying, locomotion) and short generation time of *C. elegans* (~3 days from egg to adult) make it an optimal organism for forward genetic approaches. Hermaphroditic reproduction permits line and mutation propagation without staged crosses and also simplifies isolation of homozygous mutants, thus random mutagenesis of a parental group of animals yields 25% of F2 progeny that are homozygous for any given mutation. These mutants are then screened for a particular phenotype of interest. As a result, forward genetic screens have been the technique of choice for nematode biologists for many years to impartially isolate any number of participants in a given pathway. Screens isolating mutants that phenocopy a known mutant, such as abnormal egg-laying and touch

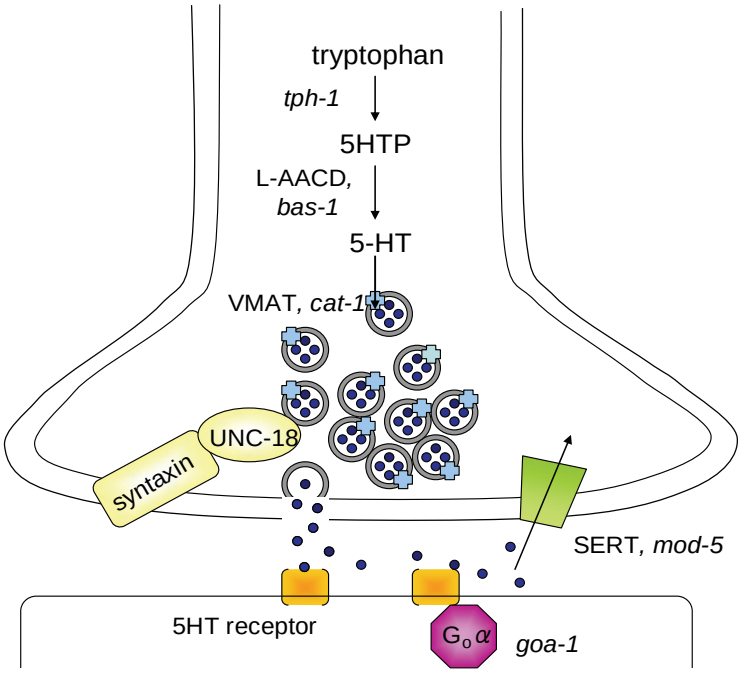

Figure 1 | **5-HT biosynthesis is conserved from *C. elegans* to man**[18]. 5-HT is packaged into vesicles (grey spheres) through the activity of a vesicular monoamine transporter (VMAT, *cat-1*[19], pale blue plus). Synaptic vesicle release is facilitated by the well conserved SNARE complex (yellow), many of the components of this complex include the two illustrated above (UNC-64/syntaxin, UNC-18/nSec-1) were originally identified in *C. elegans*[20, 21]. As in mammals *C. elegans* 5-HT receptors are divided into metabotropic (*ser-1*[22], *ser-4*[23], *ser-5*[24], and *ser-7*[25] coupled to Gαq, Gαo, Gαs and Gαs respectively), and ionotropic (*mod-1*[26]) categories.

sensitivity, have been utilized in the past to elucidate functional components of neuronal signaling such as neurotransmitter biosynthesis and packaging, as well as led to the discovery of programmed cell death[30-32]. The tedious prospect of screening tens of thousands of random mutants in search of the few mutants of interest stresses the importance of having a phenotype that is easily observable in the laboratory and optimally amenable to a high-throughput process. Only a subset of mutants isolated in a screen will contain defects in a particular pathway of interest, for example a screen for animals defective in egg-laying may yield mutations in the nervous system as well as vulval muscle development. Potentially interesting mutants therefore must undergo further genetic or pharmacological tests to determine the deficient pathway. In the case of a abnormal egg-laying screen, animals defective in vulval formation rather than malfunction in neural circuitry are distinguished by their egg-laying responses to exogenous 5-HT[31]. Thus, a phenotype for a forward genetic screen should not only be easily scored in the laboratory but also sensitive to genetic and pharmacological tools with which to examine the integrity of these circuits. The actions of 5-HT within *C. elegans* provides insight into the potential phenotypes expressed by SERT-defective animals (which theoretically express elevated synaptic 5-HT) which may then be exploited in a screen for genes controlling SERT trafficking, localization, and activity.

C. elegans AND 5-HT

In *C. elegans* (**Figure 2a**) 5-HT is an active participant in a variety of motor and autonomic behaviors. Application of exogenous 5-HT mimics the presence of food resulting in increased egg-laying and pharyngeal pumping (the nematode feeding mechanism) and decreased locomotion[33]. Animals deficient in 5-HT synthesis display decreased male mating efficiency[34], increased reproductive lifespan, increased fat storage, increased dauer arrest, decreased egg-laying[18], and defective starvation-dependent slowing in response to food (known as "enhanced slowing")[35]. In addition, 5-HT modulates complex chemosensory[36] and olfactory learning[37] behaviors. These behaviors are thought to be regulated by eight classes of serotonergic neurons identified through anti-5-HT immunofluoresence (**Figure 2b**, **Table 1**), four of which are located in the head of the animal (see expanded view page 3). Cloning of the *tph-1* gene in *C. elegans* combined with GFP imaging has identified the NSMs, ADFs, HSNs, CPs, AIMs and RIH as 5-HT production sites[18]. Serotonergic neurons not expressing *tph-1* are presumed to obtain their serotonin through activity of the *C. elegans* serotonin transporter, *mod-5*, although this requires further investigation.

MOD-5

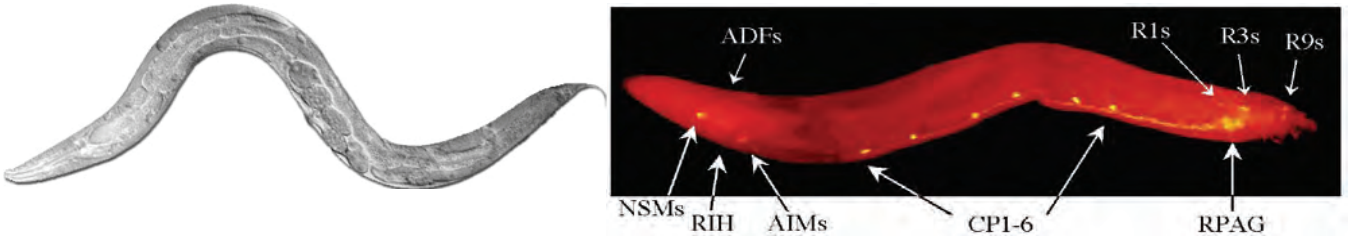

Figure 2 | ***C. elegans*. a |** Nomarski image of adult *C. elegans*. Image courtesy of the Hardin Lab. **b |** Anti-5-HT immunofluoresence of adult male *C. elegans*. Image courtesy of the Loer Lab.

Table 1 | **Serotonergic neurons in *C. elegans*.**

Class	Type	Location	Function
NSMs[38] (2, bilaterally symmetric)	Neurosecretory Motor Neuron	Anterior bulb of pharynx	Pharyngeal pumping
ADFs[19] (2, bilaterally symmetric)	Amphid sensory neuron	Nerve ring	Dauer entry
AIMs[19] (2, bilaterally symmetric)	Interneuron	Nerve ring	Unknown
RIH[19] (unpaired)	Interneuron	Nerve ring	Unknown
HSNs[39] (2, bilaterally symmetric)	Motor neuron, Hermaphrodite Specific	Vulva	Egg-laying
VC4, VC5[19] (unpaired)	Motor neuron, hermaphrodite specific	Vulva/ventral cord	Egg-laying
CP1-6[34] (unpaired)	Possible motor neuron, male specific	Ventral cord	Male mating
R1, R3, R9[34] (bilaterally symmetric)	Ray sensory neurons, male specific	Male tail/lumbar ganglia	Male mating

The *C. elegans* serotonin transporter (*mod-5)* gene encodes a protein with 44% amino acid identity with mammalian SERT proteins that confers paroxetine-sensitive 5-HT transport on nonneuronal cells after heterologous expression[40]. *mod-5* activity within the HSNs, ADFs, and NSMs is inferred from the detection of 5-HT immunofluoresence in mutants that lack the ability to synthesize 5-HT (*tph-1*) after incubation with exogenous 5-HT and which can be blocked by selective serotonin reuptake inhibitor (SSRI) fluoxetine[40]. *mod*-5 null mutants are viable and healthy, and consistent with the hypothesis that these animals express excess synaptic 5-HT these animals exhibit hyperenhanced slowing, increased egg-laying in response to 5-HT, and reduced fat content[40, 41]. The effects of exogenous 5-HT and behaviors in animals lacking of 5-HT synthesis indicate *mod-5* mutants might be expected to express dauer entry resistance and increased pharyngeal pumping, although this has not yet been characterized. In the following sections we will discuss the role of *mod-5* activity within a selection of these phenotypes easily scored in the laboratory to ascertain their suitability as a basis for a forward genetic screen.

MOD-5 AND PHARYNGEAL PUMPING

Nematodes feed by the peristaltic motion of the pharynx known as pharyngeal pumping, which serves to suck in and trap a slurry of bacteria within a bulbular extension of the pharynx, which is then ground and pushed into the intestine[42]. Worms perform this motion about 40 times a minute in the absence of food and greater than 200 times a minute in the presence of food[43]. Traditional methods of measuring pharyngeal pumping involve manual quantification of pumping rates; hence this behavior is not frequently used in forward genetic screens. More sophisticated methods of quantifying pumping rates exploit the transparent nature of the worm, correlating pumping rate with an intake of a fluorescent reporter comparable in size to bacteria[44]. This paradigm is amenable to high-throughput methods but requires an initial investment in instrumentation capable of isolating and recording fluorescence from a single worm. Pharyngeal pumping is thought to be partly regulated by the two serotonergic neurosecretory motor neurons (NSMs) located in the anterior bulb of the pharynx (**Figure 3**). These are the most robustly stained serotonergic neurons within the animal and send processes to the region of the pharynx where bacteria accumulate, suggesting they are the "food sensing" neurons of the worm[42]. Exogenous 5-HT increases pharyngeal pumping[33], however laser ablation of the NSMs only modestly decreases pharyngeal pumping[43]. Further ablation of all neurons within the pharynx except M4 causes only minor deficits in pharyngeal pumping[43], suggesting that an intrinsic pacemaker ability may exist within pharyngeal muscle cells and that most pharyngeal neurons are dispensable under standard laboratory conditions. Interestingly, *tph-1* mutants show wildtype pumping rates in the absence of food but deficient pumping in the presence of food[18] demonstrating serotonin is not required for basal pumping activity. *mod-5* mutants are expected to show increased pharyngeal pumping for which there is a much smaller potential pool of confounding mutants than other phenotypes. Further investigation will demonstrate the potency of this phenotype and role of *mod-5* in this behavior that has the potential to provide a basis for a screen to elucidate regulators of SERT expression and function.

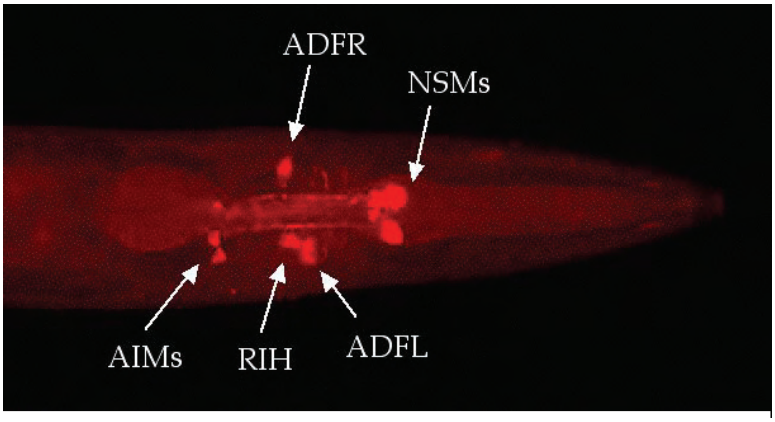

Figure 3 | **Anti-5-HT immunofluoresence in the *C. elegans* head neurons.** Photo courtesy of the Loer Lab: http://home.sandiego.edu/~cloer/loerlab/5-HTcells.html

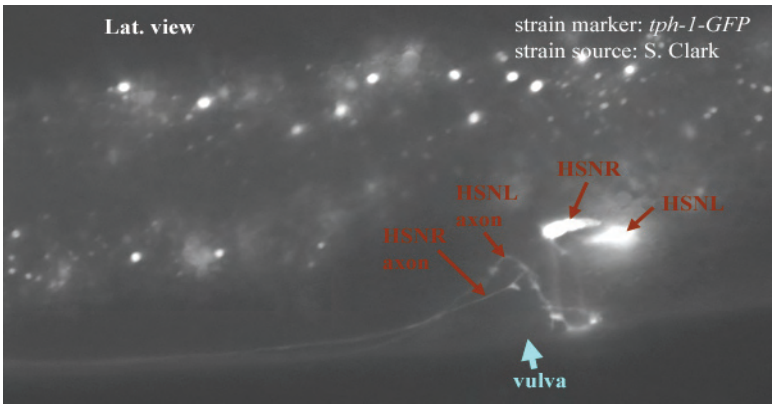

Figure 4 | **GFP imaging of the *C. elegans* vulva revealing left and right HSN cell bodies with axons synapsing the vulval musculature.** Anterior is left, ventral is down. Nonspecific fluorescence in anterior and dorsal areas is gut autofluoresence. Photo courtesy of wormbase.org.

MOD-5 AND EGG-LAYING

Egg-laying is one of the most popular phenotypes for genetic screens in *C. elegans* because abnormal egg-laying is easily observable in the laboratory with manual techniques. The effects of 5-HT and other pharmacological agents on egg-laying are readily examined by incubating a single animal in buffer containing drug and counting the number of eggs laid after a short period. Mutant animals incapable of egg-laying are easily identified within a large population as they become bloated with eggs retained in the uterus, a phenotype known as "*egl*" or more colorfully as "bag of worms," which describes the process of egg-hatching within the adult animal. Egg-laying is regulated by activity of the HSNs (**Figure 4**) and VCs, both which innervate the vulval muscle[19, 38, 39, 45]. Mutant hermaphroditic animals in which the HSNs undergo cell death display an *egl* phenotype[30], and this mutation confers resistance to fluoxetine and imipramine induced egg-laying[46], which indicates a modulatory role for *mod-5* at the HSNs in egg-laying. Consistent with the hypothesis that *mod-5* mutants express increased synaptic 5-HT, *mod-5* mutants are hypersensitive to the presence of 5-HT and lay more eggs than wildtype at a given 5-HT concentration[40]. The biggest difference between the two groups lies at a modest concentration of 5-HT (~6mM) where a wildtype worm will lay between 0 and 14 eggs within an hour, (on average about 2.5 eggs) and a *mod-5* animal under the same conditions will lay between 0 and 17 eggs, with an average of 10 eggs (unpublished data, **Figure 5**). Based on the variability observed in individual egg-laying responses, screening a mutant population for the *mod-5* egg-laying phenotype requires either generating an average egg-laying profile for clonal populations of mutagenized F2 animals (instead of assaying single mutants), thereby increasing the number of total experiments by 10-fold or the number of false positives recovered. Alternatively a screen could be envisioned utilizing the effects of SSRIs on the egg-laying system, where wildtype animals would be expected to lay eggs in response to fluoxetine, yet the drug would fail to induce egg-laying in *mod-5* mutants. However application of the antidepressants fluoxetine, imipramine, and clomipramine to both *mod-5* and *tph-1* animals results in egg-laying similar to that observed in wildtype[47] indicating these antidepressants activate alternative targets within the worm, possibly the 5-HT receptors themselves[47]. Therefore, although SSRI-induced egg-laying is HSN dependent, it is 5-HT and *mod-5* independent. Together these studies indicate the egg-laying circuitry as well as the influence of *mod-5* on egg-laying is more complex than initially envisioned. There are multiple levels for modulation of egg-laying, from neurons in the head to the vulval muscle, thus the level at which the action *mod-5* most significantly influences egg-laying is unclear. The off-target effects of SSRIs in *C. elegans* limit the potential egg-laying phenotypes of *mod-5* mutants for use in forward genetic screens and the use of these drugs to examine the integrity of *mod-5* and HSN function. However, egg-laying remains an easily identifiable, semi-high throughput, and well characterized phenotype which may be utilized to examine regulatory genes controlling SERT transporter trafficking, localization, and activity.

MOD-5 AND LOCOMOTION

Abnormal locomotor activity is another *C. elegans* behavior easily observed in the laboratory. Paralyzed animals are easily identified within a population or in response to exogenous drug, and many mutations have been characterized that result in abnormal or uncoordinated movement. Application of exogenous 5-HT results in decreased locomotion[33] and *mod-5* null mutants display increased sensitivity to 5-HT induced immobilization[40]. This phenotype could be exploited by incubating a population of mutagenized animals on a plate containing 5-HT and isolating immobilized animals. However, isolated

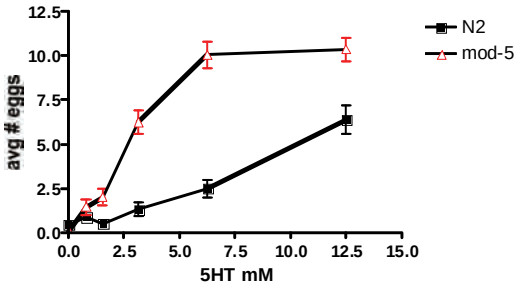

Figure 5 | **Average egg-laying response of wildtype and SERT-defective mutants in increasing concentrations of 5-HT.** n=50 for each data point.

mutants may contain defects in 5-HT reuptake as well as body muscle formation and GABA and acetylcholine synthesis and release. To prevent isolation of animals with general mutations of the motor circuit, a locomotory-based screen should require animals to move to a particular area of the plate before assessment of 5-HT induced immobilization, similar to the paradigm used to observe the enhanced slowing response. Animals starved for a brief period (30 min) display a normal locomotor rate which dramatically slows upon encountering a bacterial lawn (enhanced slowing[35]), a trait evolved presumably to protect the animal from starvation. This is observed in the laboratory by manually quantifying the locomotor rate of starved animals as they move from an area without food to a bacterial lawn. Starved animals are not hypersensitive to inhibition of locomotion by 5-HT, suggesting this behavior is modulated presynaptically. 5-HT synthesis mutants completely lack this response, a deficit that is rescued by the application of exogenous 5-HT[35]. Enhanced slowing is blocked by 5-HT receptor antagonists mianserin and methiothepin[35] further supporting the role of 5-HT in this behavior, and is potentiated by fluoxetine[40], suggesting this response is a direct measure of *mod-5* activity. *mod-5* mutants display wildtype locomotory rates under standard laboratory conditions and exhibit a hyper-enhanced slowing response. Starved wildtype animals typically slow from a rate of 60 body bends per minute to 15 body bends per minute upon encountering food, whereas *mod-5* mutants become almost immobile[40]. Enhanced slowing is partially mediated through the putative food sensing NSMs as laser ablation of these neurons significantly, but not completely, impairs the enhanced slowing response[35]. Enhanced slowing in NSM ablated animals is not potentiated by fluoxetine[35], indicating *mod-5* influences locomotion at the NSMs. These data demonstrate the important regulatory role of *mod-5* within the *C. elegans* motor circuit and the utility of this phenotype in a screen to elucidate mechanisms of SERT function. However, observation of this phenotype in the laboratory is labor intensive and would be more effective in a screen if amenable to higher throughput methods.

SUMMARY

The unique in-depth knowledge of neuronal wiring and development paired with the elegant combination of genetic tractability and simplified behavior makes the synaptically conserved *C. elegans* system amenable to many powerful approaches, particularly forward genetics. Until recently the effects of 5-HT in this system have been broadly examined through excessive exogenous application of 5-HT or a widespread loss of 5-HT synthesis. Recent characterizations of SERT-defective mutants provide phenotypes, particularly pumping and locomotion, with which to investigate endogenous regulators of SERT and 5-HT signaling. Further characterization of these mutants may reveal additional phenotypes, including resistance to dauer entry and fat accumulation, to use in a screen which may reveal the impact of SERT alleles on 5-HT transport and turnover. These approaches may provide unbiased assessments of transporter regulatory molecules both in the worm and in man, potential novel drugable targets for the treatment of many 5-HT-related disorders, and help elucidate the genetic basis of behavior.

REFERENCES

1. Wong DT, Perry KW and Bymaster FP (2005). Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat Rev Drug Discov* **4** (9): 764-774.
2. Fuller RW and Wong DT (1990). Serotonin uptake and serotonin uptake inhibition. *Ann N Y Acad Sci* **600** 68-78; discussion 79-80.
3. Rudnick G and Clark J (1993). From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim Biophys Acta* **1144** (3): 249-263.
4. Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V and Blakely RD (1993). Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci U S A* **90** (6): 2542-2546.
5. Rudnick G and Wall SC (1992). The molecular mechanism of "ecstasy" [3,4-methylenedioxy-methamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release. *Proc Natl Acad Sci U S A* **89** (5): 1817-1821.
6. Sodhi MS and Sanders-Bush E (2004). Serotonin and brain development. *Int Rev Neurobiol* **59** 111-174.
7. Hahn MK and Blakely RD (2002). Monoamine transporter gene structure and polymorphisms in relation to psychiatric and other complex disorders. *Pharmacogenomics J* **2** (4): 217-235.
8. Murphy DL, Lerner A, Rudnick G and Lesch KP (2004). Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol Interv* **4** (2): 109-123.
9. Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, Han Q, Jiang L, Li C, Folstein SE and Blakely RD (2005). Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *Am J Hum Genet* **77** (2): 265-279.
10. Ramamoorthy S, Giovanetti E, Qian Y and Blakely RD (1998). Phosphorylation and regulation of antidepressant-sensitive serotonin transporters. *J Biol Chem* **273** (4): 2458-2466.
11. Qian Y, Galli A, Ramamoorthy S, Risso S, DeFelice LJ and Blakely RD (1997). Protein kinase C activation regulates human serotonin transporters in HEK-293 cells via altered cell surface expression. *J Neurosci* **17** (1): 45-57.
12. Jayanthi LD, Samuvel DJ, Blakely RD and

Ramamoorthy S (2005). Evidence for biphasic effects of protein kinase C on serotonin transporter function, endocytosis, and phosphorylation. *Mol Pharmacol* **67** (6): 2077-2087.

13. Samuvel DJ, Jayanthi LD, Bhat NR and Ramamoorthy S (2005). A role for p38 mitogen-activated protein kinase in the regulation of the serotonin transporter: evidence for distinct cellular mechanisms involved in transporter surface expression. *J Neurosci* **25** (1): 29-41.
14. Daws LC, Gould GG, Teicher SD, Gerhardt GA and Frazer A (2000). 5-HT(1B) receptor-mediated regulation of serotonin clearance in rat hippocampus in vivo. *J Neurochem* **75** (5): 2113-2122.
15. Zhu CB, Steiner JA, Munn JL, Daws LC, Hewlett WA and Blakely RD (2007). Rapid stimulation of presynaptic serotonin transport by A(3) adenosine receptors. *J Pharmacol Exp Ther* **322** (1): 332-340.
    This paper describes several mechanisms of SERT regulation, especially the impact of PKG and p38 MAPK on SERT trafficking and activity, and demonstrates a role for adenosine A3 receptor activity in control of 5-HT clearance.
16. Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A, Mossner R, Westphal H and Lesch KP (1998). Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. *Mol Pharmacol* **53** (4): 649-655.
17. Carneiro AM, Airey DC, Thompson B, Zhu CB, Lu L, Chesler EJ, Erikson KM and Blakely RD (2009). Functional coding variation in recombinant inbred mouse lines reveals multiple serotonin transporter-associated phenotypes. *Proc Natl Acad Sci U S A* **106** (6): 2047-2052.
18. Sze JY, Victor M, Loer C, Shi Y and Ruvkun G (2000). Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. *Nature* **403** (6769): 560-564.
    Identification and cloning of the *C. elegans* 5-HT synthesis gene *tph-1* identifies the serotonergic neurons capable of 5-HT synthesis as well as several key 5-HT dependent behaviors through phenotypic analysis of *tph-1* mutants.
19. Duerr JS, Frisby DL, Gaskin J, Duke A, Asermely K, Huddleston D, Eiden LE and Rand JB (1999). The cat-1 gene of Caenorhabditis elegans encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J Neurosci* **19** (1): 72-84.
20. Hosono R and Kamiya Y (1991). Additional genes which result in an elevation of acetylcholine levels by mutations in Caenorhabditis elegans. *Neurosci Lett* **128** (2): 243-244.
21. Hosono R, Hekimi S, Kamiya Y, Sassa T, Murakami S, Nishiwaki K, Miwa J, Taketo A and Kodaira KI (1992). The unc-18 gene encodes a novel protein affecting the kinetics of acetylcholine metabolism in the nematode Caenorhabditis elegans. *J Neurochem* **58** (4): 1517-1525.
22. Olde B and McCombie WR (1997). Molecular cloning and functional expression of a serotonin receptor from Caenorhabditis elegans. *J Mol Neurosci* **8** (1): 53-62.
23. Hamdan FF, Ungrin MD, Abramovitz M and Ribeiro P (1999). Characterization of a novel serotonin receptor from Caenorhabditis elegans: cloning and expression of two splice variants. *J Neurochem* **72** (4): 1372-1383.
24. Hapiak VM, Hobson RJ, Hughes L, Smith K, Harris G, Condon C, Komuniecki P and Komuniecki RW (2009). Dual excitatory and inhibitory serotonergic inputs modulate egg laying in Caenorhabditis elegans. *Genetics* **181** (1): 153-163.
25. Hobson RJ, Hapiak VM, Xiao H, Buehrer KL, Komuniecki PR and Komuniecki RW (2006). SER-7, a Caenorhabditis elegans 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics* **172** (1): 159-169.
26. Ranganathan R, Cannon SC and Horvitz HR (2000). MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in C. elegans. *Nature* **408** (6811): 470-475.
27. Sulston JE and Horvitz HR (1977). Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. *Dev Biol* **56** (1): 110-156.
28. Sulston JE, Schierenberg E, White JG and Thomson JN (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. *Dev Biol* **100** (1): 64-119.
29. White JGS, E. Thomson, J.N. Brenner, S. (1986). The structure of the nervous system of the nematode *C. elegans. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **314** 1-340.
30. Ellis HM and Horvitz HR (1986). Genetic control of programmed cell death in the nematode C. elegans. *Cell* **44** (6): 817-829.
31. Trent C, Tsuing N and Horvitz HR (1983). Egg-laying defective mutants of the nematode Caenorhabditis elegans. *Genetics* **104** (4): 619-647.
32. Chalfie M, Horvitz HR and Sulston JE (1981). Mutations that lead to reiterations in the cell lineages of C. elegans. *Cell* **24** (1): 59-69.
33. Horvitz HR, Chalfie M, Trent C, Sulston JE and Evans PD (1982). Serotonin and octopamine in the nematode Caenorhabditis elegans. *Science* **216** (4549): 1012-1014.
34. Loer CM and Kenyon CJ (1993). Serotonin-deficient mutants and male mating behavior in the nematode Caenorhabditis elegans. *J Neurosci* **13** (12): 5407-5417.
35. Sawin ER, Ranganathan R and Horvitz HR (2000). C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* **26** (3): 619-631.
    This paper examines 5-HT modulation of locomotion, demonstrating a role for *C. elegans* SERT within specific serotonergic neurons to integrate current food availability with previous experience to impact the motor circuit.
36. Chao MY, Komatsu H, Fukuto HS, Dionne HM and Hart AC (2004). Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. *Proc Natl Acad Sci U S A* **101** (43): 15512-15517.
37. Zhang Y, Lu H and Bargmann CI (2005). Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. *Nature* **438** (7065): 179-184.
38. McIntire SL, Garriga G, White J, Jacobson D and Horvitz HR (1992). Genes necessary for directed axonal elongation or fasciculation in C. elegans. *Neuron* **8** (2): 307-322.
39. Desai C, Garriga G, McIntire SL and Horvitz HR

(1988). A genetic pathway for the development of the Caenorhabditis elegans HSN motor neurons. *Nature* **336** (6200): 638-646.

40. Ranganathan R, Sawin ER, Trent C and Horvitz HR (2001). Mutations in the Caenorhabditis elegans serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. *J Neurosci* **21** (16): 5871-5884.
Identification and cloning of the *C. elegans* SERT (*mod-5*) describes homology to mammalian SERT and pharamacological response profiles after heterologous expression. *In vivo* disruption reveals the role of *mod-5* activity in 5-HT related behaviors and phenotypes theoretically resulting from excess endogenous 5-HT. Cells expressing *mod-5* are implicated and 5-HT independent effects of fluoxetine are also examined.
41. Srinivasan S, Sadegh L, Elle IC, Christensen AG, Faergeman NJ and Ashrafi K (2008). Serotonin regulates C. elegans fat and feeding through independent molecular mechanisms. *Cell Metab* **7** (6): 533-544.
42. Albertson DG and Thomson JN (1976). The pharynx of Caenorhabditis elegans. *Philos Trans R Soc Lond B Biol Sci* **275** (938): 299-325.
43. Avery L and Horvitz HR (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. *Neuron* **3** (4): 473-485.
44. Boyd WA, McBride SJ and Freedman JH (2007). Effects of genetic mutations and chemical exposures on Caenorhabditis elegans feeding: evaluation of a novel, high-throughput screening assay. *PLoS One* **2** (12): e1259.
45. Waggoner LE, Zhou GT, Schafer RW and Schafer WR (1998). Control of alternative behavioral states by serotonin in Caenorhabditis elegans. *Neuron* **21** (1): 203-214.
46. Weinshenker D, Garriga G and Thomas JH (1995). Genetic and pharmacological analysis of neurotransmitters controlling egg laying in C. elegans. *J Neurosci* **15** (10): 6975-6985.
47. Dempsey CM, Mackenzie SM, Gargus A, Blanco G and Sze JY (2005). Serotonin (5HT), fluoxetine, imipramine and dopamine target distinct 5HT receptor signaling to modulate Caenorhabditis elegans egg-laying behavior. *Genetics* **169** (3): 1425-1436.

FURTHER INFORMATION

Randy Blakely's Lab: www.blakelylab.org

Genetics in Invertebrates: Modeling Dopaminergic Signaling and Neurodegeneration

J. Andrew Hardaway

Dopamine (DA) is an important modulatory neurotransmitter, mediating complex human processes such as arousal, learning, reward and motor control; those same behaviors that go awry in neural disorders such as attention-deficit-hyperactivity disorder(ADHD), bipolar disorder, schizophrenia and Parkinson's Disease (PD). Owing to the anatomical and genetic complexity of vertebrate model systems, the invertebrate model systems *Caenhorhabditis elegans* and *Drosophila melanogaster* are ideal systems to study genetic contributions within DA networks and, in the case of flies, genes that contribute to or suppress DA neuron degeneration. In worms, the use of forward and reverse genetics has revealed how a modest dopaminergic nervous system can regulate locomotion, and how a simple locomotory phenotype can be employed to identify novel regulatory genes and their functions within its DA network. Continued genetic study of these networks may reveal novel genes involved in regulating DA biosynthesis, release, uptake and signaling. Many genes have been identified in familial-associated PD (FAPD), and these candidate genes have been used in flies to study neurodegeneration. This review will describe how flies have been employed to dissect the function of three FAPD genes: α-synuclein, parkin, and PINK1. In the case of parkin and PINK1, reverse genetic approaches in the fly have revealed the importance of mitochondrial dynamics to DA neuron susceptibility in PD. The impact of these findings may define a true departure from classical forward and reverse genetics in invertebrates, and that a clearer understanding of DA neural networks will be revealed through the mutual employment of both with convergent genetics.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: hardawayja@gmail.com

INTRODUCTION

From worms to humans, dopamine(DA) is one of the most important neurotransmitters in regulating complex definitive behaviors. In humans, DA's actions extend to processes like arousal, learning, motor control and reward, the defects of which are evident in disorders such as ADHD, PD, bipolar disorder, and schizophrenia. Our understanding of these processes and disease states has advanced much in recent years, but our understanding of the molecular circuitry that regulate dopaminergic pathways is still incomplete. To uncover the proteins that regulate key processes such as DA biosynthesis, release, reuptake and signaling, a combination of genetic approaches must be taken to: (1) enrich our functional understanding of known genes, (2) discover novel genes, or (3) link existing genes to dopaminergic signaling processes. While still possessing the unbiased nature of classical forward genetics, forward genetics today is often paired with candidate-gene approaches, the use of which creates the opportunity to model dopaminergic states and determine, through mutagenesis, what novel genes can mimic or suppress these states. Conversely, the exclusive use of reverse genetics is, by definition, limited to the study of genes that have already been implicated in DA signaling. Drawing from both classical forward and reverse genetic approaches, "convergent genetics" may be the best way to appreciate the sophisticated gene networks that regulate DA signaling. What follows is an overview of efforts in invertebrate model systems that have augmented our understanding of dopaminergic circuitry in *C. elegans* and the functional contributions of three FAPD genes: α-synuclein, parkin, and PINK1 to dopaminergic diseases like PD using *Drosophila*.

Due to the ease of genetic manipulations, a complete genome sequence, simple behaviors, and a growing list of powerful experimental techniques, *C. elegans* is an effective system with which to study the regulation of a network of DA-releasing and responsive cells. *C. elegans* have a 100 megabase (Mb) genome that consists of five pairs of autosomal chromosomes and a pair of X chromosomes in the case of self-fertilizing hermaphrodites (XO in the rare males)[1, 2]. The transparent worm has a very precise and reproducible cell lineage program, thus, the origin and development of the cellular DA network can be traced and visualized from embryo to adult[3-6]. In addition, serial electron microscope reconstructions of the entire worm have revealed the distinct

morphology, location, electrical and synaptic connections of all neurons[7]. Therefore, the role of DA can be studied within multi-neuronal circuits and its influence mapped to specific cells without reservation about unknown connections extant in vertebrates. Moreover, genetic manipulations of *C. elegans* can reveal the cell autonomous or non-autonomous function of one gene within these simple, multi-neuronal circuits.

A forward genetic approach in the worm is not new, but its application, combined with the development of *in vivo* electrophysiology, continues to reveal genes important for neuronal function, especially those essential for synaptic transmission. Erik Jorgensen and colleagues have characterized previously identified uncoordinated(*unc*) mutants as important components of synaptic vesicle docking, priming, and fusion[8-12]. The same group has also identified the function and localization of several genes in glutamatergic and GABAergic neurons within the worm[13-15]. The functional conservation of these synaptic genes in mammals demonstrates that the use of *C. elegans* is a valid approach to identify novel dopaminergic genes *in vivo*.

The study of DA signaling in *C. elegans* began in 1975 with the work of Nobel Laureate John Sulston. Using Nomarski microscopy and a technique known as formaldehyde-induced fluorescence(FIF)[16], Sulston identified all of the dopaminergic neurons in the hermaphrodite and male worms[17]. The hermaphrodite possesses eight dopaminergic neurons consisting of two pairs of cephalic (CEP) neurons, a single pair of anterior deirid neurons (ADE), and one pair of posterior deirid (PDE) neurons[17, 18]. Sulston also noted the presence of three additional pairs within the sexually dimorphic male tail in sensory rays five, seven + nine, the loss of which were later shown to cause defects in male mating coordination[17, 19]. Through the use of transgenic DA specific reporters, these neurons have been shown to specifically express genes associated with the biosynthesis of DA such as tyrosine hydroxylase (cat-2) and its uptake via the presynaptic DA transporter (dat-1)[20, 21].

After identifying these neurons, Sulston then performed a small forward genetic screen isolating mutants that showed a decrease in FIF, mutants that he deemed *cat* or catecholamine deficient. In his screen, he isolated five mutants (cat-(1-5)) that showed a reduction or complete loss of FIF, and mapped the mutations to chromosomal intervals[17]. Despite the modesty of his screen, Sulston identified three important genes involved in the maintenance of proper DA signaling in the worm. The cloning and sequencing of the cat-1 locus demonstrated that it encodes the reserpine sensitive *C. elegans* vesicular monoamine transporter (vMAT), responsible for the packaging of biogenic amines, including DA and serotonin (5-HT), into synaptic vesicles[22]. Cat-4 encodes GTP cyclohydrolase I, a necessary cofactor that participates with the aforementioned cat-2 in the biosynthesis of biogenic amines, including DA[23]. The importance of this early work cannot be overstated, as later investigators have used these mutants to study behaviors altered by hypodopaminergic and hyposerotenergic states[23], but techniques mimicking a hyperdopaminergic state in the worm have also borne fruit.

The use of exogenous DA, in combination with genetic approaches, has revealed important components of the dopaminergic network and how they function together to produce distinct behaviors in the worm, most notably locomotion. In a landmark study by Schafer and Kenyon, it was found that wild-type worms paralyze when plated on exogenous DA or 5-HT[24]. After a four-hour period, these same worms will adapt and begin to move normally, but worms lacking a functional voltage-sensitive calcium channel subunit unc-2 fail to adapt to exogenous DA and remain paralyzed. DA regulation of worm locomotion is also evident in a behavior known as the basal slowing response, a behavior where well-fed wild type worms reduce their locomotory rate upon entering a lawn of bacteria[23]. It is believed that DA, by slowing locomotion in the presence of food, maximizes the opportunity for feeding and the continued survival of the worm – as DA also modulates food search[25]. Worms lacking DA due to a mutation in cat-2 do not slow in response to food, and a later study demonstrated that worms lacking a particular DA receptor dop-3 also fail to reduce their locomotory rate[26]. With a repertoire of dopaminergic locomotory phenotypes in hand, more detailed genetic studies of the dopaminergic network were possible.

The observation that *C. elegans* will paralyze when plated on exogenous DA offered a simple, robust phenotype for researchers to exploit using both forward and reverse genetic approaches[24, 27]. Using bioinformatics, Chase *et al.* identified a new DA receptor dop-3, characterized the locomotive behaviors of worms lacking dop-3 and other known receptors, and found that dop-3 and the antagonistic actions of dop-1, mediate DA's effects on locomotion. Worms lacking dop-3 fail to demonstrate the basal slowing response and do not paralyze on exogenous DA, but both behaviors are normal in worms lacking both receptors dop-1 and dop-3. They determined that these receptors are coexpressed within groups of cholinergic and GABAergic motor neurons along the ventral cord of the animal, acting antagonistically in the same cells to either promote or inhibit locomotion. Further experiments have demonstrated that the cholinergic neurons are the most important for DA's modulation of locomotion (Daniel Chase, personal communication). Expressing fluorescently tagged

Nomarski Microscopy
A microscopy technique that enhances contrast in an unstained, transparent sample.

Formaldehyde-induced Fluorescence
The formation of fluorescent isoquinolines upon incubation of catecholamines with gaseous formaldehyde.

dop-1 and dop-3 in these neurons has shown that dop-3 is expressed diffusely along the plasma membrane in these neurons, but dop-1 is localized at the neuromuscular junctions of these neurons, expression data that supports the hypothesis that dop-1 promotes locomotion through activation of muscle and that paralysis can occur with an excess of extrasynaptic DA that hyperactivates dop-3 (Daniel Chase, personal communication). Following description of the receptors that mediate this phenomenon, they used forward genetics to look for mutants that phenocopy the exogenous DA-resistant strain *dop-3(vs106)*. They isolated nine mutants in four genomic loci, and, using these mutants and candidate based analysis they determined that dop-1 is acting through the *C. elegans* $G\alpha_q$ protein egl-30 and dop-3 is acting through the $G\alpha_o$ protein goa-1, and mutants that decrease goa-1 or increase egl-30 signaling increase exogenous DA resistance. Having elegantly described the postsynaptic actions with a convergent genetic approach, study of the presynaptic machinery has capitalized on these findings.

The aforementioned presynaptic dopaminergic neurons express components that regulate the temporal and spatial actions of DA, most importantly, the presynaptic DA transporter (DAT) or dat-1 in *C. elegans.* The study of dat-1 in *C. elegans* began with the cloning of an antidepressant and cocaine-sensitive cDNA that exhibits DA-specific uptake and encodes a protein with 47% and 43% homology to the human norepinephrine transporter (hNET) and hDAT respectively[28]. It is expressed specifically in DA neurons and will mediate the *in vivo* transport of neurotoxins such as 6-OHDA, while worms lacking a functional dat-1 are resistant to these effects[21, 29]. Dat-1 is also important in controlling DA's influence on locomotion, but, unlike the use of exogenous DA to identify DA receptors, its influence is most apparent in regulating endogenous DA levels. When placed in a small volume of water, wild-type worms will increase their locomotory rate and thrash at a sustained level for 20-25 min., but worms lacking a functional DA transporter (*dat-1(ok157))* will swim at first and paralyze within 10 minutes[30]. This phenotype, known as swimming induced paralysis, or SWIP, is presynaptically rescued by pretreatment with the vMAT inhibitor reserpine or knockout of cat-1 or cat-2, and postsynaptically by loss of dop-3. In addition, the SWIP phenotype can be induced by the application of tricyclic anti-depressants and amphetamine, which may help reveal genes important for drug action (unpublished observations and Carvelli *et al.*, in press). This robust phenotype is now being used in forward and candidate based screens to look for novel presynaptic regulators of dat-1, DA release, and postsynaptic components in motor neurons (Hardie *et al.*, in press and Daniel Chase, personal communication). The continued application of forward genetics with a robust phenotype will yield important functional insights into presynaptic genes involved in DA release and uptake, but the application of reverse genetics in invertebrate systems enables the study of one gene of interest in DA neurons.

Beginning in the mid 1990's, several genes were identified in pedigrees of FAPD, but their role in mediating DA neuron susceptibility is still unclear. One of the pathological hallmarks of PD is the progressive age-dependent loss of dopaminergic neurons in the substantia nigra, but animal models were required to study genetic contributions to this neurodegenerative process. To that end, researchers employed invertebrate and vertebrate model systems to study these candidate genes. Although this strategy has recently been used in *C. elegans* (rev. in [31, 32]), the fruit fly, *Drosophila melanogaster*, is the most common invertebrate model for modeling PD and using genetics to understand the susceptibility of dopaminergic neurons.

With its history of use dating back to Charles Woodworth and Thomas Hunt Morgan in the early 1900s, *Drosop*hila may be the most well studied and understood of all genetic model systems. Currently, its power as a tool to study neurodegeneration stems from our understanding of the organism's genome. The fly has a fully sequenced genome of 180 Mb that is contained on three pairs of autosomes and a pair of X/Y chromosomes and is predicted to encode ~13,601 genes[33]. During development some cells replicate their DNA without undergoing cell division or separation of sister chromatids, forming giant polytene chromosomes that can be seen under a microscope and have a characteristic and reproducible black and white banding pattern, which has facilitated the mapping of mutations in mutagenesis screens[33]. *Drosophila* also possess practical advantages of other invertebrates, which are short generation times, large population sizes, and a simple and consistent nervous system. The adult fly is thought to contain around 300,000 neurons, about 10^3 fold greater than the worm, so much effort has been expended to characterize dopaminergic neural populations in the fly throughout development[34]. Earlier work used immunoreactivity to dopaminergic components such as TH or dopa decarboxylase(Ddc, a.k.a – aromatic acid decarboxylase) to identify these cells, and more recent work has used the Gal4/UAS system to specifically identify cells that express these genes using fluorescent reporters[35, 36]. In studying the adult fly brain, these studies identified six pairs of clusters of dopaminergic neurons in and around the protocerebrum of the fly and a ventral medial pair[35-38]. In addition to behavioral and survival analysis, the loss of these protocerebral dopaminergic clusters has been the primary measure of PD models in the fly.

Following the linkage of the chromosomal locus 4q21-q23 in a large Italian kindred with autosomal dominant PD[39], and its subsequent identification as α-synuclein (PARK1)[40]; many researchers sought to create animal models of PD to study α-synuclein's role in pathogenesis. Using the Gal4/UAS system in flies, Feany and Bender overexpressed human WT α-synuclein and FAPD alleles (A30P and A53T) pan-neuronally and in dopaminergic neurons[41]. In all cases, they saw an age dependent loss of dopaminergic neurons, restricted involvement to the nervous system, and the formation of filamentous inclusions reminiscent of Lewy Bodies (LBs), one of the pathological hallmarks of PD. Furthermore, the overexpression of these genes in flies caused an age-dependent decline in climbing ability, which parallels motor symptoms such as bradykinesia in patients with PD. The same group reported that the loss of DA neurons was attenuated by the overexpression of the chaperone Hsp70[42] or the pharmacological activation of chaperones[43], and also showed that chaperones localize to LBs of post-mortem PD patient samples. The post-translational modification of α-synuclein may also be important, as mutant flies overexpressing phosphorylation-null (S129A) α-synuclein do not show DA neuron degeneration and, surprisingly, show increased inclusion formation. Currently a subject of considerable debate, these results are consistent with the hypothesis that inclusions may be a neuroprotective mechanism.

Now equipped with a model that recapitulates the core pathology and behavior of the disease, many investigators sought to study the genetic influences that might suppress neurodegeneration in a model of PD. Genetic studies of FAPD have identified several genes that produce autosomal recessive juvenile parkinsonism (ARJP)[44-46], but it is the candidate gene approach in the fly that has revealed much about the function of two ARJP genes PTEN induced kinase 1 or PINK1(PARK6) and parkin(PARK2) *in vivo* and how they may regulate the susceptibility of dopaminergic neurons.

Through study of its function in *Drosophila*, we now better understand the function of PINK1 in dopaminergic neurons. PINK1 was first identified in a large Sicilian kindred with four family members afflicted by ARJP[44], who were later found to have nonsense or missense loss of-function single nucleotide polymorphisms (SNPs) in the coding region of PINK1[47]. In 2006, three groups published studies characterizing the neurodegenerative phenotypes of flies with deletions in dPINK1, and found very similar phenotypes[48-50]. These mutant strains showed decreased longevity, male sterility, and mitochondrial defects in energy demanding regions of the fly such as the male testis, thorax, and flight muscle. Such defects included vacuolarization of the mitochondria, disorganization, and a decrease in total ATP levels, suggesting that PINK1 has a role in mitochondrial maintenance. In both studies, these defects could be suppressed by the overexpression of parkin, which is consistent with an epistatic role of parkin to PINK1.

Parkin was first identified in a large deletion in a Japanese patient with ARJP, and was mapped to the long arm of chromosome 6(6q25.2-q27)[45]. The cloning and sequencing of this gene revealed an N-terminal ubiquitin-like domain, a motif similar to a RING-finger at its C-terminus, and would later be identified as a cytoplasmic E3 ubiquitin ligase[5,1]. Consistent with its role in ARJP and as a neurodegenerative suppressor, the knockdown of parkin in flies (*parkin*) could augment PaeI-R-mediated loss of dopaminergic neurons, and, conversely, its overexpression could protect against both PaeI-R and α-synuclein-mediated DA neurodegeneration[52]. A second group showed that a complete loss-of-function resulted in a reduced lifespan, locomotor defects and male sterility; the underlying basis for which was shown to be mitochondrial dysfunction[53]. Using reverse genetic approaches in an invertebrate system, these studies, and those of PINK1, have shown that mitochondrial dysfunction may underlie susceptibility of DA neurons in PD.

Mitochondria are highly dynamic organelles, and the importance of PINK1 and parkin in regulating these dynamics may be a pivotal process in maintaining neural integrity and preventing neurodegeneration (rev in. [54]). Genetic interactions have demonstrated that PINK1/parkin acts in a linear pathway in mitochondrial fission, and that mitochondrial defects and behavioral phenotypes evident in these mutants are rescued by reduced gene dosage of proteins that promote mitochondrial fusion[55]. A similar study confirmed the same phenomenon concerning these fission/fusion regulatory proteins, and, using mitoGFP, demonstrated that the loss of PINK1 causes the formation of mitochondrial aggregates and tubules not seen in WT[56]. These data suggest that mitochondrial fission is somehow neuroprotective in *Drosophila* DA neurons, or, alternatively, that neurodegeneration in the case of flies and patients with ARJP results from the inability of these neurons to adapt to their dynamic energetic needs through either fission or fusion. For example, it has been shown that mitochondrial fusion protects against neurodegeneration in the cerebellum, so the neuroprotective dynamics of mitochondria may not be so one-sided[57]. In addition, a recent study using SH-SY5Y cells showed that mitochondrial morphological defects and a decrease in ATP production upon loss of function of parkin or PINK1 were rescued by the

Lewy Bodies
Proteinaceous neural aggregates that typify PD patients, but are also seen in other neurological disorders.

Bradykinesia
Slowness in the execution of movement, one of the primary motor symptoms of PD.

E3 ubiquitin ligase
Enzyme that pairs with an E2 ubiquitin-conjugating enzyme to attach ubiquitin moieties to particular substrate lysine residues.

Mitochondrial fission
Division of mitochondria into two smaller parts.

Mitochondrial fusion
The combination of two mitochondria into one that occurs at the tips or sides of the mitochondria.

mitoGFP
Green fluorescent protein (GFP) fused to a mitochondrial localization sequence, effectively labeling only mitochondria with GFP.

overexpression of proteins that promote mitochondrial fusion, Mfn2 and Opa1, or the expression of a dominant negative Drp1, which inhibits fission[58]. The precise roles of PINK1/parkin in the fission machinery are unknown, so the continued study of these genes in invertebrates may help refine their function in regulating mitochondrial dynamics.

In summary, the use of convergent genetics in invertebrate model systems has revealed much about dopaminergic signaling components in *C. elegans* and DA neurodegenerative susceptibility genes in *Drosophila*. The ease of genetic manipulations, transgenic expression and knockdown in these systems make them a simple, tractable system to study candidate genes. Furthermore, genomic annotation, conserved anatomical phenotypes, and simple behaviors in these two systems create the opportunity to use forward genetics to identify novel components of dopaminergic networks or proteins that may suppress or induce neurodegeneration in these systems.

REFERENCES

1. Brenner S (1974). The genetics of Caenorhabditis elegans. *Genetics*. **77** (1): 71-94.
2. Hodgkin J (2005). Introduction to genetics and genomics. *WormBook*. 1-3.
3. Sulston JE, Albertson DG and Thomson JN (1980). The Caenorhabditis elegans male: postembryonic development of nongonadal structures. *Dev Biol*. **78** (2): 542-576.
4. Sulston JE and Horvitz HR (1977). Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. *Dev Biol*. **56** (1): 110-156.
5. Sulston JE, Schierenberg E, White JG and Thomson JN (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. *Dev Biol*. **100** (1): 64-119.
6. Sulston JE and White JG (1980). Regulation and cell autonomy during postembryonic development of Caenorhabditis elegans. *Dev Biol*. **78** (2): 577-597.
7. White, Southgate E, Thomson JN and Brenner S (1986). The structure of the nervous system of the nematode Caenorhabditis elegans. *Philosophical Transactions of the Royal Society of …*.
8. Richmond JE, Davis WS and Jorgensen EM (1999). UNC-13 is required for synaptic vesicle fusion in C. elegans. *Nat Neurosci*. **2** (11): 959-964.
9. Richmond JE, Weimer RM and Jorgensen EM (2001). An open form of syntaxin bypasses the requirement for UNC-13 in vesicle priming. *Nature*. **412** (6844): 338-341.
10. Weimer R, Richmond J, Davis W, Hadwiger G, Nonet M and Jorgensen E (2003). Defects in synaptic vesicle docking in unc-18 mutants. *Nat Neurosci*. **6** (10): 1023-1030.
11. Hammarlund M, Palfreyman M, Watanabe S, Olsen S and Jorgensen E (2007). Open syntaxin docks synaptic vesicles. *Plos Biol*. **5** (8): e198.
12. Hammarlund M, Watanabe S, Schuske K and Jorgensen E (2008). CAPS and syntaxin dock dense core vesicles to the plasma membrane in neurons. *The Journal of Cell Biology*. **180** (3): 483-491.
13. McIntire SL, Reimer RJ, Schuske K, Edwards RH and Jorgensen EM (1997). Identification and characterization of the vesicular GABA transporter. *Nature*. **389** (6653): 870-876.
14. Schuske K, Beg AA and Jorgensen E (2004). The GABA nervous system in C. elegans. *Trends Neurosci*. **27** (7): 407-414.
15. Schuske K, Palfreyman M, Watanabe S and Jorgensen E (2007). UNC-46 is required for trafficking of the vesicular GABA transporter. *Nat Neurosci*. **10** (7): 846-853.
16. DeLellis RA (1971). Formaldehyde-induced fluorescence technique for the demonstration of biogenic amines in diagnostic histopathology. *Cancer*. **28** (6): 1704-1710.
17. Sulston J, Dew M and Brenner S (1975). Dopaminergic neurons in the nematode Caenorhabditis elegans. *J Comp Neurol*. **163** (2): 215-226.
18. Mcdonald P, Jessen T, Field J and Blakely RD (2006). DA Signaling Architecture in Caenorhabditis elegans. *Cell Mol Neurobiol*. **26** (4-6): 591-616.
19. Liu KS and Sternberg PW (1995). Sensory regulation of male mating behavior in Caenorhabditis elegans. *Neuron(Cambridge*.
20. Lints R and Emmons SW (1999). … dopaminergic neurotransmitter identity among Caenorhabditis elegans ray sensory neurons …. *Development*.
21. Nass R, Hall DH, Miller DM and Blakely RD (2002). Neurotoxin-induced degeneration of DA neurons in Caenorhabditis elegans. *Proc Natl Acad Sci USA*. **99** (5): 3264-3269.
22. Duerr JS, Frisby DL, Gaskin J, Duke A, Asermely K, Huddleston D, Eiden LE and Rand JB (1999). The cat-1 gene of Caenorhabditis elegans encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J Neurosci*. **19** (1): 72-84.
23. Sawin ER, Ranganathan R and Horvitz HR (2000). C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron*. **26** (3): 619-631.
24. Schafer WR and Kenyon CJ (1995). A calcium-channel homologue required for adaptation to DA and serotonin in Caenorhabditis elegans. *Nature*. **375** (6526): 73-78.
25. Hills T, Brockie PJ and Maricq AV (2004). DA and glutamate control area-restricted search behavior in Caenorhabditis elegans. *J Neurosci*. **24** (5): 1217-1225.
26. Chase DL, Pepper JS and Koelle MR (2004). Mechanism of extrasynaptic DA signaling in Caenorhabditis elegans. *Nat Neurosci*. **7** (10): 1096-1103.
    Paper elegantly describes the postsynaptic actions of DA on locomotion. Important to separate DA's effects pre and post-synaptically
27. Weinshenker D, Garriga G and Thomas JH (1995). Genetic and pharmacological analysis of neurotransmitters controlling egg laying in C. elegans. *J Neurosci*. **15** (10): 6975-6985.
28. Jayanthi LD, Apparsundaram S, Malone MD, Ward E, Miller DM, Eppler M and Blakely RD (1998). The Caenorhabditis elegans gene T23G5.5 encodes an antidepressant- and cocaine-sensitive DA transporter. *Mol Pharmacol*. **54** (4): 601-609.
    Describes the initial cloning of the DAT in *C. elegans* and demonstrates its pharmacological profile. My studies are looking directly at regulation of dat-1.
29. Nass R, Hahn MK, Jessen T, McDonald PW, Carvelli L and Blakely RD (2005). A genetic screen in Caenorhabditis elegans for DA neuron insensitivity to 6-hydroxydopamine identifies DA transporter mutants impacting transporter biosynthesis and trafficking. *J Neurochem*. **94** (3): 774-785.
30. Mcdonald P, Hardie S, Jessen T, Carvelli L, Matthies D and Blakely RD (2007). Vigorous Motor Activity in

Caenorhabditis elegans Requires Efficient Clearance of DA Mediated by Synaptic Localization of the DA Transporter DAT-1. *J Neurosci*. **27** (51): 14216-14227.
Paper describes the phenotype I'm employing for my studies. It describes the importance of presynaptic regulation *in vivo*.

31. Nass R and Blakely RD (2003). T HE C AENORHABDITIS ELEGANS D OPAMINERGIC S YSTEM: Opportunities for Insights …. *Annu Rev Pharmacol Toxicol*.
32. Caldwell GA and Caldwell KA (2008). Traversing a wormhole to combat PD. *Dis Model Mech*. **1** (1): 32-36.
33. Leland H. Hartwell LH, Michael L. Goldberg, Ann E. Reynolds, Lee M. Silver, Ruth C. Veres (2008). Genetics: From Genes to Genomes, 3rd Edition. New York,NY: McGraw-Hill.
34. Wolf FW and Heberlein U (2003). Invertebrate models of drug abuse. *J Neurobiol*. **54** (1): 161-178.
35. Friggi-Grelin F, Coulom H, Meller M, Gomez D, Hirsh J and Birman S (2003). Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. *J Neurobiol*. **54** (4): 618-627.
36. Lundell MJ and Hirsh J (1994). Temporal and spatial development of serotonin and DA neurons in the Drosophila CNS. *Dev Biol*. **165** (2): 385-396.
37. Budnik V and White K (1988). Catecholamine-containing neurons in Drosophila melanogaster: distribution and development. *J Comp Neurol*. **268** (3): 400-413.
38. Nässel DR and Elekes K (1992). Aminergic neurons in the brain of blowflies and Drosophila: DA- and tyrosine hydroxylase-immunoreactive neurons and their relationship with putative histaminergic neurons. *Cell Tissue Res*. **267** (1): 147-167.
39. Polymeropoulos MH, Higgins JJ, Golbe LI, Johnson WG, Ide SE, Di Iorio G, Sanges G, Stenroos ES, Pho LT, Schaffer AA, Lazzarini AM, Nussbaum RL and Duvoisin RC (1996). Mapping of a gene for PD to chromosome 4q21-q23. *Science*. **274** (5290): 1197-1199.
40. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI and Nussbaum RL (1997). Mutation in the alpha-synuclein gene identified in families with PD. *Science*. **276** (5321): 2045-2047.
41. Feany MB and Bender WW (2000). A Drosophila model of PD. *Nature*. **404** (6776): 394-398.
Paper was the first to describe the use of *Drosophila* in modeling PD. **It also demonstrates how candidate gene approaches can be used effectively in study of DA neurodegeneration.**
42. Auluck PK, Chan HY, Trojanowski JQ, Lee VM and Bonini NM (2002). Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for PD. *Science*. **295** (5556): 865-868.
43. Auluck PK and Bonini NM (2002). Pharmacological prevention of Parkinson disease in Drosophila. *Nat Med*. **8** (11): 1185-1186.
44. 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.
45. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y and Shimizu N (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*. **392** (6676): 605-608.
46. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA and Heutink P (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science*. **299** (5604): 256-259.
47. 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, González-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 PD caused by mutations in PINK1. *Science*. **304** (5674): 1158-1160.
48. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM and Chung J (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. *Nature*. **441** (7097): 1157-1161.
49. Clark IE, Dodson MW, Jiang C, Cao JH, Huh J, Seol J, Yoo S, Hay B and Guo M (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*. **441** (7097): 1162-1166.
50. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, Yang L, Beal MF, Vogel H and Lu B (2006). Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. *Proc Natl Acad Sci USA*. **103** (28): 10793-10798.
51. Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K and Suzuki T (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat Genet*. **25** (3): 302-305.
52. Yang Y, Nishimura I, Imai Y, Takahashi R and Lu B (2003). Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. *Neuron*. **37** (6): 911-924.
53. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany M and Pallanck LJ (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci USA*. **100** (7): 4078-4083.
54. Knott A, Perkins G, Schwarzenbacher R and Bossy-Wetzel E (2008). Mitochondrial fragmentation in neurodegeneration. *Nat Rev Neurosci*. **9** (7): 505-518.
55. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ and Pallanck LJ (2008). The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci USA*. **105** (5): 1638-1643.
56. Yang Y, Ouyang Y, Yang L, Beal MF, McQuibban A, Vogel H and Lu B (2008). Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. *Proc Natl Acad Sci USA*. **105** (19): 7070-7075.
57. Chen H, McCaffery JM and Chan DC (2007). Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell*. **130** (3): 548-562.
58. Lutz AK, Exner N, Fett ME, Schlehe JS, Kloos K, Lämmermann K, Brunner B, Kurz-Drexler A, Vogel F, Reichert AS, Bouman L, Vogt-Weisenhorn D, Wurst W, Tatzelt J, Haass C and Winklhofer KF (2009). Loss of Parkin or PINK1 Function Increases Drp1-dependent Mitochondrial Fragmentation. *J Biol Chem*. **284** (34): 22938-22951.

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FURTHER INFORMATION

Randy Blakely's Lab: www.blakelylab.org

Good Looking…Better Looking! Performance Monitoring and Behavioral Adjustments in the Oculomotor System

David Godlove

Executive control refers to the process of guiding action toward goals. Successful goal seeking agents cancel actions when changing circumstances render them inappropriate. Adaptive agents also track the frequency with which reinforcement is attained in order to adjust strategies when gains are too low. Response inhibition, or the ability to cancel action, has classically been investigated using a stop-signal task. Performance monitoring, or tracking gains and making behavioral adjustments, has been investigated using tasks with variable reward contingencies. These investigations are often carried out by observing monkeys performing saccade tasks. Since the input and output properties of the macaque oculomotor system are understood in comparatively great detail, it provides a simplified and useful model for investigating aspects of executive control. Discoveries suggest that several areas of the frontal and medial cortex are involved in oculomotor control and reward processing, including the frontal eye fields, the supplementary eye fields, and the anterior cingulate cortices. Of these, the supplementary eye fields have been further implicated in implementing behavioral adjustments during complex tasks. Neural activity in these areas, particularly in the anterior cingulate cortex, may contribute to human error related EEG signals. The behavioral relevance and physiological sources of these signals are poorly understood and animal models are sorely needed. Observations of monkeys performing asymmetrically rewarded tasks suggest that the mesencephalic dopamine system and basal ganglia may interact with frontal and medial cortices implementing a broad performance monitoring system.

Saccades
Accurate, high velocity eye movements used by primates to control gaze with precision.

Oculomotor system
Broadly, the areas of the brain, the nerves, and the muscles which play a direct role in controlling eye position and producing eye movements.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: david.c.godlove@vanderbilt.edu.

THE STOP SIGNAL TASK AND OCULOMOTOR CONTROL

The stop-signal task (SST) was developed to investigate response inhibition[1-2]. In a typical version, subjects are required to discriminate between stimuli by making speeded manual responses. On a subset of trials, the stimulus to be discriminated is quickly followed by a second stimulus (the stop-signal) instructing subjects to cancel their prepared response. Logan and Cowan proposed a race model of the SST which provides a crucial estimate of the timing of the hidden, response inhibition process[3]. The ability to measure stop-signal reaction time (or SSRT) proved invaluable to the physiological investigation of cancelling action. It was subsequently demonstrated that the race model could fit behavior in a saccade version of the SST [4], and this version has been used with great success to investigate oculomotor control in monkeys [4-14]. Specifically, the logic of the race model identifies activation and timing criteria necessary for neurons to participate in preparing or cancelling eye movements. This framework allowed Hanes and colleagues to identify neural populations in the frontal eye field (FEF)[7] and superior colliculus (SC)[10] with activity necessary to produce or inhibit saccades during the SST. These findings led to the hypothesis that movement and fixation related cells in the FEF and SC may implement a process similar to the race model during the SST. By relaxing an assumption of independence, Boucher and colleagues provided an elegant link, showing that the race model can be extended in neurally plausible terms which fit both behavioral and physiological data[15]. Thus, a large body of established work provides a great deal of theoretical leverage for investigation of the oculomotor system using the SST. Special attributes of the SST which will be referred to in italics throughout the discussion also make it uniquely suited to investigate error detection and performance monitoring (**Figure 1**).

PERFORMANCE MONITORING IN EYE MOVEMENT FIELDS OF THE FRONTAL AND MEDIAL FRONTAL CORTICES

In macaque monkeys, eye movements are elicited by low-current, electrical stimulation of at least three areas of frontal and medial frontal cortex; the FEF[16], the supplementary eye field (SEF)[17], and the rostral cingulate motor area of the anterior cingulate cortex (ACC)[18]. As noted above, investigation using the SST

Race model
A class of dynamic systems models in which several processes accrue toward a threshold. The process which crosses threshold first determines the outcome of the process.

Superior colliculus
A midbrain structure where representations of visual, auditory, and tactile stimuli are combined with a map of eye movement coordiates.

Electrical stimulation
Electrical current injected (typically with a microelectrode) directly into cortex in order to cause local depolarization of neurons.

Speed accuracy tradeoffs
In many tasks, accurate performance is dictated by a balance between speed and accuracy. Slow responding ensures accuracy, but reduces the number of responses per unit time; fast responding ensures that more responses are generated, but may deteriorate accuracy. Speed and accuracy are therefore often used as measures of performance monitoring.

electroencephalogram (EEG)
A continuous record of voltage changes caused by neural activity measured at the scalp with passive electrodes.

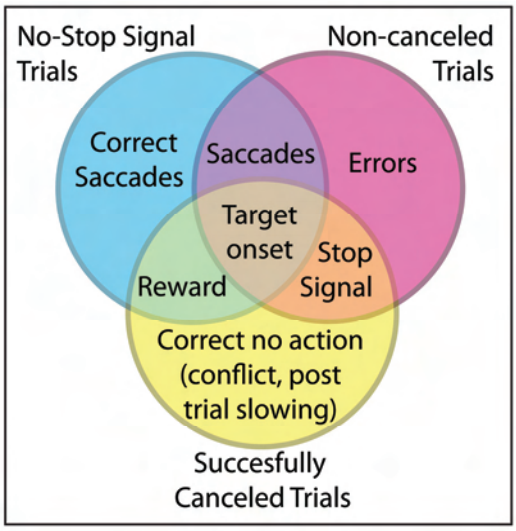

Figure 1 | **Venn diagramatic representation of trial types observed during the stop signal task.** Rings represent trial types. Areas of overlap represent commonalities between trial types. Note the response conflict and post trial slowing are observed in association with canceled trials in the stop signal task, breaking with their normal association with error trials in other tasks.

shows neural modulation in the FEF that is sufficient to play a role in producing or inhibiting eye movements [7]. Other work suggests that the FEF participates in decisions to make saccades to visual targets[19], and a direct link has been observed between the activity of movement related cells in the FEF and saccadic eye movements[8]. However, the contributions of the SEF and ACC to saccades are more nuanced.

During the SST, the majority of neurons in the SEF modulate too late to play a direct role in executing or withholding saccades. Interestingly, some neurons in the SEF exhibit post-saccadic activity when monkeys make errors in withholding saccades. Other neurons show activity before and during reinforcement on correct saccade trials, or when saccades are successfully cancelled[13]. *The SST dissociates actions from outcomes because two separate responses may be correct in different circumstances and identical responses may be either correct or erroneous.* Therefore, these error and reinforcement related signals cannot be explained as effects of visual stimuli or motor responses during the task, and they have led to the hypothesis that the SEF participates in performance monitoring of the oculomotor system[11-12,14]. Careful reading of the original description of the SEF reveals mention of reinforcement related cells, and cells which discharged rhythmically when the animal licked juice reward from a spout[17]. More than just monitoring performance, findings suggest that the SEF may play a direct role in influencing performance during saccade tasks. When monkeys make internally guided decisions during an asymmetrically rewarded free saccade task, nearly 50% of recorded visual neurons in the SEF show enhanced pre-target activity which may bias performance[20]. Moving from correlational measures to inferring causation, Stuphorn and Schall (2006) recorded behavior during the SST while delivering sub threshold intercranial stimulation to small areas within the SEF. While stimulation decreased reaction times (RTs) during simple visually guided saccades, RTs increased and overall accuracy improved in the context of the SST[13]. Taken together, these results suggest that the SEF plays a role in monitoring the outcome of saccades and making behavioral adjustments such as speed accuracy tradeoffs when necessary[11-12].

Cells in the ACC also show activity during errors and in relation to reinforcement, although the specific conditions under which they respond vary slightly from those observed in the SEF. Half of the observed ACC neurons which display error related modulation also display modulation when reward is unexpectedly withheld on correct trials. Of the neurons which respond to reinforcement, some respond when juice is delivered on correct trials, some respond to unexpected juice delivery, and some are modulated in both reinforcement conditions [9]. Thus, neuronal responses in the ACC tend to depend less on the animals behavior and more on trial outcome than those of the SEF. In humans, errors committed during speeded response tasks elicit a characteristic pattern of event related potential (ERP) waveforms (reviewed in more detail below) known as the error related negativity (ERN) and error related positivity (Pe)[21-22]. Dipole source localization has implicated the ACC as the probable source of the ERN signal[23], and local field potentials (LFPs) recorded from the macaque ACC show error related components with a form and time course similar to that of the human ERN[6]. Monkey homologues of human ERPs have been demonstrated in the past [24]. It remains to be seen if monkeys exhibit a homologue of the human ERN and Pe, but if so, the LFP data recorded in ACC may provide a crucial link between human EEG recordings and single cell recordings in monkeys. This development could pave the way for precise physiological characterization of the error related processes apparent in human EEG traces. We will now briefly discuss error related EEG components identified in humans.

THE ERROR RELATED NEGATIVITY AND POSITIVITY

When humans commit errors while performing speeded response tasks, a negative ERP component with a frontocentral scalp distribution can be observed[21-22]. This ERN typically peaks around 100ms after the erroneous response and cannot be

Event related potential
A waveform created by aligning many EEG epochs to a common task related event (such as response) and then collapsing across epochs to produce a single averaged waveform. ERPs minimize random trial to trial fluctuations in the EEG through this averaging process to highlight task related components.

Dipole source localization
General term describing several methods used to localize EEG voltage fluctuations to one or several areas of the brain. Dipole source localization results must be interpreted with care since they are results of mathematically "ill posed, inverse" problems.

Local field potentials
Low frequency voltage fluctuations produced by the ensemble activity of local neurons recorded intercranially.

N2 and **P3**
Stimulus related ERP components. The N2 refers to the second negative component (usually occurring around 200ms) in a stimulus aligned ERP trace, and the P3 follows similar nomenclature. Both the N2 and the P3 really constitute families of components which can be observed in response to a wide variety of stimuli in multiple modalities.

explained in terms of task related stimulus or motor related processing[25]. A similar ERP component dubbed the feedback related negativity can be observed when subjects are informed of a failure to earn reinforcement[25]. The time course of the ERN is very similar to the time course observed in error related cellular modulation in the SEF and the ACC described above [6,9,13]. Dipole source localization generally implicates areas in or around the ACC as the ERN locus, and investigation using concurrent EEG and fMRI recordings show error related hemodynamic signals which fluctuate in correlation with trial to trial ERN variation[23]. It is generally accepted that the ERN and error related hemodynamic responses observed in the posterior medial frontal cortex reflect similar neural responses to errors [25]. The Pe follows the ERN, peaking at around 300 ms. It has a more parietal scalp distribution and also seems to be related to internal error processing [25]. Less is currently known concerning the anatomical source or hemodynamic correlates of the Pe.

Since their discovery, it has been hypothesized that error ERPs may reflect the activity of a neural network that is also involved in behavioral compensation. In one of the first descriptions of the ERN, Gehring and colleagues provided evidence that its amplitude could be attenuated when subjects were instructed to place emphasis on speed rather than accuracy, and that ERN amplitude also correlated with the force of manual response errors, as well as the probability of correcting an error or initiating a correct response on subsequent trials[22]. However, methodological concerns blunt the force of these findings, and attempts to replicate them have varied in their success [25]. Subsequent investigation has suggested that the amplitude of the ERN may be positively correlated with subsequent RTs on ambiguous trials[23,26]. In contrast to this finding, Nieuwenhuis and coworkers found RT adjustments that were correlated with the amplitude of the Pe, not the ERN. Furthermore, RT adjustments and Pe amplitude changes were only observed in association with trials on which participants reported awareness of errors[27]. The authors suggested that the ERN reflects error monitoring outside of conscious awareness, but recent findings by Woodman challenge this interpretation[28]. Klein and coworkers showed that activation of the supplementary motor area was associated with post-error slowing, reminiscent of the stimulation studies in SEF mentioned above[29]. Kerns and colleagues found evidence that error related activity in the ACC correlates with measures of behavioral adjustment independent of RT[30]. Adopting a different approach, Ridderinkhof and coworkers, found that correct trials preceding errors were characterized by greater positivity, which they interpreted as a failure of behavioral performance monitoring by the same network which gives rise to the ERN[31]. In sum, evidence for a link between error related activity and behavioral adjustments is currently contradictory and somewhat underwhelming given the scope of research. This may stem in part from task differences and variations in the operational definitions of behavioral adjustments themselves.

Error related components elicited during the SST have been identified, but varying degrees of rigor have been applied in these studies complicating their interpretation. Ridderinkhof and coworkers investigated stimulus driven ERPs (N2 and P3 components) elicited by the stop-signal itself on canceled and non-canceled trials. These investigators found larger amplitude components with longer latencies when subjects failed to cancel their responses[32]. Further investigation showed that decreasing the percentage of stop-signal trials caused subjects to speed up and the P3 to increase in amplitude[33]. These studies were taken as evidence that the P3 component plays a role in stopping behavior, but a role for the N2 and P3 in error monitoring was also considered, and it was suggested that they may overlap with ERN and Pe components in the SST. Interesting as these findings are, it must be acknowledged that motor response related EEG activity on non-cancelled trials was not removed by the subtraction procedure utilized in these studies which may have influenced the results[32]. Later research was carried out using "ignore" stimuli on trials without stop signals to control for stimulus related confounds introduced by stop-signal presentation. These studies demonstrated robust error related components untainted by stimulus or motor responses[34-35]. Other investigators have attempted to characterize the ERN in the SST in terms of its relationship to autonomic responses[36] or awareness[37], but failed to control for stimulus related confounds of stop-signal presentation (see **Figure 1**). Thus, while error related effects are readily apparent in the stop-signal paradigm, we know very little about their relation to behavior in this task. This is particularly unfortunate since *the unique structure of the SST causes participants to slow responses after successfully cancelled trials instead of errors*[5], a dissociation which may prove useful in correlating error ERPs with behavior.

Several theories have been proposed concerning the cognitive and physiological mechanisms reflected by the ERN. In one of the first descriptions of the ERN, Falkenstein and coworkers suggested that it indexed processing of the mismatch between executed and appropriate responses[21]. This mismatch is essentially one definition of an error. The source of the putative correct response representation during error commission is, however, uncertain. Current

Executive control
The set of cognitive functions which allow complex behavior to be generated beyond simple stimulus and response arcs.

Movement and fixation related cells
Neurons in the SC and FEF which fire maximally before the eyes move or while they are still respectively.

Mesencephalic dopamine system
A collection of cells in the midbrain which provide diffuse input of the modulatory neurotransmitter dopamine to frontal and medial cortices.

Basal ganglia
Several collections of cell bodies which lie at the base of the cerebrum and form a system crucial to generation of movement as well as normal emotional function and cognition.

Mesolimbic Dopamine pathways
A collection of cells in the midbrain which provide diffuse input of the modulatory neurotransmitter dopamine to various areas classically defined as "limbic" areas.

Self-stimulation studies
Experiments in which subjects are implanted with stimulating electrodes which allow them to deliver current intercranially to themselves by carrying out behaviors (such as pressing levers).

Memory guided saccade task
A task in which subjects must make an eye movement to the remembered location of a visual stimulus at the end of a delay period.

theories seek to describe the ERN in terms of more general monitoring processes. Of these, the response conflict theory[38-39] and dopamine (DA) related reinforcement learning theories [40-42] have had broad influence. The response conflict theory suggests that uncertain circumstances produce coactivation of conflicting responses along with high probabilities of errors. This response conflict is continuously monitored by the ACC, which outputs the signal to areas in the prefrontal cortex in order to recruit increasing or decreasing levels of executive control as the situation warrants[38]. An intuitively appealing model of ACC function, conflict monitoring also provides satisfactory fits to behavioral data, as well as explaining many puzzling aspects of observed ERN activity[39]. But it is not entirely clear how this model generalizes to tasks which may not engender conflicting responses. *The SST provides a dissociation of error and response conflict since movement and fixation neurons are maximally coactivated on correct canceled trials*[7]. Studies using the SST have found conflict monitoring responses in the SEF[14], but direct physiological evidence for conflict monitoring in the ACC is lacking[6,9]. Reinforcement learning theories of ACC function take an alternate approach by suggesting that phasic signals from the mesencephalic DA system are responsible for the observed ERN[40]. It is well known that the mesencephalic DA system exhibits a phasic decrease in DA signaling in conjunction with failure to obtain reinforcement[43] (discussed below). Through projections to the ACC, this phasic decrease may disinhibit apical dendrites of pyramidal neurons and mediate synaptic plasticity[40]. The supposed DA "training signal" may then be used by ACC neurons to provide motor influence to an appropriate cortical controller[40] or to learn context dependant predictions of error likelihood[42]. Models of reinforcement learning are successful in fitting behavioral data, and make many predictions concerning ERN signaling which have been observed in the laboratory[41-42]. Additionally, this theory dovetails with physiological findings suggesting a key role for the basal ganglia in oculomotor performance monitoring.

THE BASAL GANGLIA PLAY AN IMPORTANT ROLE IN OCULOMOTOR PERFORMANCE MONITORING

The basal ganglia (BG) form reentrant loops with virtually every area of cortex through the thalamus, and project to several midbrain and brainstem nuclei [44-45]. Consensus has emerged that behaviorally relevant information converges with control over motor output in the BG, placing them in a key position to guide goal directed responses. In addition to their well-known role in orchestrating movements of the trunk and limbs through the skeletomotor circuit, the BG contribute to normal emotional and cognitive function, and they are vital in regulating saccadic eye movement. The oculomotor circuit of the BG exerts tonic, GABAergic inhibition on the SC via the substantia nigra pars reticulata (SNpr). The caudate (CD) can release tonic inhibition on the SC through the "direct" pathway or potentiate inhibition through the "indirect" pathway which projects through the subthalamic nucleus (STN)[46]. DA cells of the substantia nigra pars compacta (SNpc) project to the CD. They facilitate signaling along the direct pathway and inhibit signaling along the indirect pathway by targeting projection neurons with D1 like and D2 like receptors respectively. The FEF and SEF project excitatory input to the CD as well as directly to the STN. This so-called "hyper-direct" pathway may provide fast, potent, cortically driven inhibition of the SC through the STN which may aide in canceling planned responses[47]. Taken together, these observations describe a mechanism by which dopaminergic signaling in the BG can influence oculomotor output[48].

A large body of evidence suggests that the mesoncephalic and mesolimbic DA pathways play a role in signaling the presence of reward and facilitating motivated behavior. Qualitatively, patients with Parkinson's disease (which depletes DA) exhibit paucity of spontaneously generated movements including gaze shifts[48]. Empirically, the classic medial-septal area, self stimulation studies of Olds and Milner are generally taken to demonstrate the ability of the DA system to reinforce behavior[49]. More recently, Schultz and colleagues have provided evidence that the DA systems may provide an ongoing "reward-prediction error signal" by comparing the probability of reward given an animal's behavior in the current context to actual outcomes and signaling deviations[43]. The phasic increases and decreases that DA cells exhibit in response to reward or its absence resemble the output of a simple machine learning algorithm called the method of temporal differences, and could be used to increase the frequency of behaviors leading to reward and update future reward prediction[50]. Redgrave and Gurney have put forth the alternate view that phasic DA responses signal the presence of biologically salient events, including reward related and unexpected stimuli. They argue that contextual and motor cues coexist in the BG and the phasic DA signal serves to synaptically strengthen responses in the current context when they produce unexpected events. Thus, the DA signal may allow an animal to discover new actions which lead to novel outcomes and expand its behavioral repertoire[51-52]. Although the scope of the phasic DA signal is still in question, it is clear that DA signaling is related to reward.

Receptive fields
The spatial extent in which a stimulus encourages a neuron to fire action potentials.

In an important series of experiments, Hikosaka and coworkers showed that visual and saccade related neurons in the CD modulate their firing rates to reflect changing reward contingencies in a memory guided saccade task[53-55]. Animals were required to make memory guided saccades to one of 2 or 4 target locations, but during one condition only a single target location was rewarded. Animals made saccades with shorter latencies and higher peak velocities to rewarded locations, and the receptive fields of visual and memory related neurons in the CD shifted dramatically in response to changing reward contingencies[54]. Moreover, changes in discharge rate of saccade related CD cells were temporally correlated with reward related behavioral adjustments made by the animal, suggesting that CD cell responses could help facilitate the observed changes in saccade speed and latency[55]. Not only are cellular responses in the CD enhanced when receptive fields contain rewarded targets, but CD neurons often exhibit responses that specify the size of the upcoming reward. These effects may be mediated by DA dependent long term potentiation arising from SNpc input[53]. If this were verified, it would provide an elegant example of goal directed behavior under the guidance of dopaminergic signaling through the BG. Variants of a sequential probability ratio test are commonly used to model decision making processes[56-57]. These models maximize reward rates when decision thresholds are set optimally [58-59]. Behaviorally, this is equivalent to making speed/accuracy tradeoffs, and there is circumstantial evidence to suggest that the BG may implement equations required to make these adjustments through a hard-wired circuit[60].

CONCLUDING REMARKS

In sum, intercranial recordings from monkeys guided by computational modeling efforts are providing an increasingly comprehensive view of oculomotor performance monitoring circuits. This work may shed much needed light on human error related activity observed in EEG and fMRI recordings. The SST provides several unique behavioral dissociations related to performance monitoring. Its creative use will continue to provide compelling tests of model predictions and answer lingering questions about error detection and behavioral adjustments.

REFERENCES

1. Helson H and Steger JA (1962). On the inhibitory effects of a second stimulus following the primary stimulus to react. *J of Exp Psy.* **64** (3): 201-205.
2. Lappin JS and Eriksen CW (1966). Use of a delayed signal to stop a visual reaction-time response. *J of Exp Psy.* **72** (6): 805-811.
3. Logan GD and Cowan WB (1984). On the ability to inhibit thought and action: a theory of an act of control. *Psy Rev.* **91** (3): 295-327.
4. Hanes DP and Carpenter RHS (1999). Coutermanding saccades in humans. *Vis Res.* **39**: 2777-2791.
5. Emeric EE, Brown JW, Boucher L, Carpenter RHS, Hanes DP, Harris R, Logan GD, Mashru RN, Pare` M, Pouget P, Stuphorn V, Taylor T and Schall JD (2007). Influence of history on saccade countermanding performance in humans and macaque monkeys. *Vis Res.* **47**: 35-49.
6. Emeric EE, Brown JW, Leslie M, Pouget P, Stuphorn V and Schall JD (2008). Performance monitoring local field potentials in the medial frontal cortex of primates: anterior cingulate cortex. *J Neurophysiol.* 99: 759-772.
   Demonstrates that local field potentials in the anterior cingulate cortex exhibit error related components that are similar in timing and appearance to the error related negativity and positivity observed in humans. So doing, it goes a long way toward bridging the gap between intracranial single cell recordings of error and reinforcement related activity in monkeys and error relate ERP components observed in humans.
7. Hanes DP, Patterson WF and Schall JD (1998). Role of frontal eye fields in countermanding saccades: visual, movement, and fixation activity. *J Neurophysiol.* **79**: 817-834.
8. Hanes DP and Schall JD (1996). Neural control of voluntary movement initiation. *Science*. **274**: 427-430.
9. Ito S, Stuphorn V, Brown JW and Schall JD (2003). Performance monitoring by the anterior cingulate cortex during saccade countermanding. *Science*. **302**: 120-122
10. Pare` M and Hanes DP (2003). Controlled movement processing: superior colliculus activity associated with countermanded saccades. *J Neurosci*. **23**(16): 6480-6489.
11. Schall JD and Boucher L (2007). Executive control of gaze by the frontal lobes. *Cog, Affect, & Behav Neurosci*. 7(4): 396-412.
    Reviews findings from a range of investigations using the stop-signal task in order to build a broad theory of executive control in the oculomotor system carried out by the frontal lobes. Integrates data from physiological recordings with the neurally plausible "interactive race model" of Boucher, Palmeri, Logan, and Schall (2007).
12. Schall JD, Stuphorn V and Brown JW (2002). Monitoring and control of action by the frontal lobes. *Neuron*. **36**: 309-322.
13. Stuphorn V & Schall JD (2006). Executive control of countermanding saccades by the supplementary eye field. *Nat Neurosci*. **9**(7): 925-931.
14. Stuphorn V, Taylor TL and Schall JD (2000). Performance monitioring by the supplementary eye field. *Nature*. **408**: 857-860.
15. Boucher L, Palmeri T, Logan GD and Schall JD (2007). Inhibitory control in mind and brain: an interactive race model of countermanding saccades. *Psy Rev*. **114**(2): 376-397.

16. Bruce CJ and Goldberg ME (1985). Primate frontal eye fields. I. single neurons discharging before saccades. *J Neurophysiol*. **53**(3): 603-635.
17. Schlag J and Schlag-Rey M (1987). Evidence for a supplementary eye field. *J Neurophysiol.* **57**(1): 179:200.
18. Mitz AR and Wise SP (1987). The somatotopic organization of the supplementary motor area: intracortical microstimulation mapping. *J Neurosci*. **7**(4): 1010-1021.
19. Schall JD and Thompson KG (1999). Neural selection and control of visually guided eye movements. *Annu Rev Neurosci*. **22**: 241-259.
20. Coe B, Tomihara K, Matsuzawa M and Hikosaka O (2002). Visual and anticipatory bias in three cortical eye fields of the monkey during an adaptive decision-making task. *J Neurosci*. **22**(12): 5081-5090.
21. Falkenstein M, Hohnsbein J, Hoormann J and Blanke L (1990) Effects of errors in choice reaction tasks on the ERP under focused and divided attention. *Psychophysiol Brain Res*. **1**: 192-195.
22. Gehring WJ, Goss B, Coles MGH, Meyer DE and Donchin E (1993). A neural system for error detection and compensation. *Psy Science*. **4**(6): 385-390.
23. Debener S, Ullsperger M, Siegel M, Fiehler K, von Cramon D and Engel AK (2005). Trial-by-trial coupling of concurrent electroencephalogram and functional magnetic resonance imaging identifies the dynamics of performance monitoring. *J Neurosci.* **25**(50): 11730-11737.
24. Woodman GF, Kang MK, Rossi AF and Schall JD (2007). Nonhuman primate event-related potentials indexing covert shifts of attention. *PNAS*. **104**(38): 15111-15116.
25. Ridderinkhof KR, Ullsperger M, Crone E and Niewenhuis S (2008). The role of the medial frontal cortex in cognitive control. *Science*. **306**: 443-447.
26. West R and Travers S (2008). Tracking the temporal dynamics of updating cognitive control: and examination of error processing. *Cerebral Cortex*. **18**: 1112-1124.
27. Nieuwenhuis S, Ridderinkhof KR, Blom J, Band GPH and Kok A (2001). Error-related brain potentials are differentially related to awareness of response errors: evidence from an antisaccade task. *Psychophysiol.* **38**: 752-760.
28. Woodman GF (in press). Masked targets trigger event-related potentials indexing shifts of attention but not error detection. *Psychophysiology*.
29. Klein TA, Endrass T, Kathmann N, Neumann J, von Cramon DY and Ullsperger M (2007). Neural correlates of error awareness. *NeuroImage*. **34**: 1774-1781.
30. Kerns JG, Cohen JD, MacDonald AW, Cho RY, Stenger VA and Carter CS (2004). Anterior cingulate conflict monitoring and adjustments in control. *Science*. **303**: 1023-1026.
31. Ridderinkhof KR, Nieuwenhuis S and Bashore TR (2003). Errors are foreshadowed in brain potentials associated with action monitoring in cingulate cortex in humans. *Neurosci Letters*. **348**: 1-4.
32. Kok A, Ramautar JR, De Ruiter MB, Band GPH and Ridderinkhof KR (2004). ERP components associated with successful and unsuccessful stopping in a stop-signal task. *Psychophysiol*. **41**: 9-20.
33. Ramautar JR, Kok A and Ridderinkhof KR (2004). Effects of stop-signal probability in the stop-signal paradigm: the N2/P3 complex further validated. *Brain and Cog*. **56**: 234-252.
34. Stahl J and Gibbons H (2007). Dynamics of response-conflict monitoring and individual differences in response control and behavioral control: an electrophysiological investigation using a stop-signal task. *Clinical Neurophysiol*. **118**: 581-596.
35. Dimoska A, Johnstone SJ and Barry RJ (2006). The auditory-evoked N2 and P3 components in the stop-signal task: indices of inhibition, response-conflict or error-detection?. *Brain and Cog*. 62: 98-112.
Shows clear error related negativity and positivity in the event related potentials of humans performing a stop-signal task with appropriate controls. Thus the peculiar behavioral dissociations of the stop signal task which make it an attractive paradigm for studying performance monitoring do not render it incompatible with error ERP studies.
36. van Boxtel GJM, Molen MW, Jennings JR and Bruian CHM (2001). A psychophysiological analysis of inhibitory motor control in the stop-signal paradigm. *Bio Psy*. **58**: 229-262.
37. Endrass T, Franke C and Katmann N (2005). Error awareness in a saccade countermanding task. *J Psychophysiol*. **19**(4): 275-280.
38. Botvinick MM, Braver TS, Carter CS and Cohen JD (2001). Conflict monitoring and cognitive control. *Psy Rev.* **108**(3): 624-652.
39. Yeung N, Botvinick MM, Cohen JD (2004). The neural basis of error detection: conflict monitoring and the error-related negativity. *Psy Rev.* **111**(4): 931-959.
40. Holroyd CB and Coles MGH (2002). The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. *Psy Rev*. **109**(4): 679-709.
41. Nieuwenhuis S, Holroyd CB, Mol N, Coles MGH (2004). Reinforcement-related brain potentials from medial frontal cortex: origins and functional significance. *Neurosci and Biobehav Rev*. **28**: 441-448.
42. Brown JW, Braver TS (2008). Learned predictions of error likelihood in the anterior cingulate cortex. *Science*. **307**: 1118-1121.
43. Schultz W, Dayan P and Montague R (1997). A neural substrate of prediction and reward. *Science*. **275**: 1593-1599.
44. DeLong MD (2000). The basal ganglia. In: *Principles of Neural Science* 4th ed, (Kandel ER, Schwartz JH and Jessell), 853-867. New York: McGraw-Hill.
45. Mink JW (2008). The basal ganglia. In: *Fundamental Neuroscience* 3rd ed, (Squire LR, Berg D, Bloom FE, du Lac S, Ghosh A and Spitzer NC), 725-750. Amsterdam: Elsevier.
46. Hikosaka O and Wurtz RH (1989). The basal ganglia: In *The Neurobiology of Saccadic Eye Movements*, (Wurtz RH and Goldberg ME). Amsterdam: Elsevier.
47. Aaron AR and Poldrack RA (2006). Cortical and subcortical contributions to stop signal response inhibition: role of the subthalamic nucleus. *J Neurosci*. **26**(9): 2424-2433.
48. Hikosaka O, Bromberg-Martin E, Hong S and Masayuki M (2008). New insights on the subcortical representation of reward. *Curr Op in Neurobio*. **18**: 203-208.

49. Olds J and Milner P (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Exp Psy*. **47**(6):419-427.
50 Motague PR, Hyman SE and Cohen JD (2004). Computational roles for dopamine in behavioral control. *Nature*. **431**: 760-767.
51. Redgrave P, Gurney K and Reynolds J (2008). What is reinforced by phasic dopamine signals?. *Brain Res Rev*. **58**:322-339.
52. Redgrave P and Gurney K (2006). The short-latency dopamine signal: a role in discovering novel actions?. *Nat Rev Neurosci*. **7**: 967-975.
53. Ding L and Hikosaka O (2006). Comparison of reward modulation in the frontal eye field and caudate of the macaque. *J Neurosci*. **26**(25): 6695-6703.
54. Kawagoe R, Takikawa Y and Hikosaka O (1998). Expectation of reward modulates cognitive signals in the basal ganglia. *Nat Neurosci*. **1**(5): 411-416.
55. Lauwereyns J, Watanabe K, Coe B and Hikosaka O (2002). A neural correlate of response bias in monkey caudate nucleus. *Nature*. 418: 413-417.
Although not the first use of the "one directional reward" (1DR) task this study demonstrates how powerful the paradigm can be. A simple manipulation of spatial reward contingencies in a visually guided saccade task leads to anticipatory activity in caudate neurons which may be sufficient to explain speeded reaction time effects.
56. Ratcliff R and McKoon G (2008). The diffusion decision model: theory and data for two-choice decision tasks. *Neural Computation*. **20**: 873-922.
57. Roitman JD and Shadlen MN (2002). Response of neurons in the lateral intraparietal area during a combined visual discrimination reaction time task. *J Neurosci*. **22**(21): 9475-9489.
58. Simen P, Cohen JD and Holmes P (2006). Rapid decision threshold modulation by reward rate in aneural network. *Neural Networks*. **19**: 1013-1026.
59. Sugrue LP, Corrado GS and Newsome WT. (2009). Matching behavior and the representation of value in the parietal cortex. *Science*. **304**: 1782-1787.
60. Bogacz R, Brown E, Moehlis J, Holmes P and Cohen J (2006). The physics of optimal decision making: a formal analysis of models of performance in two-alternative forced-choice tasks. *Psy Rev*. **113**(4): 700-765.

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FURTHER INFORMATION

Jeff Schall's Lab:
http://www.psy.vanderbilt.edu/faculty/schall/

Amphetamine-fueled insights into dopaminergic diseases: the protein kinase Akt drives responses to psychostimulants

Michael Siuta

Amphetamine (AMPH) is a psychostimulant that exerts its behavioral effects, in part, through release of pre-synaptic dopamine (DA) via reversal of the dopamine transporter (DAT) at mesostriatal synapses. Due to the characteristic and robust release of DA in response to AMPH, this drug is often used to study animal models of diseases where DA dysfunction at mesostriatal synapses is implicated, namely schizophrenia, Parkinson's disease, and drug addiction. Interestingly, the function of the protein kinase Akt (also known as protein kinase B) has recently been associated, in both human and animal studies, with both the pathogenesis and treatment of these DA-related diseases. Akt is stimulated by phosphotidylinositol 3-kinase (PI3K) signaling, which itself is activated by growth factors (such as brain derived neurotrophic factor) and hormones (such as insulin) through receptor tyrosine kinases (RTKs). Many of these growth factors and hormones also influence the actions of psychostimulants through cellular and molecular mechanisms that include promotion of DAT trafficking, increased axonal innervation of the striatum, and enhanced synthesis of pre-synaptic dopamine. Recent evidence suggests that many of these mechanisms may be profoundly regulated by Akt. Collectively, studies of the activation and inhibition of PI3K/Akt signaling, through pharmacologic, genetic, or viral manipulations, suggest a prominent role for Akt signaling in neuronal growth, neuronal migration, and regulation of DA neurotransmission. These findings hold promise for development of future strategies aimed at more directly influencing Akt signaling in the brain in order to treat dopaminergic diseases.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: michael.siuta@vanderbilt.edu.

Psychostimulants like amphetamine (AMPH) are used to study behavior and physiology in animal models of Parkinson's disease, schizophrenia, and addiction [1, 2, 3]. While the symptoms of these diseases are quite disparate in humans, they are all, to some degree, linked to the function of dopaminergic systems in brain. Recent evidence suggests that common intracellular signaling pathways may be important in the treatment and pathogenesis of these diseases. One such pathway involves the serine/threonine protein kinase Akt. Human studies demonstrate that genetic variation in the isoform Akt1 influences dopamine-associated structures and functions in humans[4], and, potentially, the risk for schizophrenia, methamphetamine abuse[5], and Parkinson's disease[6]. Human studies have also discovered defects in phosphorylation of Akt related to mental illness diagnoses[7, 8, 9], suggesting that activators of Akt, like the phosphotidylinositol 3-kinase (PI3K) proteins, also modulate dopamine (DA) in brain. PI3K is activated by receptor tyrosine kinases (RTKs), which, in turn, are activated by a diverse set of hormones, including insulin[10], and growth factors, including brain-derived neurotrophic factor[11] (BDNF). Intriguingly, many RTK ligands, along with PI3K/Akt itself, influence the actions of AMPH and other psychostimulants[12-17].

One of the most studied functions of AMPH is its ability to increase synaptic DA. AMPH accomplishes this by multiple mechanisms, including DA-efflux through reversal of the dopamine transporter (DAT), the major protein involved in synaptic clearance of DA. AMPH is also capable of entering the cell to trigger release of DA from pre-synaptic vesicle stores, again by reversal of transporter function. Trafficking of the DAT to the cell surface has recently shown to be dependent on RTKs[18], PI3K[19], and Akt[20], providing a molecular mechanism to explain the potential for hormones and growth factors to modulate DA systems and responses to stimulants.

In addition to surface levels of DAT, the magnitude of DA release elicited by AMPH, and the effects on consequent behaviors, are also governed by the amount of pre-synaptic DA available. Pre-synaptic DA can be influenced by several factors, including DA synthesis, the health of DA neurons, and the density of DA terminals, processes where PI3K/Akt also plays a role[13]. Thus, the goals of the present review are to (1) model the regulation of the DAT and responses to psychostimulants by PI3K/Akt, (2) review the activators of PI3K/Akt in brain, and analyze their PI3K/Akt dependent functions, and (3)

integrate evidence from animal and culture studies to assess mechanisms underlying the relationship between RTKs, PI3K/Akt signaling, and responses to psychostimulants. As activation or inhibition of PI3K/Akt signaling profoundly influences DA-related behaviors, understanding the different levels (cellular and molecular) at which Akt modulates AMPH actions provides insights into how this pathway regulates both pre-synaptic DA and the DAT, an important pharmacological target. Understanding AMPH responses may help to inform ways to target Akt for the treatment of psychiatric and neurologic diseases.

PI3K/AKT SIGNALING, DAT SURFACE EXPRESSION, AND RESPONSES TO PSYCHOSTIMULANTS

The PI3K/Akt signaling cascade can be activated following stimulation of RTKs[21]. The tyrosine-phosphorylated protein products of receptor stimulation interact with the SH2 domain on growth factor sensitive isoforms of PI3K, stimulating its lipid kinase activity. PI3K then catalyzes phosphorylation of phosphoinositides at the 3-position in the inositol ring, causing an increase in the generation of PIP2 and PIP3. The Pleckstrin homology (PH) domain of Akt interacts with these phosphorylated phosphoinositide byproducts, which causes membrane translocation of Akt. This translocation allows Akt to be phosphoryated itself at the Threonine-308 and Serine-473 residues by phosphoinositide-dependent kinase 1 (PDK1) and the mammalian target of rapamycin (mTOR) complex 2 (mTORC2). Phosphorylation of Akt at the 308 and 473 residues is necessary for full activation of the enzyme's kinase function.[21]

Inhibition of PI3K pharmacologically with LY294002 decreases cell surface expression of the DAT both *in vitro*, in heterologous cell culture lines, and *ex vivo*, in striatal synaptosomes[22]. Stimulation of PI3K activity with either insulin pretreatment or constitutively active PI3K results in an enhancement of DA uptake[22]. A direct role for Akt in these effects is suggested by studies *in vitro* where AMPH-induced internalization of the DAT, and consequent reductions in DA uptake, are blocked by a virus expressing constitutively active Akt or insulin stimulation, in a PI3K- and Akt-dependent manner[20]. Compelling *in vivo* evidence to support the relationship between PI3K/Akt signaling and the DAT comes from studies in hypoinsulinemic animals, which show reduced Akt activity in brain along with reduced DAT cell surface expression, DA clearance, and amphetamine-induced efflux of DA[12]. Pharmacologic inhibition of PI3K in the rodent striatum causes a parallel reduction in AMPH-induced DA efflux, and local pretreatment with insulin restores the effects of DA clearance and AMPH-induced efflux in hypoinsulinemic mice[12]. Together, this evidence suggests that local activitation of RTK/PI3K/Akt signaling is the mediator of these effects in hypoinsulinemic animals.

The decreased DAT cell surface expression and AMPH-induced DA efflux with PI3K inhibition provides a potential mechanism to explain how Akt activation and inhibition affects psychostimulant- and reward-related behaviors observed in other studies. Hypoinsulinemic animals show diminished self-administration of AMPH[3], consistent with the diminished availability of surface DAT to promote DA release with drug use. In a similar fashion, administration of the PI3K inhibitor LY294002 reduces the sensitizing effects of cocaine[16]. In addition to addiction models, Parkinson's disease models also often rely on AMPH-induced behavioral endpoints to track functional effects of various lesions and treatments. Usually, these models involve AMPH-induced locomotor rotations following unilateral lesions or treatments to DA cell bodies in the substantia nigra. A unilateral 6-hydroxydopamine lesion (6-OHDA) to the substantia nigra, for example, results in differential AMPH-induced release of DA between the lesioned and unlesioned sides of brain, and this functional asymmetry is reflected in increased turning behavior toward (ipsiversive) the lesioned side. Unilateral injections of associated adenovirus vectors (AAVs) expressing myristolated Akt (myr-Akt), a constitutively active form of Akt, results in contraversive turning behaviors. This suggests a relative increase in AMPH-induced DA in the myr-Akt expressing side. This enhanced AMPH response is likely due at least in part to elevated nigral DA associated with myr-Akt expression, which supports the overall ability of Akt signaling to promote the actions of AMPH[13].

Characteristic cellular changes associated with Akt signaling also reflect differences in reward sensitivity and responses to stimulants observed with Akt modulation. Withdrawal periods following chronic opiate administration, for example, cause diminished sensitivity to opiate reward (as measured by conditioned place preference (CPP)), reductions in Akt phosphorylation, and decreased midbrain DA neuron size [23]. The cellular basis of the effects on sensitivity to reward are emphasized in this particular study, as viral inhibition of PI3K/Akt signaling in the midbrain itself reduces cell body size and CPP, suggesting the Akt downregulation is sufficient to cause the observed cellular and behavioral responses to chronic opiates. Viral enhancement of the pathway, conversely, reverses the effects of chronic opiates on cell size and reward-related behaviors[23]. Similarly, myr-Akt injections, which increase responses to AMPH[13], as stated above, also enlarge tyrosine hydroxylase (TH) neuron cell bodies in

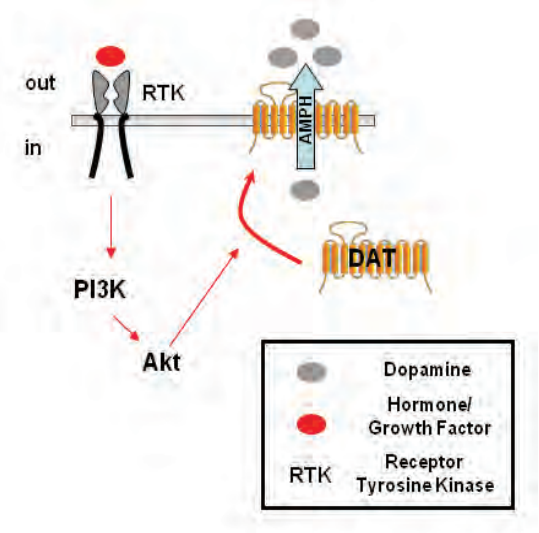

Figure 1 | **Model of PI3K/AKT influence on the dopamine transporter.**

midbrain[13, 24] and increase the density of striatal TH terminals[13]. Indeed, oftentimes it is difficult to disentangle the potential cellular versus molecular influences of Akt on responses to psychostimulants, unless the effects evaluated are compared on an acute time scale (where molecular effects like trafficking presumably predominate) versus a chronic time scale, when the trophic influence of Akt become prominent. RTK activators, which have a growing number of documented PI3K-dependent effects, have long been studied as modulators of responses to psychostimulants in different contexts. Thus, findings from these studies provide insight into the mechanisms whereby Akt signaling in brain can promote DA release in response to psychostimulants (See model in **Figure 1**).

PI3K/AKT-DEPENDENT INFLUENCES OF RTKS ON DOPAMINE SYSTEMS

RTKs that stimulate Akt signaling in brain: Insulin stimulates PI3K/Akt signaling through activation of a receptor tyrosine kinase (RTK) and promotes DAT trafficking to the plasma membrane[20]. While the insulin receptor is widely distributed in brain[25], there are many other RTKs in brain which affect DA systems that also have PI3K-dependent effects. Among the RTK ligands also capable of inducing Akt phosphorylation are nerve growth factor[26](NGF), brain-derived neurotrophic factor[11](BDNF), glial-derived neurotrophic factor[27](GDNF), fibroblast growth factor [28](FGF), and the epidermal growth factor (EGF) family of proteins, which includes neuregulin-1[8](NRG-1). A role for many of these RTKs has been postulated in either the pathogenesis or treatment of schizophrenia[29], psychostimulant addiction[30] and Parkinson's[31], suggesting that RTKs influence dopaminergic systems in a similar fashion to PI3K/Akt signaling.

PI3K-dependent cellular influences of RTKs: An increasing number of PI3K-dependent effects of RTK ligands have recently been uncovered, largely focused on the trophic effects of Akt. For example, the promotion of neurite outgrowth in dopaminergic cell lines by NGF is partly inhibited by the PI3K inhibitor LY290042[32]. In addition, the ability of NRG-1 to induce chemotactic migration is blocked by inhibition of PI3K and the NRG-1-associated RTK, erbB2[33]. IGF-1 stimulation of growth cone expansion in cultured neurons is also attenuated by treatment with LY294002[34]. Intriguingly, myr-Akt expression in the substantia nigra, described above, results in increased tyrosine hydroxylase positive terminals in the striatum without changing cell density in the nigra itself. This suggests that the increased terminal density is not due to changes in cell number but changes in target innervation[13]. These findings suggest that one potential mechanism for the influence of Akt on DA systems is through promotion of axonal outgrowth from DA cell bodies, resulting in increased DA terminal density. Together with evidence supporting the influence of Akt on cell size, mentioned above, and the PI3K-dependence of BDNF, IGF-1, and estrogen on neuroprotection *in vitro*[27] and *in vivo*[10], Akt seems to be a powerful positive modulator of DA systems[13].

RTKs, PI3K, and DA synthesis and release: In addition to cellular events, which occur over a longer time course, RTKs also promote short-term modulation of DA systems through PI3K/Akt signaling. In PC12 cells, NGF, EGF, and IGF-1 enhance stimulated release of DA release in a manner subject to inhibition of PI3K[35, 36]. Recent evidence implicates that this effect is true in brain also, as treatment with BDNF in striatal slice preparations also enhances stimulated release of DA, and this effect is blocked by LY249002 administration[37]. The mechanisms underlying the enhanced release of DA by RTKs is unknown, but they are believed to be presynaptic[37], and could potentially involve a combination of factors including stimulation of DA synthesis by TH[13], enhancement of calcium-responsible secretory vesicles[35], and promotion of DA recycling via DAT trafficking to the cell surface[20]. These mechanisms are all consistent with the overall effect of myr-Akt viruses in the dopaminergic midbrain- a promotion of pre-synaptic DA function reflected by increased cell size, terminal density, total nigrostriatal dopamine content, and AMPH-induced behaviors[13, 24]. These mechanisms, in conjunction with promotion of cell surface DAT, contribute to the ability of Akt to promote DA release in response to AMPH.

ACTIVATORS OF PI3K/AKT SIGNALING AND RESPONSES TO PSYCHOSTIMULANTS

RTKs in DAT trafficking: According to the model provided in **Figure 1**, RTK activators will promote DAT cell surface expression, DA uptake, and responses to stimulants, and inhibitors, such as LY249002, will diminish these effects. One study supporting this model showed that, in rat striatal synaptosomes, both RTK inhibition (with genistein and tyrphostin) and PI3K inhibition led to a rapid downregulation of DA clearance and DAT cell surface expression[18]. Conversely, acute growth factor (BDNF) treatment increased DA uptake, and this increase is prevented upon co-treatment with the PI3K inhibitor LY294002[18], paralleling previous findings on the effects of insulin. In addition, the effect of RTKs on DA uptake in this study are primarily dependent on the Vmax for uptake, as opposed to the Km. Thus, this effect of RTKs on DA clearance is attributable to the total number of available DAT, rather than a change in affinity[18].

BDNF and responses to stimulants: Thus, the regulation of the DAT by RTKs directly parallels the modulation of DAT by insulin[12], and which is dependent in part on PI3K. This is significant for the established role of BDNF in the regulation of DA release and related behaviors in response to psychostimulants[15, 38]. Both intra-NAc or intra-VTA infusions of BDNF enhance locomotor responses to cocaine[15], consistent with the model in **Figure 1** of increased DAT availability and overall promotion of pre-synaptic DA by Akt. Several studies support this relationship between BDNF and psychostimulant behaviors, with anti-BDNF antibodies decreasing and viral enhancement of BDNF increasing locomotor activity in response to methamphetamines[38,39]. In line with these findings, antibodies directed against either BDNF or its RTK also diminish DA release in response to methamphetamine[38], suggesting BDNF promotes mechanisms related to increasing stores of pre-synaptic dopamine.

GDNF-related responses to psychostimulants: Interestingly, BDNF and GDNF seem to have opposite effects on reward-related behaviors, as studies show that GDNF *decreases* cocaine and opiate conditioned place preference[40], while BDNF *increases* drug reward and promotes self-administration of stimulants[30]. While the effects of GDNF seem contrary to our model, studies that measure GDNF effects on AMPH-induced release of D, support our model, with GDNF stimulation increasing and GDNF inhibition decreasing AMPH-induced DA efflux[41, 42]. This is in direct parallel to the proposed influence of BDNF on methamphetamine-induced efflux[38], suggesting that BDNF and GDNF may not ultimately have entirely opposite effects on responses to AMPH. Other findings on GDNF in support of our model include pronounced enhancements of DA uptake in GDNF-treated midbrain neuron cultures[43] and enhanced AMPH-induced locomotion with single nigral injections of GDNF [44]. Studies in animals with nigrostriatal lesions show that GDNF treatment enhances striatal DA content[45] and increases cell surface labeling of the DAT by radioligands[46, 47], suggesting an overall support of pre-synaptic DA function by GDNF. GDNF thus appears to enhance locomotor effects of stimulants, although conditioned place preference is diminished in treated animals.

Potential role of BDNF in cocaine sensitization: BDNF, in contrast to GDNF, is theorized to have an important role in the initiation of drug addiction[30]. The role of BDNF in models of psychostimulant addiction is particularly intriguing, as cocaine self-administration has been shown to increase midbrain BDNF levels[30]. In mice trained to self-administer cocaine, local deletion of BDNF in the nucleus accumbens, through conditional knockout strategies, diminishes cocaine self-administration[30]. The dynamics of BDNF signaling in the acquisition of cocaine addiction are therefore in line with our model. In normal animals, upregulation of BDNF with cocaine administration[30], according to our model, would lead to net activation of PI3K/Akt signaling. This, in turn, would stimulate DAT trafficking, providing an increased numbers of substrate for cocaine to bind to with repeated drug administration and also promoting replenishment of pre-synaptic DA. Intact Akt signaling, we hypothesize, is required for appropriate reuptake and recycling of DA into pre-synaptic terminals with DA release. Future biochemical and physiological studies are needed to determine the validity of this model.

Other RTKs and modulation of DAT: There are many other RTKs that may influence DA function similarly, including IGF-1, estrogen, FGF, and EGF. Some evidence already exists for modulation of DA function by these RTK ligands. Both FGF and epidermal growth factor (EGF) increase DA uptake in cultured cells[48], and FGF acutely enhances DAT cell surface expression[28]. However, the Akt dependence of these effects have yet to be determined.

CONCLUSIONS

The consensus in the literature on overall effects of Akt on DA systems is toward a promotion of DA release and DA-related behaviors in response to AMPH. Growth factor and hormonal signaling through RTKs is an increasingly well understood mechanism for regulation of nigrostriatal DA with therapeutic implications. The multiple mechanisms whereby RTKs and Akt potentially enhance AMPH actions converge at the promotion of are pre-synaptic DA function, causing increases in cell size, axonal

density, DAT trafficking and, potentially, upregulation of tyrosine hydroxylase. Animal models that focus on the temporal relationship between RTK signaling and DAT dynamics are warranted in order to separate contributions of DAT trafficking (on an acute time course) and cellular trophism (on a chronic time course) to AMPH actions; the Akt-dependence of any observed effects should also be established. Future studies in humans will bear out the potential of these mechanisms to translate into treatments of dopaminergic diseases.

REFERENCES

1. Smith AD, Kozlowski DA, Bohn MC and Zigmond MJ (2005). Effect of AdGDNF on dopaminergic neurotransmission in the striatum of 6-OHDA-treated rats. *Exp Neurol*. **193** (2): 420-426.
2. Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M and Gogos JA (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat Genet*. **36** (2): 131-137.
3. Galici R, Galli A, Jones DJ, Sanchez TA, Saunders C, Frazer A, Gould GG, Lin RZ and France CP (2003). Selective decreases in amphetamine self-administration and regulation of dopamine transporter function in diabetic rats. *Neuroendocrinology*. **77** (2): 132-140.
4. Tan HY, Nicodemus KK, Chen Q, Li Z, Brooke JK, Honea R, Kolachana BS, Straub RE, Meyer-Lindenberg A, Sei Y, Mattay VS, Callicott JH and Weinberger DR (2008). Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. *J Clin Invest*. **118** (6): 2200-2208.
5. Ikeda M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshiya Y, Sekine Y, Iyo M, Harano M, Komiyama T, Yamada M, Sora I, Ujike H, Inada T and Ozaki N (2006). Positive association of AKT1 haplotype to Japanese methamphetamine use disorder. *Int J Neuropsychopharmacol*. **9** (1): 77-81.
6. Xiromerisiou G, Hadjigeorgiou GM, Papadimitriou A, Katsarogiannis E, Gourbali V and Singleton AB (2008). Association between AKT1 gene and Parkinson's disease: a protective haplotype. *Neurosci Lett*. **436** (2): 232-234.
7. Karege F, Perroud N, Burkhardt S, Schwald M, Ballmann E, La Harpe R and Malafosse A (2007). Alteration in Kinase Activity But Not in Protein Levels of Protein Kinase B and Glycogen Synthase Kinase-3[beta] in Ventral Prefrontal Cortex of Depressed Suicide Victims. *Biological Psychiatry*. **61** (2): 240-245.
8. Keri S, Seres I, Kelemen O and Benedek G (2009). Neuregulin 1-stimulated phosphorylation of AKT in psychotic disorders and its relationship with neurocognitive functions. *Neurochem Int*.
9. Keri S, Seres I, Kelemen O and Benedek G (2009). The Relationship Among Neuregulin 1-Stimulated Phosphorylation of AKT, Psychosis Proneness, and Habituation of Arousal in Nonclinical Individuals. *Schizophr Bull*. sbp063.
10. Quesada A, Lee BY and Micevych PE (2008). PI3 kinase/Akt activation mediates estrogen and IGF-1 nigral DA neuronal neuroprotection against a unilateral rat model of Parkinson's disease. *Dev Neurobiol*. **68** (5): 632-644.
11. Bogush A, Pedrini S, Pelta-Heller J, Chan T, Yang Q, Mao Z, Sluzas E, Gieringer T and Ehrlich ME (2007). AKT and CDK5/p35 Mediate Brain-derived Neurotrophic Factor Induction of DARPP-32 in Medium Size Spiny Neurons in Vitro. *J Biol Chem*. **282** (10): 7352-7359.
12. Williams JM, Owens WA, Turner GH, Saunders C, Dipace C, Blakely RD, France CP, Gore JC, Daws LC, Avison MJ and Galli A (2007). Hypoinsulinemia regulates amphetamine-induced reverse transport of dopamine. *PLoS Biol*. 5 (10): e274.
    This paper is among the first *in vivo* evidence in support of our model- that deficits in PI3K/Akt signaling lead to decreases in DAT surface expression and concomitantly reduced efflux of DA in response to AMPH. The biochemical and *in vivo* electrochemical methods associated with this paper are relevant to the outlined specific aims.
13. Ries V, Henchcliffe C, Kareva T, Rzhetskaya M, Bland R, During MJ, Kholodilov N and Burke RE (2006). Oncoprotein Akt/PKB induces trophic effects in murine models of Parkinson's disease. *Proc Natl Acad Sci U S A*. 103 (49): 18757-18762.
    The characteristic cellular and psychostimulant-induced behavioral effects of Akt enhancement are demonstrated here, in addition to viral methods that are relevant to the specific aims. While this paper does not measure cell surface DAT expression and AMPH-induced DA efflux, we would expect them to be increased with viral Akt enhancement, according to our model. The findings of increased nigrostriatal DA content following myr-Akt treatment does reflect the influence of Akt on promotion of AMPH actions through mechanisms that, overall, promote pre-synaptic DA.
14. Flores C, Samaha A-N and Stewart J (2000). Requirement of Endogenous Basic Fibroblast Growth Factor for Sensitization to Amphetamine. *J Neurosci*. **20** (2): 55RC-.
15. Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ and Taylor JR (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci*. **19** (10): 4110-4122.
16. Izzo E, Martin-Fardon R, Koob GF, Weiss F and Sanna PP (2002). Neural plasticity and addiction: PI3-kinase and cocaine behavioral sensitization. *Nat Neurosci*. **5** (12): 1263-1264.
17. Owens WA, Sevak RJ, Galici R, Chang X, Javors MA, Galli A, France CP and Daws LC (2005). Deficits in dopamine clearance and locomotion in hypoinsulinemic rats unmask novel modulation of dopamine transporters by amphetamine. *J Neurochem*.
18. Hoover BR, Everett CV, Sorkin A and Zahniser NR (2007). Rapid regulation of dopamine transporters by tyrosine kinases in rat neuronal preparations. *J Neurochem*. 101 (5): 1258-1271.
    This is the first paper to demonstrate that inhibition of receptor tyrosine kinases (RTKs) themselves are sufficient to decrease cell surface expression of the DAT and DA uptake in neuronal preparations, and to show that BDNF itself is capable of influencing DA uptake in a PI3K-dependent fasion. This paper allows the potential to expand our model to include the effects of

other relevant growth factors on DA clearance and AMPH actions. Evidence from the effects of other growth factors on AMPH actions is largely in support of our models, although the effects of these RTKs on acute regulation of DAT cell surface expression are still limited, aside from where cited in the review.

19. 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.
20. 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. *Mol Pharmacol*. **68** (1): 102-109.
21. Rameh LE and Cantley LC (1999). The Role of Phosphoinositide 3-Kinase Lipid Products in Cell Function. *J Biol Chem*. **274** (13): 8347-8350.
22. Lucia C, José AM, Kristopher MK, Jasmine VF, Namita S, James DL, Leeb-Lundberg LMF, Gerald M, Eileen ML, Lisa MB, Toni SS, Jonathan AJ, Richard ZL and Aurelio G (2002). PI 3-kinase regulation of dopamine uptake. *Journal of Neurochemistry*. **81** (4): 859-869.
23. Russo SJ, Bolanos CA, Theobald DE, DeCarolis NA, Renthal W, Kumar A, Winstanley CA, Renthal NE, Wiley MD, Self DW, Russell DS, Neve RL, Eisch AJ and Nestler EJ (2007). IRS2-Akt pathway in midbrain dopamine neurons regulates behavioral and cellular responses to opiates. *Nat Neurosci*. **10** (1): 93-99.
24. Ries V, Cheng HC, Baohan A, Kareva T, Oo TF, Rzhetskaya M, Bland RJ, During MJ, Kholodilov N and Burke RE (2009). Regulation of the postnatal development of dopamine neurons of the substantia nigra in vivo by Akt/protein kinase B. *J Neurochem*. **110** (1): 23-33.
25. 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 Res*. **964** (1): 107-115.
26. Madziar B, Shah S, Brock M, Burke R, Lopez-Coviella I, Nickel AC, Cakal EB, Blusztajn JK and Berse B (2008). Nerve growth factor regulates the expression of the cholinergic locus and the high-affinity choline transporter via the Akt/PKB signaling pathway. *J Neurochem*. **107** (5): 1284-1293.
27. Perez-Garcia MJ, Cena V, de Pablo Y, Llovera M, Comella JX and Soler RM (2004). Glial cell line-derived neurotrophic factor increases intracellular calcium concentration. Role of calcium/calmodulin in the activation of the phosphatidylinositol 3-kinase pathway. *J Biol Chem*. **279** (7): 6132-6142.
28. Murase S and McKay RD (2006). A specific survival response in dopamine neurons at most risk in Parkinson's disease. *J Neurosci*. **26** (38): 9750-9760.
29. Mei L and Xiong W-C (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci*. **9** (6): 437-452.
30. Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M and Self DW (2007). Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci*. 10 (8): 1029-1037.
    This paper studies the dynamics of BDNF, another RTK ligand, on responses to the psychostimulant cocaine, with the paper suggesting BDNF is essential for cocaine sensitization based on findings from anti-BDNF antibodies and conditional knockout studies. Cocaine use tends to increase levels of BDNF in wild type mice, which, according to our model, would also result in activation of Akt through RTK stimulation of PI3K, and thus promote subsequent trafficking of the DAT to the plasma membrane. The upregulation of BDNF, Akt, and the DAT would act to promote both recycling of DAT back into the pre-synaptic cleft, preventing depeltion of neurotransmitter, and increasing availabilty of the DAT for binding psychostimulants. Similar findings directly implicating PI3K inhibition in cocaine sensitization have also been published, but this paper was given preference due to the use of conditional knockout technology and viral methods aimed at deleting BDNF selectively in the midbrain.
31. Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN and Heywood P (2003). Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. **9** (5): 589-595.
32. Kim Y, Seger R, Suresh Babu CV, Hwang SY and Yoo YS (2004). A positive role of the PI3-K/Akt signaling pathway in PC12 cell differentiation. *Mol Cells*. **18** (3): 353-359.
33. Kanakry CG, Li Z, Nakai Y, Sei Y and Weinberger DR (2007). Neuregulin-1 regulates cell adhesion via an ErbB2/phosphoinositide-3 kinase/Akt-dependent pathway: potential implications for schizophrenia and cancer. *PLoS ONE*. **2** (12): e1369.
34. Laurino L, Wang XX, de la Houssaye BA, Sosa L, Dupraz S, Caceres A, Pfenninger KH and Quiroga S (2005). PI3K activation by IGF-1 is essential for the regulation of membrane expansion at the nerve growth cone. *J Cell Sci*. **118** (16): 3653-3662.
35. Amino S, Itakura M, Ohnishi H, Tsujimura J, Koizumi S, Takei N and Takahashi M (2002). Nerve growth factor enhances neurotransmitter release from PC12 cells by increasing Ca(2+)-responsible secretory vesicles through the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase. *J Biochem*. **131** (6): 887-894.
36. Itakura M, Yamamori S, Kuwahara R, Sekiguchi M and Takahashi M (2005). Two distinct regulatory mechanisms of neurotransmitter release by phosphatidylinositol 3-kinase. *J Neurochem*. **94** (2): 502-509.
37. Goggi J, Pullar IA, Carney SL and Bradford HF (2003). Signalling pathways involved in the short-term potentiation of dopamine release by BDNF. *Brain Research*. **968** (1): 156-161.
38. Narita M, Aoki K, Takagi M, Yajima Y and Suzuki T (2003). Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. *Neuroscience*. **119** (3): 767-775.
39. Bahi A, Boyer F, Chandrasekar V and Dreyer JL (2008). Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats. *Psychopharmacology (Berl)*. **199** (2): 169-182.
40. Messer CJ, Eisch AJ, Carlezon WA, Jr., Whisler K, Shen L, Wolf DH, Westphal H, Collins F, Russell DS

and Nestler EJ (2000). Role for GDNF in biochemical and behavioral adaptations to drugs of abuse. *Neuron*. **26** (1): 247-257.
41. Cass WA, Walker DJ and Manning MW (1999). Augmented methamphetamine-induced overflow of striatal dopamine 1 day after GDNF administration. *Brain Res*. **827** (1-2): 104-112.
42. Hebert MA, Van Horne CG, Hoffer BJ and Gerhardt GA (1996). Functional effects of GDNF in normal rat striatum: presynaptic studies using in vivo electrochemistry and microdialysis. *J Pharmacol Exp Ther*. **279** (3): 1181-1190.
43. Consales C, Volpicelli F, Greco D, Leone L, Colucci-D'Amato L, Perrone-Capano C and di Porzio U (2007). GDNF signaling in embryonic midbrain neurons in vitro. *Brain Research*. **1159**: 28-39.
44. Hudson J, Granholm A-C, Gerhardt GA, Henry MA, Hoffman A, Biddle P, Leela NS, Mackerlova L, Lile JD, Collins F and Hoffer BJ (1995). Glial cell line-derived neurotrophic factor augments midbrain dopaminergic circuits in vivo. *Brain Research Bulletin*. **36** (5): 425-432.
45. Kirik D, Rosenblad C, Bjorklund A and Mandel RJ (2000). Long-Term rAAV-Mediated Gene Transfer of GDNF in the Rat Parkinson's Model: Intrastriatal But Not Intranigral Transduction Promotes Functional Regeneration in the Lesioned Nigrostriatal System. *J Neurosci*. **20** (12): 4686-4700.
46. Connor B, Kozlowski DA, Unnerstall JR, Elsworth JD, Tillerson JL, Schallert T and Bohn MC (2001). Glial Cell Line-Derived Neurotrophic Factor (GDNF) Gene Delivery Protects Dopaminergic Terminals from Degeneration. *Experimental Neurology*. **169** (1): 83-95.
47. Batchelor PE, Liberatore GT, Porritt MJ, Donnan GA and Howells DW (2000). Inhibition of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor expression reduces dopaminergic sprouting in the injured striatum. *European Journal of Neuroscience*. **12** (10): 3462-3468.
48. Inazu M, Takeda H, Ikoshi H, Uchida Y, Kubota N, Kiuchi Y, Oguchi K and Matsumiya T (1999). Regulation of dopamine uptake by basic fibroblast growth factor and epidermal growth factor in cultured rat astrocytes. *Neuroscience Research*. **34** (4): 235-244.

FURTHER INFORMATION

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

Neuroprotection by Physical Activity

Amanda C Mitchell

Physical activity is neuroprotective, lowering the risk of neurological diseases, increasing overall brain health, and leading to specific gene expression changes throughout the brain. In particular it upregulates growth factors, immediate early genes, immune genes, synaptic trafficking genes, neurotransmitter systems, and activates the extracellular signal-regulated kinases 1 and 2 (ERK1/2) and protein kinase B (PKB/AKT) signal transduction pathways. The beneficial effects of physical activity are supported in animal models of Parkinson's disease (PD). The unilateral 6-hydroxydopamine (6-OHDA) rat and bilateral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse PD models show behavioral and biochemical sparing in the striatum after forced limb use, and forced treadmill running.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: amanda.c.mitchell@vanderbilt.edu.

PHYSICAL ACTIVITY BENEFICIALLY AFFECTS THE BRAIN

A growing body of evidence suggests that mild[1], moderate, and vigorous physical activity[2] are neuroprotective, decreasing the risk of many brain disorders including ischemic stroke[3,4], Alzheimer's disease[1,5], and Parkinson's disease (PD)[2]. Many clinicians routinely recommend physical activity for those suffering from the effects of these diseases. In PD patients physical activity has been shown to improve gait, tremor, grip strength, balance, and motor coordination[6,7]. Regardless of disease presence physical activity can improve sleep[8], cognition[9,10], and decrease depression[11-13]. Supporting animal data show that exercise and environmental enrichment enhance learning and memory, increase neuronal survival, increase resistance to brain insults, trigger synaptogenesis, promote brain angiogenesis, and promote neurogenesis[14,15]. We believe physical activity affects the entire brain and have previously studied the effects of physical activity on the brain alone (unpublished) and in an Alzheimer's disease model[14].

Exercise gene expression changes have been studied largely in the hippocampus, a site of neurogenesis crucial in spatial learning and memory[16-18]. Initial examinations showed increases in nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) throughout the brain with the most dramatic increases in the hippocampus and posterior cortex[16]. In the hippocampus voluntary wheel running also increases the expression of phosphoinositide kinase 3 (PI3K), protein kinase B (PKB/AKT), BDNF, cAMP response element binding (CREB), and tyrosine kinase B (TrkB, the BDNF receptor)[19]. It is now understood that physical activity modulates the BDNF system through intracellular signaling systems such as AKT and extracellular signal-regulated kinases 1 and 2 (ERK1/2) with endpoint effects on the production, phosphorylation, and function of CREB[20] (**Figure 1**). AKT also phosphorylates forkhead box O3 (FOXO3), a transcription factor, causing its retention in the cytoplasm. When in the nucleus, FOXO3 likely triggers apoptosis by inducing the expression of genes critical for cell death[21]. Keeping FOXO3 in the cytoplasm, therefore, may promote cell survival.

Two DNA microarray studies comparing voluntary running rats to their sedentary counterparts revealed the upregulation of genes involved in neuronal activity, synaptic structure, and neuronal plasticity in the hippocampus[22, 23]. These genes included: neurotrophins, immediate early genes (IEGs), immune genes, and trafficking proteins[22]. The second study also revealed the upregulation of neurotrophic factors (NGF, BDNF, and basic fibroblast growth factor, FGF-2) as well genes involved in synaptic trafficking (syntaxin, synapsin I, and synaptotagmin), neutrotransmitter systems (ionotropic glutamate receptor subunits NR2A and NR2B, excitatory amino-acid carrier 1 (EAAC1), γ-aminobutyric-acid receptor β3 ($GABA_A$ β3), and glutaminic acid decarboxylase (GAD65)), and signal transduction pathways (ERK1/2, and protein kinase C (PKC))[23]. They furthered showed that CaMKIIδ was more highly expressed during acute exercise (3 days) and that ERK1/2 was more highly expressed during chronic exercise (28 days)[23]. CaMKII is activated by increases in Ca^{2+} (**Figure 1**) and phosphorylates many substrates including components of the ERK1/2 signal transduction pathway. **Figure 1** shows the BDNF – TrkB interaction, but most growth factors, including glial derived neurotrophic factor (GDNF), NGF, and FGF-2, activate the same signaling cascades[24-26].

Immediately following both voluntary wheel running and treadmill running in rodents there is an increase of corticosterone (indicative of the stress response) along with a decrease in phosphorylated CREB (pCREB) with treadmill running animals displaying a higher elevation of corticosterone and

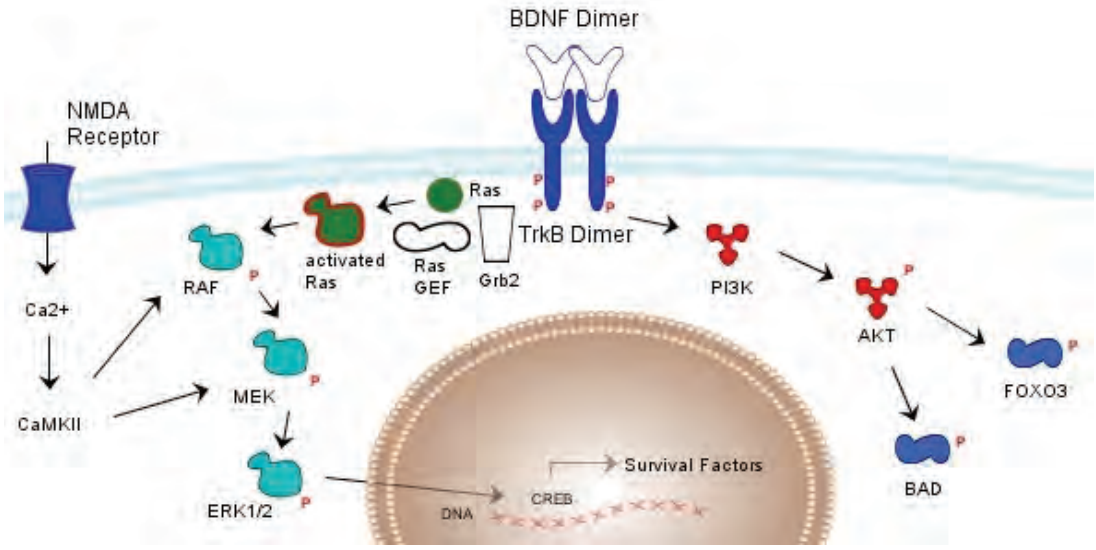

Figure 1 | **BDNF Signaling Pathways**. BDNF activates the AKT and ERK1/2 pathways. PI3K indirectly causes the phosphorylation of AKT, which phosphorylates and inhibits death proteins (FOXO3 and BAD). ERK1/2 is phosphorylated by a kinase cascade (RAF to MEK to ERK1/2) that is activated by RAS, which is activated by RAS-GEF binding to Grb2 bound to phosphorylated TrkB dimers. This pathway also can be phosphorylated by CaMKII[19, 21-23].

decrease in pCREB[27]. Though of small effect, corticosterone is known to decrease the expression of BDNF in the dentate gyrus (DG) of the hippocampus[28]. If corticosterone is administered subcutaneously to adrenalectomized animals, there is a transient decrease in BDNF at 4 and 6 hours and an increase in its TrkB receptor at 6 and 12 hours[29]. This immediate stress response, however, is likely specific to acute exercise and may diminish with repeated exercise exposures[27]. Studies have shown that the increases in corticosterone do fall off over time[30,31]. After five weeks of wheel running, there is no difference in corticosteroid response in a 20 minute restraint stress test between exercised and sedentary animals[31]. Lastly, in a study that standardized the distance ran between rats, it was shown that voluntary exercisers ran more rapidly for a shorter time than forced exercisers and had less bromodeoxyuridine (BrdU) incorporation into the DNA of hippocampal slices (an indication of neurogenesis)[32]. These studies show that although forced exercise may transiently activate the stress response, long term forced exercise may be more beneficial.

PARKINSON'S DISEASE MODELS

After extensive investigation of the hippocampus our attention has turned to brain areas related to PD. PD is characterized by tremor at rest, muscle rigidity, postural instability, and a slowing of physical movement (bradykinesia) that can progress to a complete loss of movement (akinesia)[33]. As disabling motor symptoms are managed with medications (such as L-3, 4-dihydroxyphenylalanine, L-DOPA), other symptoms become more apparent. These include depression, high level cognitive dysfunction, and subtle language problems[34,35]. It is thought that symptoms emerge from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). At the onset of motor symptoms dopaminergic neuron loss is already 60-80%. These neurons normally project to the striatum forming the nigrostriatal dopaminergic pathway[33]. Insufficient action of dopamine (DA) on the striatum is believed to lead to decreased stimulation of the motor cortex and PD symptoms[34-38].

PD is also often characterized by the presence of Lewy bodies. These proteinaceous cytoplasmic inclusions composed of α-synuclein, are present in the locus ceruleus, nucleus basalis of Meynert, dorsal motor nucleus of the vagus, hypothalamus, and other sites of some PD patients, but 20-40% patients with neuronal loss in the SNpc have no Lewy bodies raising the question of whether Lewy bodies are markers of presymptomatic PD or a feature of normal aging[39]. Common treatments aim to replace and stabilize dopamine. The most common is L-DOPA, which crosses the blood brain barrier and is converted to DA. Neuroprotective strategies, such as physical activity, however, aim to slow dopaminergic neuron loss and lead to improved functioning of the remaining neurons[33].

It is difficult to model the progressive nature of PD in animals, but two models, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), are able to model some of the pathology and symptoms. 6-OHDA causes the degeneration of catecholaminergic neurons (DA, norepinephrine, and epinephrine) when applied to the

brain. For localized dopaminergic degeneration it is stereotactically injected into the SNpc, the nigrostriatal tract (medial forebrain bundle), or the striatum of one brain hemisphere[33]. Dopaminergic neurons start degenerating within 24 hours and striatal DA is depleted 80-90% 2-3 days later corresponding with bradykinesia, impairment of movement initiation, and skilled motor functions on the contralateral side of the body[33,39]. SNpc degeneration also causes the upregulation of post synaptic DA receptors in the striatum. In a unilateral model this upregulation causes contralateral rotations with administration of a DA receptor agonist, apomorphine[36].

MPTP crosses the blood brain barrier and is metabolized by monoamine oxidase B (MAO-B) to 1-methyl-4-phenyl-2, 3-dihydropyridinium ion (MPP+). MPP+ is selectively taken up by the DA transporter (DAT) where it inhibits complex I of the mitochondrial electron transport chain, which mirrors the 30-40% decrease in mitochondrial electron chain complex I activity in the SNpc of PD patients[33]. Increased reactive oxygen species (hydrogen peroxide, superoxide, peroxyl radicals, nitric oxide, and hydroxyl radicals) caused by a dysfunctional complex I react with nucleic acids, proteins, lipids, and other molecules altering their structure, causing damage, and eventually leading to axon degeneration and neuron loss[40].

Acute bilateral MPTP exposure leads to 50-93% loss of cells in the SNpc and more than 99% loss of DA in the striatum leading to akinesia, rigidity, and in some species tremor[33]. A stable early stage unilateral model of PD (MPTP) was developed in middle-aged monkeys[41]. The most pronounced difference from acute bilateral models is the preservation of dopaminergic fiber projections to the caudate nucleus and putamen. Other studies support the concept that cell bodies of DA neurons can be maintained in the substantia nigra for long periods following axonal loss in the striatum[42]. The early stage model with greater preservation of nigrostriatal projections could be useful for testing neuroprotective strategies, such as exercise, to preserve and restore dopaminergic innervation to the striatum.

PHYSICAL ACTIVITY PROMOTES BEHAVIORAL AND BIOCHEMICAL SPARING

Increases in dendritic arborization and synapse number in the cortex have been associated with motor training[43-45]. Hence, Tillerson et al hypothesized that motor training might retard the loss of dopaminergic neuron projections from the SNpc to the striatum in a unilateral 6-OHDA rat model. After infusing 6-OHDA into the medial forebrain bundle (MFB), they forced the use of the impaired limb by casting the unimpaired limb on days 1-7, 3-9, or 7-13 after lesioning. Apomorphine-induced contralateral rotations and DA levels were used as a measure of SNpc dopaminergic neuron loss. Animals receiving a cast on days 1-7 and 3-9 did not show step or forelimb asymmetry, rotated significantly different from sham animals in response to apomorphine, and had significantly different levels of DA, DOPAC, or HVA from sham animals. Timing of exercise matters; early forced use (days 1-7 and 3-9), but not late forced use (days 7-13), of the impaired limb attenuated movement asymmetry and dopamine loss[45].

Tillerson switched to a forced treadmill running paradigm, as unilateral forced use is an exercise modality not commonly practiced in humans. They believed that treadmill running, like forced use, would attenuate DA loss and behavior. Rats were given either 6-OHDA and mice were given MPTP and forced to run until day 12 or 30 for behavioral tests and sacrificed for biochemical analysis. Moderate forced treadmill running reversed 6-OHDA movement impairments in rats after one day with 450 m/day of treadmill running, reversed MPTP movement impairments in mice after three days with 50 m/day, and attenuated striatal DA loss and DA terminal marker loss (DAT, VMAT, tyrosine hydroxylase (TH)) in both models[46]. Treadmill running, like forced use, attenuated both movement impairments and dopamine loss.

It is believed that mild stress can cancel the effect of neuroprotection. Both voluntary and forced exercise have been associated with mild stress. Howell et al addressed this issue by looking at the effect of stress on voluntary exercise. Animals were placed into three groups: runners allowed access to a running wheel, stressed runners allowed access to a running wheel (stressed with one hour of wheel immobilization a day, food deprivation, and a shift in the light dark cycle), and nonrunners. Both stressed runners and nonrunners had significantly more apomorphine rotations than runners alone with no difference in TH staining suggesting that mild stress can cancel the affect of exercise[47]. Earlier studies indicate that corticosterone, produced immediately following exercise, may diminish neuroprotection; these effects, however, wear off after five weeks[30-31]. Numerous other studies demonstrate that voluntary and forced exercise can ameliorate the behavioral and biochemical consequences of 6-OHDA and MPTP PD models[47-52] though the time, amount, method of exercise, and type of lesion do affect the behavioral and biochemical outcome.

In all of the studies the number of SNpc cells did not change with exercise[47-52]; rather, we believe behavioral and biochemical sparing comes from sparing of SNpc axons and terminals projecting to the striatum[51]. It has been hypothesized that forced use ameliorates the behavioral and biochemical effects of

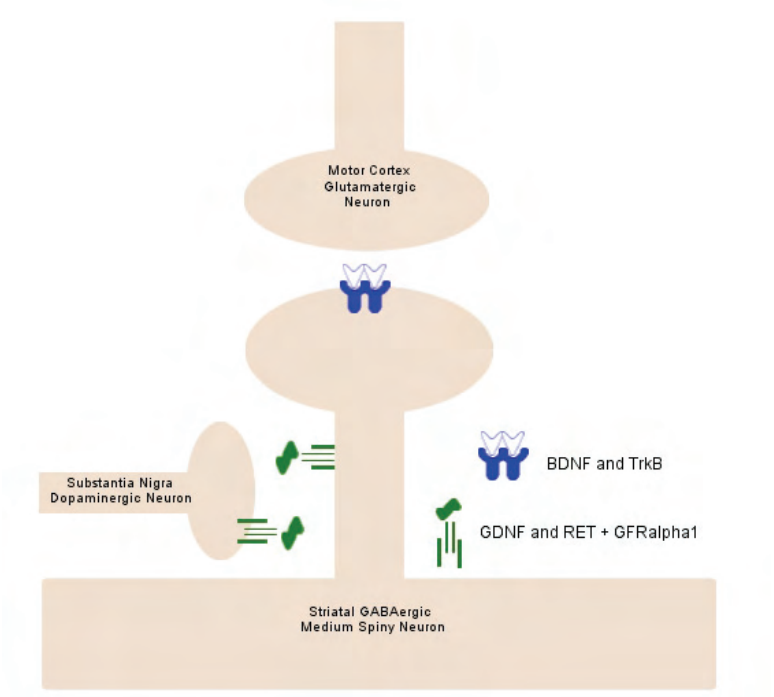

Figure 2 | **Sparing with Exercise.** Exercise results in an increase of neurotrophic factors and their receptors. Both BDNF and GDNF are increased with exercise. In the striatum we believe cortical BDNF increases the survival of striatal spines, while GDNF increases the survival of both SN terminals and striatal spines.

6-OHDA and MPTP through a cascade of events that involves GDNF[53], a potent survival factor for DA neurons[54]. There is a significant increase of striatal GDNF 24 and 72 hours after using a non-impaired limb[55]. In the striatum ERK1/2 activation by GDNF remains elevated up to 1 month afterwards[55,56]. The medium spiny neurons of the striatum receive BDNF from cortical input. They receive and produce GDNF. GDNF homodimers bind two GFRα1 receptors, which then bind two RET (Rearranged during Transfection) receptors, which cross phosphorylate each other in the striatum and SNpc. BDNF increases the survival of striatal spines, while GDNF increases the survival of both SNpc terminals and striatal spines (**Figure 2**). Exercise affects the entire brain with the upregulation of growth factors[16], and it seems most probable that the effects of exercise in PD would emerge at the intersection of the SNpc and motor cortex in the striatum.

CONCLUSIONS

Physical activity in PD has been investigated over the past decades. The 6-OHDA rat and MPTP mouse Parkinson's disease models show behavioral and biochemical sparing in the striatum after voluntary wheel running, forced limb use, and forced treadmill running though the most beneficial time (before or after lesioning), amount, and method (voluntary versus forced) of exercise for neuroprotection are still under investigation. Evidence suggests that reduced nigrostriatal degeneration is due in part to the upregulation of neurotrophic factors, one of the many affects of exercise, acting at the striatum.

REFERENCES

1. Yaffe K, Barnes D, Nevitt M, Lui LY and Covinsky K (2001). A Prospective Study of Physical and Cognitive Decline in Elderly Women: Women Who Walk. *Archives of Internal Medicine.* **161** (14): 1703-1708.
2. Thacker EL, Chen H, Patel AV, McCullough ML, Calle EL, Thun MJ, Schwarzschild MA and Ascherio A (2008). Recreational Physical Activity and Risk of Parkinson's Disease. *Mov Disord.* **23** (1):69-74.
3. Hu FB, Stampfer MJ, Colditz GA, Ascherio A, Rexrode KM, Willett WC and Manson JE (2000). Physical Activity and Risk of Stroke in Women. *JAMA.* **283**:2961-2967.
4. Lee IM and Paffenbarger RS (1998). Physical Activity and Stroke Incidence. The Harvard Alumni Health Study. *Stroke.* **29**: 2049–2054.
5. Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P and Kukull W (2006). Exercise is Associated with Reduced Risk for Incident Dementia Among Persons 65 Years of Age and Older. *Ann Intern Med.* **144** (2):73-81.
6. Hirsch MA, Toole T, Maitland CG and Rider RA (2003). The Effects of Balance Training and High-Intensity Resistance Training on Persons with Idiopathic Parkinson's Disease. *Arch Phys Med Rehabil.* **84**:1109-1117.
7. Palmer SS, Mortimer JA, Webster DD, Bistevins R and Dickinson GL (1986). Exercise Therapy for Parkinson's Disease. *Archives of Physical Medicine & Rehabilitation.* **67**:741-745.
8. Driver HS and Taylor SR (2000). Exercise and Sleep. *Sleep Med Rev.* **4** (4):387-402.
9. Colcome S and Kramer AF (2003). Fitness Effects on the Cognitive Function of Older Adults: A Meta-Analytic Study. *Psychol Sci.* **14** (2):125-130.
10. Weuve J, Kang JH, Manson JE, Breteler MM, Ware JH and Grodstein F (2004). Physical Activity, Including Walking, and Cognitive Function in Older Women. *JAMA.* **292** (12):1454-1461.
11. Dunn AL, Trivedi MH, Kampert JB, Clark CG and Chambliss HO (2005). Exercise Treatment for Depression: Efficacy and Dose Response. *Am J Prev Med.* **28** (1): 1-8.
12. Lawlor DA and Hopker SW (2001). The Effectiveness of Exercise as an Intervention in the Management of Depression: Systematic Review and Meta-Regression Analysis of Randomised Controlled Trials. *BMJ.* **322** (7289):763-767.
13. Reuter I, Engelhardt M, Stecker K and Baas H (1999). Therapeutic Value of Exercise Training in Parkinson's Disease. *Medicine & Science in Sports & Exercise.* **31**:1544-1549.
14. Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnics Z, Lee VM, Hersh LB, Sapolsky RM, Mirnics K and Sisodia SS (2005). Environmental Enrichment Reduces Aβ levels and Amyloid Deposition in Transgenic Mice. *Cell.* **120**:701-713.
15. van Praag H, Shubert T, Zhao C and Gage FH (2005). Exercise Enhances Learning and Hippocampal Neurogenesis in Aged Mice. *J. Neurosci.* **25** (38):8680-8685.

16. Neeper SA, Gomez-Pinilla F, Choi J and Cotman CW (1996). Physical Activity Increases mRNA for Brain-Derived Neurotrophic Factor and Nerve Growth Factor in Rat Brain. *Brain Res.* **726** (1-2):49-56.
17. van Praag H, Kempermann G and Gage FH (2000). Neural Consequences of Environmental Enrichment. *Nature Neuroscience*. **1**:191-198.
18. Li Y, Mu Y and Gage FH (2009). Development of Neural Circuits in the Adult Hippocampus. *Current Topics in Developmental Biology*. **87**: 149-174.
19. Chen MJ and Russ-Neustadt AA (2005). Exercise Activates the Phosphatidylinositol 3-Kinase Pathway. *Brain Res Mol Brain Res.* **135** (1-2): 181-193.
20. Vaynman S, Ying Z and Gomez-Pinilla F (2004). Hippocampal BDNF Mediates the Efficacy of Exercise on Synaptic Plasticity and Cognition. *Eur J Neurosci.* **20** (10):2580-2590.
21. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J and Greenberg ME (1999). Akt Promotes Cell Survival by Phosphorylating and Inhibiting a Forkhead Transcription Factor. *Cell.* **96**: 857-868.
22. Tong L, Shen H, Perreau VM, Balazs R and Cotman CW (2001). Effects of Exercise on Gene-Expression Profile in the Rat Hippocampus. *Neurobiol Dis.* **8** (6): 1046-1056.
23. Molteni R, Ying Z and Gómez-Pinilla F (2002). Differential Effects of Acute and Chronic Exercise on Plasticity-Related Genes in the Rat Hippocampus Revealed by Microarray. *Eur J Neurosci.* **16** (6):1107-1116.
This study explores gene expression changes in the hippocampus of rats after physical activity.
24. Hauck SM, Kinkl N, Deeg CA, Swiatek-de Lange M, Schoffmann S and Ueffing M (2006). GDNF Family Ligands Trigger Indirect Neuroprotective Signaling in Retinal Glial Cells. *Molecular and Cellular Biology.* **26** (7): 2746-2757.
25. Tsang M and Dawid (2004). Promotion and Attenuation of FGF Signaling Through the Ras-MAPK Pathway. *Sci. STKE.* **228**:17.
26. Yuan J and Yankner BA (2000). Neuronal Survival Pathways Induced by the Binding of NGF to its Receptor TrkA. *Nature.* **407**:802-809.
27. Ploughman M, Granter-Button S, Chernenko G, Attwood Z, Tucker BA, Mearow KM and Corbett D (2007). Exercise Intensity Influences the Temporal Profile of Growth Factors Involved in Neuronal Plasticity Following Focal Ischemia. *Brain Res.* **1150**: 207–216.
28. Smith MA, Makino S, Kvetnansky R and Post RM (1995). Stress and Glucocorticoids Affect the Expression of Brain Derived Neurotrophic Factor and Neurotrophin 3 mRNAs in the Hippocampus. *J of Neuroscience.* **15**: 1768-1777.
29. Schaaf M, de Jong J, de Kloet E and Vreugdenhil E (1998). Downregulation of BDNF mRNA and Protein in the Rat Hippocampus by Corticosterone. *Brain Res.* **813**: 112–120.
30. Ploughman M, Granter-Button S, Chernenko G, Tucker BA, Mearow KM and Corbett D (2005). Endurance Exercise Regimens Induce Differential Effects on Brain-Derived Neurotrophic Factor, Synapsin-I and Insulin-like Growth Factor I After Focal Ischemia. *Neuroscience.* **136**: 991–1001.
31. Fediuc S, Campbell JE and Riddel MC (2006). Effect of Voluntary Wheel Running on Circadian Corticosterone Release and on HPA Axis Responsiveness to Restraint Stress in Sprague-Dawley Rats. *J Appl Physiol*. **100**: 1867-1875.
32. Leasure JL and Jones M (2008). Forced and Voluntary Exercise Differentially Affect Brain and Behavior. *Neuroscience.* **156** (3):456-465.
33. Betarbet R, Sherrer TB and Greenamyre JT (2002). Animal Models of Parkinson's Disease. *BioEssays*. **24**:308-318
34. Brown JA, Lutsep HL, Weinand M and Cramer SC (2006). Motor Cortex Stimulation for the Enhancement of Recovery from Stroke: A Prospective, Multicenter Safety Study. *Neurosurgery.* **58** (3):464-473.
35. Dagher A, Owen AM, Boecker H and Brooks DJ (2001). The Role of the Striatum and Hippocampus in Planning: A PET Activation Study in Parkinson's Disease. *Brain.* **124** (5): 1020-1032.
36. Burke RE, Cadet JL, Kent JD, Karanas AL and Jackson-Lewis V (1990). An Assessment of the Validity of Densitometric Measures of Striatal Tyrosine Hydroxylase-Positive Fibers: Relationship to Apomorphine-Induced Rotations in 6-Hydroxydopamine Lesioned Rats. *J. Neurosci Methods.* **35** (1):63-73.
37. Cioni B (2007). Motor Stimulation for Parkinson's Disease. *Acta Neurochir Suppl.* **97**:233-238.
38. Rowe J, Klaas ES, Friston K, Frackowiak R, Lees A and Passingham R (2002). Attention to Action in Parkinson's Disease: Impaired Effective Connectivity Among Frontal Cortical Region. *Brain*. **125** (2): 276-289.
39. Gelb DJ, Oliver E and Gilman S (1999). Diagnostic Criteria for Parkinson's Disease. *Arch Neurol.* **56** (1): 33-39.
40. Mann VM, Cooper JM, Krige D, Daniel SE, Schapira AH and Marsden CD (1992). Brain, Skeletal Muscle and Platelet Homogenate Mitochondrial Function in Parkinson Disease. *Brain.* **115**: 333–342.
41. Ding F, Luan L, Ai Y, Walton A, Gerhardt GA, Gash DM, Grondin R and Zhang Z (2008). Development of a Stable, Early Stage Unilateral Model of Parkinson's disease in Middle-Aged Rhesus Monkeys. *Exp Neurol.* **212** (2): 421-439
We are using the early stage unilateral model of Parkinson's disease in Middle-Aged Rhesus Monkeys.
42. Kirik D, Rosenblad C and Bjorklun A (2000). Preservation of a Functional nigrostriatal Dopamine Pathway by GDNF in the Intrastriatal 6-OHDA Lesion Model Depends on the Site of Administration of the Trophic Factor. *Eur J. Neurosci.* **12** (11): 3871-3882.
43. Jones TA and Schallert T (1992). Overgrowth and Pruning of Dendrites in Adult Rats Recovering from Neocortical Damage. *Brain Res.* **581**:156-160.
44. Kleim JA, Lussnig E, Schwarz ER, Comery TA and Greenough WT (1996). Synaptogenesis and Fos Expression in the Motor Cortex of the Adult Rat After Motor Skill Learning. *J Neurosci.* **16** (14):4529-35.
45. Tillerson JL, Cohen AD, Philhower J, Miller GW, Zigmond MJ and Schallert T (2001). Forced Limb-Use Effects on the Behavioral and Neurochemical Effects of 6-Hydroxydopamine. *J Neurosci.* **21** (12):4427-4435.
46. Tillerson JL, Caudle MW, Reverón ME and Miller GW (2003). Exercise Induces Behavioral Recovery and Attenuates Neurochemical Deficits in Rodent Models of Parkinson's Disease. *Neuroscience.* **119** (3): 899-911.

This study shows behavioral and biochemical sparing after forced physical activity in both the 6-OHDA rat and MPTP mouse Parkinson's disease models.

47. Howell FM, Russell VA, Mabandla MV and Kellaway LA (2005). Stress Reduces the Neuroprotective Effect of Exercise in a Rat Model for Parkinson's Disease. *Behav Brain Res.* **165** (2):210-20.
48. Caudle MW, Tillerson JL, Reverón ME and Miller GW (2006). Use-Dependent Behavioral and Neurochemical Asymmetry in MPTP Mice. *Neuroscience Letters.* **418** (3); 211-212
49. Mabandla M, Kellaway L, St Clair Gibson A, Russell VA (2004). Voluntary Running Provides Neuroprotection in Rats after 6-Hydroxydopamine Injection into the Medial Forebrain Bundle. *Metab Brain Dis* **19**:43-50.
50. O'Dell SJ, Gross NB, Fricks AN, Casiano BD, Nguyen TB and Marshall JF (2007). Running Wheel Exercise Enhances Recovery from Nigrostriatal Dopamine Injury Without Inducing Neuroprotection. *Neuroscience.* **144**:1141-1151.
51. Petzinger GM, Walsh JP, Akopian G, Hogg E, Abernathy A, Arevalo P, Turnquist P, Vuckovic M, Fisher BE, Togasaki DM, and Jakowec MW. Effects of Treadmill Exercise on Dopaminergic Transmission in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Lesioned Mouse Model of Basal Ganglia Injury. *Neurobiology of Disease.* **27** (20):5291-5300.
52. Poulton NP and Muir GD (2005). Treadmill Training Ameliorates Dopamine Loss but not Behavioral Deficits in hemi-Parkinsonian Rats. *Exp Neurol.* **193**:181-197.
53. Ries V, Cheng HC, Baohan A, Kareva T, Oo TF, Rzhetskaya M, Bland RJ, During MJ, Kholodilov R and Burke RE (2009). Regulation of the Postnatal Development of Dopamine Neurons of the Substantia Nigra *In Vivo* by Akt/Protein Kinase B. *J. Neurochem.* **110** (1): 22-33.
54. Cohen AD, Tillerson JL, Smith AD, Schallert T, and Zigmond MJ (2003). Neuroprotective Effects of Prior Limb Use in 6-Hydroxydopamine-Treated Rats: Possible Role of GDNF. *Journal of Neurochemistry.* **85**: 299-305.
    This study shows GDNF increases affect limb use in rats.
55. Lin E, Cavanaugh JE, Leak RK, Perez RK and Zigmond MJ (2007). Rapid Activation of ERK by 6-Hydroxydropamine Promotes Survival of Dopaminergic Cells. *J Neuroscience Research.* **86**:108-117.
56. Lindgren N, Leak RK, Carlson KM, Smith AD and Zigmond MJ (2008). Activation of the Extracellular Signal-regulated Kinases 1 and 2 by Glial Cell Line-Derived Neurotrophic Factor and its Relation to Neuroprotection in a Mouse Model of Parkinson's Disease. *J Neuroscience Research.* **86** (9):2039-2049.
57. Shen H, Tong L, Balazs R and Cotman CW (2001). Physical Activity Elicits Sustained Activation of Cyclic AMP Response Element-binding Protein and Mitogen-Activated Protein Kinase in the Rat Hippocampus. *Neuroscience*. **107** (2):219-229.
58. Benjamini Y and Hochberg Y (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing, *J. R. Stat. Soc., B.* **57**: 289–300.
59. Mirnics K (2001). Microarrays in Brain Research: The Good, the Bad and the Ugly. *Nature Reviews Neuroscience.* **2** (6):444-447.
60. Karssen AM, Li JZ, Her S, Patel PD, Meng F, Evans SJ, Vawter MP, Tomita H, Choudary PV, Bunney WE, Jones EG, Watson SJ, Akil H, Myers RM, Schatzberg AF and Lyons DM (2006). Application of Microarray Technology in Primate Behavioral Neuroscience Research. *Methods.* **38** (3): 227-234.

FURTHER INFORMATION

Karoly Mirnics' Lab:
http://mirnicslab.vanderbilt.edu/mirnicslab/

The Influence of GABA Metabolism on GABA Neurotransmission: The Role of Metabolic Regulatory Points and a Neuronal Glutamate Transporter

Ernesto Solis, Jr.

Excessive excitatory drive in the brain is thought to underlie diseases such as epilepsy. One approach in the development of novel treatments for conditions characterized by hyper-excitability is the enhancement of GABA-mediated inhibition. While most current medical interventions target GABAergic neurotransmission postsynaptically (e.g. benzodiazepines, barbiturates), much less is known about potential presynaptic therapeutic targets at the GABAergic synapse. This review describes recent findings that have exemplified presynaptic mechanisms that may provide the basis for the development of novel treatments to alter inhibitory neurotransmission. GABA metabolism is summarized with an emphasis on the role of presynaptic regulatory points in GABA synthesis. In addition, the excitatory amino acid transporter 3 (EAAT3), which is thought to provide the substrate for GABA synthesis, will be described in detail. Finally, EAAT3 is presented as a potential therapeutic target to modulate GABA-mediated inhibition presynaptically, and the most recent findings on EAAT3's functional regulation by several key players are reviewed.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: ernesto.solis@vanderbilt.edu.

GLUTAMATE AND GABA METABOLISM

Glutamate and GABA are the major excitatory and inhibitory neurotransmitters in the brain, respectively. Unlike other neurotransmitter systems, such as monoamines, reuptake and recycling of glutamate and GABA does not appear to be as important as new synthesis to replenish the pool of neurotransmitter for synaptic vesicle filling. Despite the critical importance of neurotransmitter supply, either to prevent depletion and maintain stable transmission or perhaps to dynamically adjust in response to demand, the metabolic pathways by which these transmitters are continuously supplied to synaptic terminals have not been resolved.

GABA is synthesized by the decarboxylation of glutamate, which is catalyzed by the enzyme glutamic acid decarboxylase (GAD). Neurons are not capable of synthesizing glutamate[1] on their own; therefore inhibitory neurons, like excitatory neurons, need a supply of glutamate. At least two possible pathways through which glutamate may be acquired exists: (1) the direct uptake of extracellular glutamate or (2) the uptake of glutamine, which can be converted to glutamate by neurons. Transporters serving both of these roles are expressed by GABAergic neurons, and both have been demonstrated to play roles in the synthesis of GABA[2-5].

GABA synthesis and synaptic vesicle filling are tightly coupled processes as revealed by biochemical assays. GAD65, the synaptically localized isoform of GAD, is associated with a complex of proteins on synaptic vesicles that includes the vesicular GABA transporter[6]. GABA synthesized from glutamate is taken up into synaptic vesicles preferentially over pre-existing GABA[7]. Electrophysiological studies demonstrated that inhibiting GAD results in a reduction in the size of miniature synaptic events, which represent the amount of GABA released from a single synaptic vesicle[8]. In contrast, knock-out of the predominant membrane transporter for GABA reuptake does not influence the size of these miniature events[9]. Taken together, these findings suggest that new synthesis is more important than recycling of existing GABA. Moreover, they demonstrate that any factors influencing GABA synthesis are likely to play an important role in maintaining, and possibly regulating, inhibitory synaptic transmission. Finally, GABA is catabolized by the action of GABA transaminase (GABA-T), which deaminates GABA to make succinic semialdehyde (SSA), and then SSA dehydrogenase (SSADH) converts SSA to succinate, which enters the TCA cycle. SSA can also be converted to γ–hydroxybutyrate (GBH) by the action of SSA reductase[10] (**Figure 1**).

HOW IS SUBSTRATE OBTAINED FOR PRODUCTION OF GLUTAMATE AND GABA?

Supply of substrate to inhibitory neurons for GABA synthesis is mediated by highly regulated, sodium-dependent solute transporters, which carry metabolites against their concentration gradients. EAAT3, a member of the high affinity glutamate transporter (excitatory amino acid transporter, EAAT) family, is expressed on somatodendritic

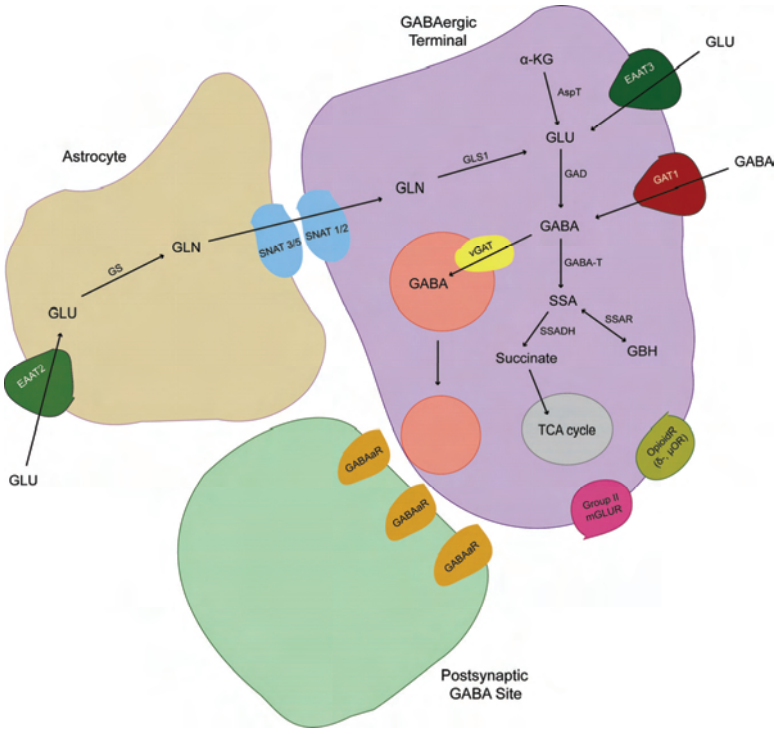

Figure 1. | **Metabolic pathways of GABA.** Acquisition of substrate for GABA synthesis, GABA packaging into synaptic vesicles, and breakdown of GABA by respective proteins.

compartments of excitatory neurons, and at axon terminals of GABAergic neurons[11, 12]. SNAT1 and 2 are members of the sodium coupled neutral amino acid transporter (SNAT) family and are expressed on excitatory and inhibitory neurons[13]. Because of their substrate affinity and the high ambient concentration of glutamine in the brain, SNAT1 and 2 are likely the major glutamine uptake pathway for neurons[14].

Astrocytes express glutamate transporters EAAT1 and 2 (also known as GLAST and GLT-1, respectively), of which EAAT2 is the major transporter for the clearance of synaptically released glutamate[15], as well as SNAT3 and 5[13]. A glutamine-glutamate-GABA cycle has been proposed[1] in which glutamate released from neurons is taken up by astrocytes, converted to glutamine with the enzyme glutamine synthetase and subsequently transported back to neurons (**Figure 1**). In neurons, glutaminase type I (GLS1) has been proposed to be the enzyme that converts glutamine to glutamate [1]. Studies have suggested that the majority of neurotransmitter glutamate is recycled by GLS1 with minimal contribution of *de novo* synthesis of glutamate from α-ketoglutarate in excitatory neurons[16, 17]. Interestingly, when GLS1 was knocked out, GABA levels were not reduced[18], suggesting alternative metabolic pathways for GABA synthesis must be present in inhibitory neurons.

In acute hippocampal slices, pharmacological inhibition of either EAATs or SNATs results in a rapid reduction of GABA vesicle content, as measured by changes in miniature inhibitory postsynaptic current (mIPSC) amplitudes[3, 19]. These results suggest that a dynamic equilibrium exists between GABA synthesis and vesicular filling, and consequently that inhibitory synaptic strength is directly regulated by two different substrate supply pathways. These results also suggest that substrate supply is a key regulatory point in the determination of inhibitory synaptic strength.

THERAPEUTIC IMPLICATIONS OF REGULATING INHIBITORY NEUROTRANSMISSION: WHAT KNOCKOUT STUDIES TELL US ABOUT THE ROLE OF KEY PROTEINS INVOLVED IN THE REGULATION OF GABA SYNTHESIS

(summarized in **Table 1**)

The importance of the regulatory points of GABA synthesis is revealed by knock-out mouse studies. After glutamate is cleared by glutamate transporters on glia, glutamine synthetase (GS) converts glutamate to glutamine to recycle neurotransmitter for both excitatory and inhibitory neurotransmission. In the context of an entire organism, the conversion of glutamate to glutamine results in detoxification of ammonia. As expected, knocking out GS has a severe phenotype, with death occurring at embryonic day 3.5[20]. Since GS has a global role beyond the central nervous system, it is not likely to be a good therapeutic target to alter GABA metabolism.

As mentioned above, GLS1 is the enzyme thought to convert glutamine to glutamate in neurons. The GLS1 knock-out mouse dies within a day of birth, is slightly smaller than wild type, has impaired respiratory function, and is deficient in goal-directed behavior[18]. It is thought that respiratory acidosis causes respiratory impairment and subsequent death.

There are 2 isoforms of GAD, GAD65 and GAD67, named after their molecular weights of 65 and 67 kDa, respectively. These 2 isoforms are encoded by independent genes and have different subcellular localizations in inhibitory neurons. As mentioned earlier, GAD65 is found at synapses. One study showed that GAD65 knock-out mice are more likely to develop seizures than wild type mice[21]. A different GAD65 knock-out mouse had an epileptic phenotype characterized by spontaneous seizures that led to death[22]. This mouse also showed increased anxiety-like behaviors and diminished response to anxiolytics[23], pre-pulse inhibition deficits[24], upregulation of the vesicular GABA transporter, and increased cytosolic GABA transport into synaptic vesicles[25].

GAD67 localizes to the cell soma of inhibitory neurons. The GAD67 knock-out mouse shows a

reduction in GABA levels throughout the brain, a reduction in GAD activity, and severe cleft palate, which leads to death within 24 hours of birth[26]. It is thought that the reduction of GABA levels in the GAD67 knock-out mouse brainstem to 30% of wild type leads to a malfunction in the respiratory control system and subsequent death[27]. The GAD65/GAD67 double knock-out mouse dies after birth due to cleft palate. GABA levels are low in this mouse[28] and another study with a different GAD65/GAD67 double knock-out mouse determined that GABA synthesis is absent[29].

The vesicular GABA transporter (vGAT) fills synaptic vesicles with both inhibitory neurotransmitters GABA and glycine. Study of a vGAT knock-out mouse showed that this mouse is incapable of executing vesicular release of GABA and glycine[29].

GAT1 is the predominant transporter responsible for GABA reuptake into inhibitory terminals, which allows for termination of GABA transmission. The GAT1 knock-out mouse showed reduced anxiety-like and depression-like behaviors[30], as well as decreased aggression[31], tremor, ataxia, nervousness, and increased extracellular GABA levels, which led to enhanced tonic inhibition and diminished phasic inhibition[9]. GAT1 has been targeted therapeutically by drugs, such as tiagabine, to treat epilepsy and anxiety[32].

GABA-T and SSADH perform a 2-step enzymatic breakdown of GABA. While there is no knock-out mouse for GABA-T, drugs that inhibit GABA-T, such as vigabatrin, have been used to increase GABA levels in the brain. Vigabatrin is not a drug of choice for epilepsy treatment because it often causes visual field defects[33]. The SSADH knock-out mouse exhibits ataxia, absence-like seizures with ictal behavior characterized by facial myoclonus, vibrissal twitching, and frozen immobility at 2 weeks. At this time, the absence seizures become more severe evolving into generalized convulsive seizures that progress into lethal status epilepticus[34, 35]. This mouse provides a good model for SSADH deficiency seen in humans[36], which is characterized by absence seizures and mental retardation.

THE ROLE OF EAAT3 ON INHIBITORY NEUROTRANSMISSION, SEIZURES, AND EPILEPSY

A role for EAAT3 as a critical regulator of neuronal excitability *in vivo* was demonstrated using antisense knock-down of the transporter[37-42]. In this study, antisense oligonucleotides to EAAT3 were infused into the lateral ventricles of rats, adjacent to the hippocampus. The animals developed spontaneous seizures corresponding to the time course of EAAT3 protein level reduction. Biochemical analysis confirmed reduced GABA content and impaired GABA synthesis in hippocampal tissue from treated animals. These results suggest that EAAT3 mediates an endogenous negative feedback mechanism whereby increased extracellular glutamate enhances GABA synthesis and inhibitory synaptic strength. The EAAT3 knock-out mouse did not have an epileptic phenotype, but it did develop dicarboxylic aminoaciduria and behavioral abnormalities[43]. In addition, this mouse develops glutathione deficiency and shows age-dependent

Table 1 | **Summary of knock-out studies of key regulatory points in GABA metabolism.**

Protein(s)	Cellular Localization	Function	KO Phenotype
Glutamic Acid Decarboxylase (GAD65)	Inhibitory neuron terminals	decarboxylates glutamate to synthesize GABA	increased seizure susceptible, epilepsy (spontaneous seizures leading to death), increased anxiety, LTD absence in visual cortex, PPI deficits, vGAT upregulation, increased cytosolic GABA transport into synaptic vesicles
Glutamic Acid Decarboxylase (GAD67)	Inhibitory neuron soma	decarboxylates glutamate to synthesize GABA	reductions in GABA levels throughout the brain, reduced GAD activities, cleft palate leading to death on P0, reduction of GABA levels in brainstem to 30% of wild type leading to abnormal respiratory patterns
GAD65/67	Inhibitory neurons	decarboxylates glutamate to synthesize GABA	die after birth due to cleft palate, GABA levels are scarce, absence of GABA synthesis
Vesicular GABA transporter (vGAT)	Inhibitory neuron terminals	transports GABA and glycine into synaptic vesicles	absence of GABA and glycine vesicular release
GABA transporter (GAT1)	Inhibitory neuron axons and terminals	GABA reuptake, terminate GABA transmission	reduced depression- and anxiety-like behaviors, decreased agression; tremor, ataxia, nervousness, increased GABA-induced tonic conductance in cerebellum, altered behavioral responses to alcohol, enhanced extracellular GABA levels, enhanced tonic, diminished phasic inhibition
GABA transaminase (GABA-T)	Inhibitory neuron terminals	breaks down GABA into succinic semialdehyde (SSA)	N/A
Succinic Semialdehyde Dehydrogenase (SSADH)	Inhibitory neuron terminals	terminates GABA catabolism by breaking down SSA into succinate	ataxia, absence-like seizures with ictal behavior characterized by facial myoclonus, vibrissal twitching, and frozen immobility at 2 weeks, absence seizures become more severe evolving into generalized convulsive seizures that progress into lethal status epilepticus
Glutamine Synthetase (GS)	Astrocytes	removes ammonia and glutamine (converts glutamate to glutamine)	dies at embryonic day 3.5
Glutaminase (GLS1)	Excitatory and inhibitory neurons	converts glutamine to glutamate	dies within 24 hours of birth, slightly smaller, impaired respiratory function, deficient in goal-directed behavior
SNAT3/5	Astrocytes	transports glutamine out of astrocytes	N/A
SNAT1/2	Neurons	takes up glutamine into neurons	N/A
Excitatory Amino Acid Transporter 3 (EAAT3)	Excitatory and inhibitory neurons	glutamate reuptake, provides substrate for GABA synthesis	decarboxylic aminoaciduria, behavioral abnormalities, glutathione deficiency, age-dependent neurodegeneration
Excitatory Amino Acid Transporter 2 (EAAT2)	Astrocytes	clears and recycles most of the extracellular glutamate	seizures, epilepsy

neurodegeneration[44]. The discrepancy in the phenotypes between the knock-out mouse and the knock-down rats, in particular the epileptic phenotype, has been attributed to a compensatory upregulation of a glutamate transporter homologous to EAAT3 in the knock-out mouse[4].

Several studies have investigated changes in EAAT3 expression in a variety of chronic epilepsy models and in human epileptic brain. Most studies of seizure models have looked at EAAT3 expression changes at least 24 hours after seizure induction[45], and these studies have examined changes at the tissue level which would predominantly reflect expression changes in the more numerous excitatory neurons. Moreover, the results of these studies were inconsistent, possibly due to differences in the epilepsy models, measurements (mRNA vs. protein) and regions examined. The only study of acute changes showed that EAAT3 protein in hippocampal pyramidal neurons appears to internalize 6 hours after kainic acid seizure induction[46]. No study has examined the expression of EAAT3 by inhibitory neurons in seizures and epilepsy. Because EAAT3 is expressed at postsynaptic sites on excitatory neurons and presynaptically on inhibitory neurons, it seems reasonable to hypothesize that seizure activity will have distinct effects on these two pools of transporters.

Recent evidence suggests that one function of glutamate transporters on inhibitory neurons, potentially EAAT3, may be the dynamic regulation of inhibition by extracellular glutamate levels[47]. Glutamate has been reported to increase extracellularly prior to seizure onset in human brain[48]. Therefore, EAAT3 may function to prevent the onset of seizures or to curtail seizure activity once started through enhancement of inhibition.

REGULATION OF EAAT3 SURFACE EXPRESSION AND GLUTAMATE UPTAKE BY SIGNALING CASCADE MOLECULES

Signaling cascade-mediated regulation of EAAT3 in inhibitory neurons could allow for modulation of inhibitory neurotransmission. A number of studies reported that EAAT3 activity is regulated by signaling cascade molecules. Most of these studies used heterologous settings including the C6 glioma cell line, which expresses EAAT3 endogenously. Regulation of neuronal EAAT3 in an endogenous setting and its effects on neuronal glutamate uptake are less well described. One study demonstrated functional upregulation of EAAT3 activity following induction of long term potentiation in the hippocampus and subsequent translocation of the protein from a cytosolic to a membrane compartment[49].

Glutamate uptake via EAAT3 is increased by non-specific activation of protein kinase C (PKC) with phorbol-12,13-myristate (PMA) in C6 glioma cells[50] and in primary neuronal cultures[46, 50]. This increase in glutamate uptake is associated with a rapid increase in EAAT3 surface expression in both C6 glioma cells and neurons[46]. Bisindolylmaleimide II (Bis II), a PKC inhibitor, completely blocks the PMA-induced glutamate uptake, but has no effect on basal glutamate uptake levels[51]. PKC activation mimicked the LTP induced upregulation of EAAT3[49].

PKC activity is mediated by a family of three subgroups (classic, novel, and atypical PKCs) each having unique properties. Classic PKC (cPKC) subtypes, which include three members (α, β, and γ), require calcium as a co-factor and are activated by diacylglycerol (DAG) and phorbol esters. In both C6 glioma cells and cortical neurons, the PMA-induced increase in EAAT3 activity was blocked with Gö6976 (10 μM), a selective inhibitor of cPKC subtypes[52]. Of the three cPKC subtypes, C6 glioma cells only express PKCα, suggesting that PKCα is the cPKC subtype that plays a role in the regulation of EAAT3 activity. In addition, C6 glioma cells treated with PMA showed a direct interaction between PKCα and EAAT3 on the cell surface[52]. Rat brain synaptosomes show basal EAAT3-PKCα association in the absence of PMA, while PMA treatment induced additional EAAT3-PKCα association. Both effects in synaptosomes are blocked by PKC antagonists suggesting the association may be triggered by endogenous stimulation of PKC activity under physiological conditions[53].

Phospatidylinositol 3-kinase (PI3K) has also been shown to regulate EAAT3 activity. Wortmannin, an irreversible PI3K inhibitor, decreases glutamate uptake and EAAT3 cell surface expression in C6 glioma cells within minutes. Platelet-derived growth factor (PDGF), which stimulates PI3K activity, increases both the activity and cell surface expression of EAAT3. The PDGF-mediated increase in EAAT3 activity is not blocked by the PKC antagonist BisII, and the PMA-mediated increase in glutamate uptake is not blocked by wortmannin suggesting that at least two independent signaling pathways regulate EAAT3 activity[50].

Protein kinase A (PKA) has been shown to regulate EAAT3 activity. In primary neuronal cultures glutamate uptake and EAAT3 surface expression decrease after treatment with H89, a PKA inhibitor. The H89-mediated decrease in glutamate uptake was counteracted by pre-treating cells with forskolin, a PKA activator[54].

As mentioned earlier, EAAT3 is expressed at GABAergic terminals and glutamatergic postsynaptic sites, but there are few studies that examined the functional regulation of EAAT3, and these studies have primarily looked at the postsynaptically

localized EAAT3. To our knowledge, no functional studies of EAAT3 at GABAergic terminals have been conducted.

REGULATION OF EAAT3 ACTIVITY BY PRESYNAPTIC RECEPTORS AT GABAERGIC TERMINALS: METABOTROPIC GLUTAMATE RECEPTORS AND OPIOID RECEPTORS

Metabotropic glutamate receptors (mGluRs) have multiple effects on interneurons through their actions on somata and axon terminals. In general, group I mGluRs (mGlu1 and mGlu5) are located on somatodendritic compartments, and group II/III mGluRs are located on presynaptic terminals, although there are many exceptions[55]. Presynaptic mGluRs on inhibitory terminals are activated by glutamate that is released from neighboring excitatory synapses[56]. When extracellular glutamate levels are sufficient to reach transporters on inhibitory terminals, it is likely that presynaptic mGluRs would be activated as well. mGlu1 agonists activate PKC in hippocampal pyramidal cells[57], but the signaling pathways activated in interneurons are not known. Interestingly, group I agonists are generally pro-convulsant *in vitro* and in animal models[58]. Investigation of possible regulation of EAAT3 activity on GABAergic neurons by mGluRs may provide important insights into the endogenous signaling mechanisms underlying a crosstalk between excitatory and inhibitory neurotransmission.

Opioid receptors are members of the superfamily of G-protein-coupled receptors that utilize inhibitory G-proteins ($G_{i/o}$). After $G_{i/o}$ are stimulated by opioid receptors, multiple effectors are activated including adenylyl cyclase and mitogen-activated protein kinase[54, 59]. Also, activation of $G_{i/o}$ leads to inhibition of cAMP production and PKA activity[60]. Inhibition of PKA has been associated with decreases in glutamate uptake and glutamate transporter surface expression levels by neurons[50]. Of the three well-characterized opioid receptors (Mu-, Delta-, and Kappa-opioid receptors), Mu-opioid receptor (MOR) and Delta-opioid receptor (DOR) are highly expressed in cortex[61, 62] and hippocampus[63-65]. While it seems that DOR is expressed by both excitatory and inhibitory neurons[66], MOR is preferentially expressed in axonal and somatodendritic compartments of GABAergic neurons in the hippocampus. In addition, both MOR and DOR localize to GABAergic neurons in dissociated cortical and hippocampal cultures[67].

Recently, it was shown in EAAT3-expressing *Xenopus* oocytes that co-expressing increasing amounts of DOR decreased glutamate uptake and EAAT3-mediated currents. In addition, DOR and EAAT3 can be co-immunoprecipitated and co-localized in both *Xenopus* oocytes and in rat cultured hippocampal neurons, suggesting a direct interaction between EAAT3 and DOR. Activation of DOR with pre-treatment of [D-Pen2,5]-enkephalin (DPDPE), a DOR agonist, counteracted the reduction in glutamate uptake and EAAT3-mediated current in *Xenopus* oocytes, and co-localization in both *Xenopus* oocytes and hippocampal neurons[68]. It is possible that DOR inactivates EAAT3 by its direct interaction and when DOR is stimulated by DPDPE, EAAT3 is released and allowed to increase its activity. This is further indication that EAAT3 could be regulated by G-protein coupled receptors. In addition, strong evidence suggests that protein kinase B (Akt), a downstream target of PI3K, regulates EAAT3 activity[69]. Akt is a downstream target of DOR in T cells making DOR a strong candidate for the regulation of EAAT3[70]. Whether MOR also regulates EAAT3 activity has not been investigated, however, its selective expression by GABAergic neurons suggests the possibility that, if it does, it may provide a signaling mechanism to selectively regulate EAAT3 on inhibitory neurons. Therefore, from a potential therapeutic perspective, MOR may be the most interesting candidate for the regulation of glutamate transporter activity in GABAergic neurons.

CONCLUSIONS

This review highlights the importance of the regulation of neurotransmitter GABA metabolism. In order to make progress towards the development of novel therapeutic targets for inhibitory neurotransmission, candidate therapeutic targets at GABAergic terminals, such as EAAT3, must be studied. Through EAAT3's possible regulation by signaling cascade molecules and specific receptors, the activity of EAAT3 could be manipulated in order to alter glutamate uptake, GABA synthesis, and ultimately, inhibitory neurotransmission. Additionally, regulatory points in GABA synthesis, such as GAD and vGAT, which can adjust GABA levels and alter inhibitory neurotransmission, need to be further explored.

REFERENCES

1. Bak LK, Schousboe A and Waagepetersen HS (2006). The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem*. **98** (3): 641-653.
2. Liang SL, Carlson GC and Coulter DA (2006). Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci*. **26** (33): 8537-8548.
   A recent study that shows glutamine transporters play a role in the synthesis of neurotransmitter GABA under certain stimulation protocols.
3. Mathews GC and Diamond JS (2003). Neuronal glutamate uptake Contributes to GABA synthesis and inhibitory synaptic strength. *J Neurosci*. **23** (6): 2040-2048.
   Physiological study, which shows glutamate

uptake by neurons leads to increased GABA synthesis.

4. Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA and Rothstein JD (2002). A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. *J Neurosci*. **22** (15): 6372-6379.
This study shows that knocking down EAAT3 yields an epileptic phenotype, which might be associated with decreased GABA levels in the hippocampus.
5. Fricke MN, Jones-Davis DM and Mathews GC (2007). Glutamine uptake by System A transporters maintains neurotransmitter GABA synthesis and inhibitory synaptic transmission. *J Neurochem*. **102** (6): 1895-1904.
6. Hsu CC, Davis KM, Jin H, Foos T, Floor E, Chen W, Tyburski JB, Yang CY, Schloss JV and Wu JY (2000). Association of L-glutamic acid decarboxylase to the 70-kDa heat shock protein as a potential anchoring mechanism to synaptic vesicles. *J Biol Chem*. **275** (27): 20822-20828.
7. Jin H, Wu H, Osterhaus G, Wei J, Davis K, Sha D, Floor E, Hsu CC, Kopke RD and Wu JY (2003). Demonstration of functional coupling between gamma -aminobutyric acid (GABA) synthesis and vesicular GABA transport into synaptic vesicles. *Proc Natl Acad Sci U S A*. **100** (7): 4293-4298.
In addition to the finding that GABA synthesis is coupled to vGAT, this study shows that GABA that is newly synthesized from glutamate is taken up into synaptic vesicles preferentially over pre-existing GABA.
8. Engel D, Pahner I, Schulze K, Frahm C, Jarry H, Ahnert-Hilger G and Draguhn A (2001). Plasticity of rat central inhibitory synapses through GABA metabolism. *J Physiol*. **535** (Pt 2): 473-482.
9. Jensen K, Chiu CS, Sokolova I, Lester HA and Mody I (2003). GABA transporter-1 (GAT1)-deficient mice: differential tonic activation of GABAA versus GABAB receptors in the hippocampus. *J Neurophysiol*. **90** (4): 2690-2701.
10. Meldrum B (1982). Pharmacology of GABA. *Clin Neuropharmacol*. **5** (3): 293-316.
11. He Y, Janssen WG, Rothstein JD and Morrison JH (2000). Differential synaptic localization of the glutamate transporter EAAC1 and glutamate receptor subunit GluR2 in the rat hippocampus. *J Comp Neurol*. **418** (3): 255-269.
12. Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N and Kuncl RW (1994). Localization of neuronal and glial glutamate transporters. *Neuron*. **13** (3): 713-725.
13. Mackenzie B and Erickson JD (2004). Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. *Pflugers Arch*. **447** (5): 784-795.
14. Bröer A and Brookes N (2001). Transfer of glutamine between astrocytes and neurons. *J Neurochem*. **77** (3): 705-719.
15. Danbolt NC (2001). Glutamate uptake. *Prog Neurobiol*. **65** (1): 1-105.
16. Hertz L (2004). Intercellular metabolic compartmentation in the brain: past, present and future. *Neurochem Int*. **45** (2-3): 285-296.
17. Peng L, Hertz L, Huang R, Sonnewald U, Petersen SB, Westergaard N, Larsson O and Schousboe A (1993). Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. *Dev Neurosci*. **15** (3-5): 367-377.
18. Masson J, Darmon M, Conjard A, Chuhma N, Ropert N, Thoby-Brisson M, Foutz AS, Parrot S, Miller GM, Jorisch R, Polan J, Hamon M, Hen R and Rayport S (2006). Mice lacking brain/kidney phosphate-activated glutaminase have impaired glutamatergic synaptic transmission, altered breathing, disorganized goal-directed behavior and die shortly after birth. *J Neurosci*. **26** (17): 4660-4671.
A surprising finding in this study was that the glutamine-glutamate pathway to recycle excitatory neurotransmitter does not seem to be as crucial as was expected for excitatory neurotransmission.
19. Fricke MN, Jones-Davis DM and Mathews GC (2007). Glutamine uptake by System A transporters maintains neurotransmitter GABA synthesis and inhibitory synaptic transmission. *J Neurochem*.
20. He Y, Hakvoort TB, Vermeulen JL, Lamers WH and Van Roon MA (2007). Glutamine synthetase is essential in early mouse embryogenesis. *Dev Dyn*. **236** (7): 1865-1875.
21. Asada H, Kawamura Y, Maruyama K, Kume H, Ding R, Ji FY, Kanbara N, Kuzume H, Sanbo M, Yagi T and Obata K (1996). Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. *Biochem Biophys Res Commun*. **229** (3): 891-895.
22. Kash SF, Johnson RS, Tecott LH, Noebels JL, Mayfield RD, Hanahan D and Baekkeskov S (1997). Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci U S A*. **94** (25): 14060-14065.
23. Kash SF, Tecott LH, Hodge C and Baekkeskov S (1999). Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci U S A*. **96** (4): 1698-1703.
24. Heldt SA, Green A and Ressler KJ (2004). Prepulse inhibition deficits in GAD65 knockout mice and the effect of antipsychotic treatment. *Neuropsychopharmacology*. **29** (9): 1610-1619.
25. Wu H, Jin Y, Buddhala C, Osterhaus G, Cohen E, Jin H, Wei J, Davis K, Obata K and Wu JY (2007). Role of glutamate decarboxylase (GAD) isoform, GAD(65), in GABA synthesis and transport into synaptic vesicles-Evidence from GAD(65)-knockout mice studies. *Brain Res*. **1154**: 80-83.
26. Asada H, Kawamura Y, Maruyama K, Kume H, Ding RG, Kanbara N, Kuzume H, Sanbo M, Yagi T and Obata K (1997). Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci U S A*. **94** (12): 6496-6499.
27. Kuwana S, Okada Y, Sugawara Y, Tsunekawa N and Obata K (2003). Disturbance of neural respiratory control in neonatal mice lacking GABA synthesizing enzyme 67-kDa isoform of glutamic acid decarboxylase. *Neuroscience*. **120** (3): 861-870.
28. Ji F, Kanbara N and Obata K (1999). GABA and histogenesis in fetal and neonatal mouse brain lacking both the isoforms of glutamic acid decarboxylase. *Neurosci Res*. **33** (3): 187-194.
29. Fujii M, Arata A, Kanbara-Kume N, Saito K, Yanagawa Y and Obata K (2007). Respiratory activity in brainstem of fetal mice lacking glutamate

decarboxylase 65/67 and vesicular GABA transporter. *Neuroscience*. **146** (3): 1044-1052.
30. Liu GX, Cai GQ, Cai YQ, Sheng ZJ, Jiang J, Mei Z, Wang ZG, Guo L and Fei J (2007). Reduced Anxiety and Depression-Like Behaviors in Mice Lacking GABA Transporter Subtype 1. *Neuropsychopharmacology*. **32** (7): 1531-1539.
31. Liu GX, Liu S, Cai GQ, Sheng ZJ, Cai YQ, Jiang J, Sun X, Ma SK, Wang L, Wang ZG and Fei J (2007). Reduced aggression in mice lacking GABA transporter subtype 1. *J Neurosci Res*. **85** (3): 649-655.
32. Schwartz TL and Nihalani N (2006). Tiagabine in anxiety disorders. *Expert Opin Pharmacother*. **7** (14): 1977-1987.
33. Kalviainen R, Nousiainen I, Mantyjarvi M, Nikoskelainen E, Partanen J, Partanen K and Riekkinen P, Sr. (1999). Vigabatrin, a gabaergic antiepileptic drug, causes concentric visual field defects. *Neurology*. **53** (5): 922-926.
34. Gibson KM, Schor DS, Gupta M, Guerand WS, Senephansiri H, Burlingame TG, Bartels H, Hogema BM, Bottiglieri T, Froestl W, Snead OC, Grompe M and Jakobs C (2002). Focal neurometabolic alterations in mice deficient for succinate semialdehyde dehydrogenase. *J Neurochem*. **81** (1): 71-79.
35. Hogema BM, Gupta M, Senephansiri H, Burlingame TG, Taylor M, Jakobs C, Schutgens RB, Froestl W, Snead OC, Diaz-Arrastia R, Bottiglieri T, Grompe M and Gibson KM (2001). Pharmacologic rescue of lethal seizures in mice deficient in succinate semialdehyde dehydrogenase. *Nat Genet*. **29** (2): 212-216.
36. Cortez MA, Wu Y, Gibson KM and Snead OC, 3rd (2004). Absence seizures in succinic semialdehyde dehydrogenase deficient mice: a model of juvenile absence epilepsy. *Pharmacol Biochem Behav*. **79** (3): 547-553.
37. Crino PB, Jin H, Shumate MD, Robinson MB, Coulter DA and Brooks-Kayal AR (2002). Increased expression of the neuronal glutamate transporter (EAAT3/EAAC1) in hippocampal and neocortical epilepsy. *Epilepsia*. **43** (3): 211-218.
38. Doi T, Ueda Y, Tokumaru J and Willmore LJ (2005). Molecular regulation of glutamate and GABA transporter proteins by clobazam during epileptogenesis in Fe(+++)-induced epileptic rats. *Brain Res Mol Brain Res*. **142** (2): 91-96.
39. Ghijsen WE, da Silva Aresta Belo AI, Zuiderwijk M and Lopez da Silva FH (1999). Compensatory change in EAAC1 glutamate transporter in rat hippocampus CA1 region during kindling epileptogenesis. *Neurosci Lett*. **276** (3): 157-160.
40. Gorter JA, Van Vliet EA, Proper EA, De Graan PN, Ghijsen WE, Lopes Da Silva FH and Aronica E (2002). Glutamate transporters alterations in the reorganizing dentate gyrus are associated with progressive seizure activity in chronic epileptic rats. *J Comp Neurol*. **442** (4): 365-377.
41. Miller HP, Levey AI, Rothstein JD, Tzingounis AV and Conn PJ (1997). Alterations in glutamate transporter protein levels in kindling-induced epilepsy. *J Neurochem*. **68** (4): 1564-1570.
42. Ueda Y, Doi T, Tokumaru J, Yokoyama H, Nakajima A, Mitsuyama Y, Ohya-Nishiguchi H, Kamada H and Willmore LJ (2001). Collapse of extracellular glutamate regulation during epileptogenesis: down-regulation and functional failure of glutamate transporter function in rats with chronic seizures induced by kainic acid. *J Neurochem*. **76** (3): 892-900.
43. Peghini P, Janzen J and Stoffel W (1997). Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. *Embo J*. **16** (13): 3822-3832.
44. Aoyama K, Suh SW, Hamby AM, Liu J, Chan WY, Chen Y and Swanson RA (2006). Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. *Nat Neurosci*. **9** (1): 119-126.
This study shows the role of EAAT3 in glutathione deficiency and in neurodegeneration over time
45. Furuta A, Noda M, Suzuki SO, Goto Y, Kanahori Y, Rothstein JD and Iwaki T (2003). Translocation of glutamate transporter subtype excitatory amino acid carrier 1 protein in kainic acid-induced rat epilepsy. *Am J Pathol*. **163** (2): 779-787.
46. Davis KE, Straff DJ, Weinstein EA, Bannerman PG, Correale DM, Rothstein JD and Robinson MB (1998). Multiple signaling pathways regulate cell surface expression and activity of the excitatory amino acid carrier 1 subtype of Glu transporter in C6 glioma. *J Neurosci*. **18** (7): 2475-2485.
This study shows that EAAT3 can be regulated by at least 2 independent signaling pathways, one involves PKC and the other involves PI3K.
47. Hartmann K, Bruehl C, Golovko T and Draguhn A (2008). Fast homeostatic plasticity of inhibition via activity-dependent vesicular filling. *PLoS One*. **3** (8): e2979.
48. During MJ and Spencer DD (1993). Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet*. **341** (8861): 1607-1610.
49. Levenson J, Weeber E, Selcher JC, Kategaya LS, Sweatt JD and Eskin A (2002). Long-term potentiation and contextual fear conditioning increase neuronal glutamate uptake. *Nat Neurosci*. **5** (2): 155-161.
50. Guillet BA, Velly LJ, Canolle B, Masmejean FM, Nieoullon AL and Pisano P (2005). Differential regulation by protein kinases of activity and cell surface expression of glutamate transporters in neuron-enriched cultures. *Neurochem Int*. **46** (4): 337-346.
51. Gonzalez MI, Kazanietz MG and Robinson MB (2002). Regulation of the neuronal glutamate transporter excitatory amino acid carrier-1 (EAAC1) by different protein kinase C subtypes. *Mol Pharmacol*. **62** (4): 901-910.
Interesting article which shows a direct interaction between EAAT3 and PKCα not just in C6 glioma, but also in neurons.
52. Gonzalez MI, Bannerman PG and Robinson MB (2003). Phorbol myristate acetate-dependent interaction of protein kinase Calpha and the neuronal glutamate transporter EAAC1. *J Neurosci*. **23** (13): 5589-5593.
53. Sims KD, Straff DJ and Robinson MB (2000). Platelet-derived growth factor rapidly increases activity and cell surface expression of the EAAC1 subtype of glutamate transporter through activation of phosphatidylinositol 3-kinase. *J Biol Chem*. **275** (7):

5228-5237.
54. Connor M and Christie MD (1999). Opioid receptor signalling mechanisms. *Clin Exp Pharmacol Physiol*. **26** (7): 493-499.
55. Conn PJ (2003). Physiological roles and therapeutic potential of metabotropic glutamate receptors. *Ann N Y Acad Sci*. **1003**: 12-21.
56. Mitchell SJ and Silver RA (2000). Glutamate spillover suppresses inhibition by activating presynaptic mGluRs. *Nature*. **404** (6777): 498-502.
57. Bashir ZI, Bortolotto ZA, Davies CH, Berretta N, Irving AJ, Seal AJ, Henley JM, Jane DE, Watkins JC and Collingridge GL (1993). Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature*. **363** (6427): 347-350.
58. Wong RK, Bianchi R, Taylor GW and Merlin LR (1999). Role of metabotropic glutamate receptors in epilepsy. *Adv Neurol*. **79**: 685-698.
59. Tso PH and Wong YH (2003). Molecular basis of opioid dependence: role of signal regulation by G-proteins. *Clin Exp Pharmacol Physiol*. **30** (5-6): 307-316.
60. Marinissen MJ and Gutkind JS (2001). G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci*. **22** (7): 368-376.
61. Drake CT and Milner TA (2002). Mu opioid receptors are in discrete hippocampal interneuron subpopulations. *Hippocampus*. **12** (2): 119-136.
62. Drake CT and Milner TA (1999). Mu opioid receptors are in somatodendritic and axonal compartments of GABAergic neurons in rat hippocampal formation. *Brain Res*. **849** (1-2): 203-215.
63. Bausch SB, Patterson TA, Appleyard SM and Chavkin C (1995). Immunocytochemical localization of delta opioid receptors in mouse brain. *J Chem Neuroanat*. **8** (3): 175-189.
64. Ding YQ, Kaneko T, Nomura S and Mizuno N (1996). Immunohistochemical localization of mu-opioid receptors in the central nervous system of the rat. *J Comp Neurol*. **367** (3): 375-402.
65. Mansour A, Fox CA, Burke S, Akil H and Watson SJ (1995). Immunohistochemical localization of the cloned mu opioid receptor in the rat CNS. *J Chem Neuroanat*. **8** (4): 283-305.
66. Commons KG and Milner TA (1997). Localization of delta opioid receptor immunoreactivity in interneurons and pyramidal cells in the rat hippocampus. *J Comp Neurol*. **381** (3): 373-387.
67. Eriksson PS, Hansson E and Ronnback L (1990). Opiate receptors in neuronal primary cultures. *Neuropharmacology*. **29** (9): 799-804.
68. Xia P, Pei G and Schwarz W (2006). Regulation of the glutamate transporter EAAC1 by expression and activation of delta-opioid receptor. *Eur J Neurosci*. **24** (1): 87-93.
Recent study that shows in C6 glioma cells there is a functional interaction between DOR and EAAT3. It is suggested that this interaction might also occur in neurons.
69. Krizman-Genda E, Gonzalez MI, Zelenaia O and Robinson MB (2005). Evidence that Akt mediates platelet-derived growth factor-dependent increases in activity and surface expression of the neuronal glutamate transporter, EAAC1. *Neuropharmacology*. **49** (6): 872-882.
70. Shahabi NA, McAllen K and Sharp BM (2006). delta opioid receptors stimulate Akt-dependent phosphorylation of c-jun in T cells. *J Pharmacol Exp Ther*. **316** (2): 933-939.

FURTHER INFORMATION

Ernesto Solis's Web Page:
http://www.people.vanderbilt.edu/~ernesto.solis

Modulation of Neuronal Differentiation by Dopamine Receptors

Fazal Arain

Brain development is a prolonged process that requires a tight regulation of spatiotemporal events. Neuropsychiatric disorders, like schizophrenia and bipolar disorder have been hypothesized to have a developmental etiology despite the fact that the manifestations of the symptoms of these diseases occur later in life. It has been suggested that developmental perturbation of neuronal architecture in specific regions of the forebrain is responsible for the manifestations of these diseases. Dopamine is a neurotransmitter that appears prior to synaptogenesis and expression of the dopamine system is particularly high in the regions implicated in neuropsychiatric disorders. The receptors for dopamine are classified into two sub-families which trigger unique intracellular signaling cascades and modulation of the physiology of these receptors appear to result in permanent alteration in the morphology of dopaminoceptive neurons. A number of approaches, that include modulation of activity of these receptors by different chemical compounds, altered receptor trafficking and developmental deletion of dopamine receptors, have complemented these conclusions. It appears that modulation of neuronal morphology depends upon the type of dopamine receptor activated, the distinct intracellular signaling cascade triggered and the region of the brain that express these neurons. The cellular mechanisms involved in the modulation of neuronal morphology are still under investigation. Alterations in the intracellular calcium ion concentration upon activation of dopamine receptors could lead to the triggering of down stream signals linked to the modulation of neuronal cytoskeleton and hence neuronal morphology. Studies conducted on receptor-specific modulation of neuronal differentiation could help us better understand the pathophysiology of neuropsychiatric disorders.

Neuroscience Graduate Program, Vanderbilt University School of Medicine, U1205 Medical Center North, Nashville, TN 37232, USA.
Correspondence e-mail: fazal.m.arain.1@vanderbilt.edu.

Brain development is a prolonged and dynamic process that begins in the prenatal period and continues through adolescence[1, 2]. This phenomenon can be appreciated by looking at the examples of spatiotemporal dynamics of dendritic differentiation and synaptogenesis[3]. Therefore these dynamics need to be considered when studying brain development.

Both genetic and environmental factors have profound effects on the formation and integrity of brain architecture. Abnormalities in the development of the brain are linked to neurological and psychiatric disorders, despite the fact that these diseases manifest their symptoms later on in life[4]. It is now believed that abnormalities in the brain architecture occur much earlier then the actual appearance of the symptoms of these diseases[5]. Therefore it can be postulated that the manifestations of these diseases could be the result of convergence of many different developmental pathological processes linked to these diseases[6].

As the brain develops the roles played by neurotransmitters change significantly[7]. In the adult nervous system, neurotransmitters are primarily involved in the transmission of information across synapses[8]. On the one hand, they perform a much more complicated role of modulating the connectivity and subsequent function of different regions of the brain during development[8]. Therefore the perturbation of the biology of neurotransmitters during development could be associated with alterations in the brain architecture and consequently with neurological and psychiatric diseases.

Two areas of the brain, namely medial prefrontal cortex (MFC) and striatum (STR) have been linked to complex brain functions such as cognition[9], motivation and planning etc. These two areas have also been implicated in neuropsychiatric disorders[6, 10, 11]. Therefore the neurotransmitter systems expressed during and after development of MFC and STR, could play a crucial role in the development of these neuropsychiatric disorders.

DOPAMINE: THE NEUROTRANSMITTER

Dopamine is a neurotransmitter that is synthesized in dopaminergic neurons from the amino acid tyrosine via a series of reactions, the rate limiting step of which is mediated by the enzyme tyrosine hydroxylase. The principle dopaminergic fiber systems in the brain include the nigrostriatal (that connects the substantia nigra pars compacta to the dorsal striatum), mesolimbic pathway (that connects ventral tegmental area to the ventral striatum) and the mesocortical pathway (that connects ventral tegmental area to the prefrontal cortex). Receptors for dopamine

are expressed in particularly high concentration in two regions of the brain; namely the MFC and STR. The role of dopamine in these areas has been linked to cognition, reward, learning and movement[12].

Dopamine can exert an excitatory or an inhibitory influence on its target cell by binding to the appropriate receptor subtypes. The action of dopamine is terminated by reuptake into the presynaptic neuron by the action of dopamine transporter; following which it can be either repackaged into vesicles or degraded by the enzymes monoamine oxidase and catechol-O-methyltransferase. Dopamine binds to five distinct receptors called dopamine receptor 1, 2, 3, 4 and 5 (D1-5). These are transmembrane receptors that are coupled to G protein, and hence they belong to G protein coupled receptors (GPCR) family. Based on intracellular signaling mechanisms, subtypes of the coupled G proteins and sequence of homology, dopamine receptors have been classified into two subfamilies; namely the D1-like receptor subfamily (that includes D1 and D5) and D2-like receptor subfamily (that includes D2, D3 and D4)[13]. D1 receptor expressing neurons co-express the neuropeptides dynorphin and substance P, while the D2 receptor expressing neurons co-express enkephlins[14, 15]. The D1-like receptors couple to $G_{\alpha s/olf}$ proteins and the D2-like receptors couple to $G_{\alpha o/i}$ protein. The activation of D1 and D2-like receptor subfamilies trigger intracellular signaling cascades that eventually result in accumulation and depletion of intracellular cAMP respectively. It is reported that there also exists another category of dopamine receptors, namely the D1 and D2 receptor heterodimers, that are coupled to $G_{\alpha q/11}$ protein and are linked to the phospholipase C pathway[16]. The signaling transduction through the $G_{\alpha q/11}$ has been shown to result in the release of calcium ions (Ca^{2+}) from intracellular stores[17].

It is traditionally believed that the D1 and D2 receptors are expressed by distinct neurons. Studies using insitu-hybridization techniques have shown that majority of D1 and D2 receptors are expressed on distinct neurons[18]. Segregation of neurons that express D1 and D2 receptor RNA has also been shown in striatum[18]. On the other hand evidence has been presented negating this view and introducing the idea that D1 and D2 receptor are also co-expressed on the same neurons (most probably as heterodimers). The evidence for physical interaction includes co-immunoprecipitation, western blotting and immunocytochemistry[17, 19]. Evidence has also been presented by studies that used in situ hybridization[20], single cell RT-PCR[18, 21] and electrophysiology[19] to support this model.

Studies have been conducted to define the functional characteristics of D1-D2 heterodimer expressing cells. Using HEK 293T and COS7 cells transfected with D1 and D2 cDNA, it has been shown that co-stimulation via D1 receptor agonist (SKF 81297) and D2 receptor agonist (quinpirole) can result in the stimulation of phospholipase C pathway and an increase in accumulation of intracellular Ca^{2+} ions[17]. Treatment of D1-D2 transfected HEK cells with phospholipase C inhibitor, U71322, was shown to be sufficient to deplete cytosolic Ca^{2+} ions[22]. In fact, it was shown that this Calcium signaling pathway could be blocked at different steps of the pathway, for example by $G_{q/11}$ inhibitor (YM254890), by antagonist of intracellular inositol triphosphate receptors (2-aminoethoxydiphenyl borate) and by depletion of intracellular calcium stores (by thoxydiphenyl borate)[22]. A calcium signal was also generated upon administration of dopamine receptor agonist SKF 83959 (that specifically stimulates the phospholipase C pathway), but it was not as strong as that generated by the co-administration of SKF 83959 and quinpirole. Interestingly, the administration of either SCH23390 (D1 receptor antagonist) or raclopride (D2 receptor antagonist) was sufficient to eliminate the signaling cascades triggered by co-administration of SKF 83959 and quinpirole[22]. These data support the hypothesis that D1/D2 receptor heterodimers are a distinct entity with unique activity profile.

As mentioned earlier, the dopamine system has been shown to be associated with a number of crucial physiological activities. Alterations in the dopamine system have also been implicated in neurological and psychiatric disorders including Huntington's disease, Parkinson's Disease, schizophrenia and bipolar disorder[6, 10, 11]. In fact, drugs used in the treatment of some these disorders target the dopamine system[23, 24]. It must also be appreciated that due to the complexities of these disorders, no single experimental model can address all aspects of these diseases.

TEMPORO-SPATIAL EXPRESSION OF DOPAMINE RECEPTORS

Dopamine is one of the neurotransmitters that appear very early in the developing brain[25]. In fact the expression of dopamine begins prior to synaptogenesis[26]. Dopaminergic neurons are born around the time of telencephalic vesicle formation, from neuroepithelial cells (the cell population from which precursor brain cells are derived)[26]. It has been observed that tyrosine hydroxylase expression (a marker for dopaminergic neurons) is detectable by embryonic day 11 (E11) in mice, E12-13 in rats, E14 in rabbits and E30 in monkeys[27, 28]. Although the expression of dopamine is evident in the developing striatum and cortex, its expression is also seen in close proximity of the neuroepithelial cells[28]. This unique spatiotemporal expression of dopamine

advocates its role as a modulator of neurogenesis. Although numerous studies have provided replicable evidence for the spatiotemporal expression of dopaminergic neurons in different species, the precise estimation of the dynamics of dopamine receptor expression has been a difficult task[29]. Unreliability of antibodies and the nonspecificity of chemical compounds that serve as dopamine receptor agonists and antagonists have contributed to this scenario[29]. Therefore a lot of studies have used alterative techniques such as *in situ* hybridization and RT PCR to define the expression pattern of dopamine receptors[30, 31]. Lately, using laser capture microdissection, it has been shown that the transcripts of dopamine receptors can be detected as early as E12 in mouse brain[29], which coincides with the birth of striatal neurons[32]. It was shown that the most abundant transcript of dopamine receptor during development was of D2[29]. It was also seen that while D1, D2 and D5 receptors show a progressive increase in expression with increasing age, D3 and D4 receptors have an oscillatory expression profile[29].

Following their birth, the dopaminergic neurons extend axonal branches that follow a dorsal trajectory towards the midbrain and then grow ventro-rostrally towards their targets in telencephalon, traversing the diencephalon[33]. At around E17, in rats, dopaminergic axon bundles start to enter the developing striatum and some of them continue to proceed towards the cortex and eventually reach their appropriate targets[33]. It is also worth noting that the trajectory of growing axons and dendrites is influenced by environmental cues (molecules that bind to their appropriate receptors on growth cones; an area present at the tips of axons and dendrites). These environmental cues include ephrins, netrin, semaphorins and slits[33]. In a recent study, the opposing roles of netrin (that binds to DCC/ deleted in colorectal cancer) and slits (that binds to robo) in guiding dopaminergic tract was described[34]. Using a zebrafish model it was shown that the absence of robo2 resulted in deviation of dopaminergic axons towards midline and that this defect was partially restored upon silencing netrin expression with morpholinos in these mutants[34]. These opposite roles of netrin and slit signaling are consistent with *in vitro* models[35]. DCC knockout mouse model has been relatively uninformative in this regard as the homozygous mutants die soon after birth but the heterozygous mice do show an altered dopamine expression pattern in the brain and an abnormal blunt response to amphetamine treatment[36].

Interestingly, modulation of D1 and D2 receptor expressed on GABAergic neurons can alter the migration of these neurons[37]. It was shown that D1 receptor activation caused an increase while D2 receptor activation caused a decrease in neuronal migration[37]. These results were also complemented with findings seen with tissue obtained from D1 and D2 receptor knock out mice[37]. In light of these findings it can be postulated that disruption of the dopamine system during a defined embryonic period can have significant and long lasting effects on neuronal architecture[38] that have functional implications[39].

In the adult brain, dopamine system orchestrates complex spatiotemporal sequence of neural events that allows information from cortex to flow to the basal ganglia and hence modulate complex functions such as motor control, cognition and behavior. The circuitry involved in the motoric function is well defined[40]. In short, the motor and pre-motor cortical areas send excitatory glutamatergic neuronal fibers that synapse predominantly with the medium spiny neurons of the striatum. The medium spiny neurons of striatum also receive dopaminergic input from substantia nigra pars compacta. Of these striatal medium spiny neurons, the ones that co-express substance P and D1 receptors send GABAergic fibers to globus pallidus par interna and hence become a part of the *direct pathway*. On the other hand medium spiny neurons that co-express D2 receptors and enkephlins send GABAergic fibers to globus pallidus par externa and hence become a part of the *indirect pathway*. The direct pathway eventually allows disinhibition of its thalamic and cortical targets while indirect pathway results in their inhibition. It is becoming increasingly evident that these pathways are not only involved in motoric control but also in the control of cognitive functions[40].

EVIDENCE OF DOPAMINE SYSTEM'S INVOLVEMENT IN NEURONAL DEVELOPMENT

As mentioned earlier, in light of its temporal expression pattern, dopamine appears to be a very strong candidate in modulating neuronal differentiation. In fact, there are several lines of evidence that link the dopamine system to neuronal differentiation (**Figure 1**).

1: Stimulation of D1 and D2 receptors has distinct consequences on neuronal morphology: A number of studies designed to investigate the effects of modulating the activity of D1 and D2 receptors on neuronal morphology have been conducted in an *in vitro* model. It has been seen that the application of D1 receptor agonist, SKF 38393, causes a reduction in the extent of neurite outgrowth of neurons derived from MFC[41, 42] while it causes an increase the extent of neurite outgrowth in striatal neurons[43]. Interestingly, these effects can be attenuated by the addition of SCH23390 (a D1 receptor blocker)[41, 43]. On the contrary, addition of quinpirole (a D2 receptor agonist) results in an increase in neurite outgrowth[44] and branching[45] of neurons derived from MFC.

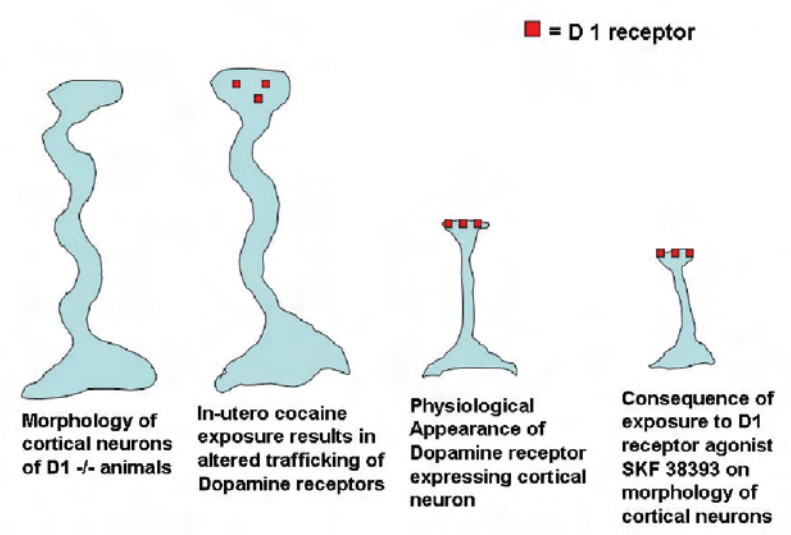

Figure 1. | **Modulation of D1 receptor causes alteration in neuronal morphology.**

Studies conducted on neuronal cell lines transfected with D2-like receptor cDNA also show an increase in neurite length and branching upon exposure to quipirole[46]. The activity of adenyl cyclase, as modulated by D1 and D2-like receptors, has been linked to these changes in neuronal morphology.

D1 receptor agonist SKF83959 that signals through the $G_{\alpha q/11}$ pathway, activates the phospholipase C pathway[16]. Although there is a controversy regarding the precise receptor population whose stimulation results in activation of $G_{\alpha q/11}$ signaling cascade[16, 47], it has been seen that exposure to SKF 83959 results in a robust increase in neurite outgrowth and a marked change in the morphology of neurons derived from MFC (Arain & Stanwood, unpublished observations). Therefore it appears that the neuronal morphology of dopaminoceptive neurons is not just modulated by the type of dopamine receptor stimulated but also by the specific intracellular signaling cascade triggered.

2: Absence of D1 receptor results in increased neurite length: Evidence for the role of dopamine receptor in modulating neuronal differentiation has also been presented using *in vivo* models. As can be predicted from the *in vitro* model (described above) which shows that since the stimulation of D1 receptor causes a decrease in neurite outgrowth, deletion of D1 receptor should have an opposite impact. This hypothesis was supported by studies done in the D1 receptor null mouse model that showed that the dendritic morphology of MFC neurons, was long and tortuous[48]. These finding were not present in the visual or parietal cortices (areas receiving little dopaminergic innervation)[48].

3: Cocaine exposure modulates dopamine receptor trafficking and neuronal morphology: In utero exposure to cocaine is associated with cognitive deficit in children[2]. Cocaine is also an inhibitor of catecholamine transporters[49]. It has been shown that exposure to cocaine during a defined developmental window causes permanent changes in the morphology of dopaminoceptive neurons[42]. These changes include elongation of dendrites and a characteristic "wavy" morphology. These changes are also accompanied by altered D1 receptor trafficking and uncoupling of D1 receptors to G protein[42, 50]. It is suggested that inhibition of dopamine transporter (by cocaine) on the presynaptic membrane results in increased concentration of dopamine in the synaptic space and this scenario is compensated by reduced surface expression of D1 receptors and their uncoupling to $G_{\alpha s}$ protein on the postsynaptic membrane. This model complements the findings seen in D1 receptor null mouse[48]. In both of these experimental models it is seen that the unavailability of functional D1 receptors result in an increase neurite outgrowth and a wavy morphology of MFC neurons. Therefore these findings are consistent with the hypothesis that inactivation (via mal-trafficking or deletion) or activation of D1 receptors has distinct impacts on the neuronal morphology.

MODULATION OF MICROTUBULE-ASSOCIATED PROTEIN-2 (MAP2) PHOSPHORYLATION RESULTS IN ALTERATION OF NEURITE MORPHOLOGY

As mentioned earlier, the stimulation of D1 receptors leads to intracellular accumulation of cAMP which leads to the activation of protein kinase A (PKA)[23]. Microtubule associated protein2 (MAP2) promotes the assembly and stabilization of microtubules[51] (that holds the cytoskeletal structure intact). MAP2 activity is modulated by phosphorylation at a number of its sites by PKA[52] and dephosphorylation by protein-phosphatase 2A (PP2A)[53]. It has been shown that one of the mechanisms involved in altering neurite length in wildtype primary neuronal cultures, following D1 receptor agonist exposure, involves the phosphorylation of amino acid residues in MAP2 by the action of PKA[54]. These findings are consistent with the observations seen in the *in vivo* model of D1 over-expressing transgenic mice[54]. Interestingly, a MAP2 deficient mouse model shows a decrease in microtubule density and a reduction in dendritic length of hippocampal neurons[55]; thus providing yet another evidence for a crucial role of MAP2 in neuronal differentiation.

ROLE OF DOWNSTREAM EFFECTORS OF Ca^{2+} SIGNALS IN NEURONAL MORPHOLOGY

As mentioned earlier, the modulation of dopamine receptors results in alteration of the Ca^{2+} signals. Therefore it is likely that Ca^{2+} signals modulates down stream signaling cascades that eventually results in altering neuronal morphology[56]. One of these down stream signals is the modulation of calcium/calmodulin-dependent protein kinase II (CamKII) activity[57]. It's been shown that constitutive activity of CamKII results in inhibition of dendritic growth, while its inhibition causes an increase in dendritic growth[57]. Furthermore, in another study it was shown that CamKII signaling involves the a number of signaling molecules that activate MAP kinase which goes on to phosphorylate the transcription factor CREB and hence eventually stimulate the transcription of Wnt-2 which directly stimulate dendritic growth[58]. Hence Ca^{2+} signaling seems to be the key architect of neuronal morphology.

CONCLUSION

The temporo-spatial pattern of dopamine system makes it an interesting candidate to study the mechanisms of brain development. The perturbation of dopamine system results in permanent alterations in neuronal morphology, the evidence for which is provided by both *in vitro* and *in vivo* models. This perturbation appears to be modulated by a number of factors that include the expression pattern of dopamine receptor subtypes, the brain region expressing these receptors and the intracellular signaling cascades triggered by them. Alteration of neuronal morphology appears to include both the extent of neurite outgrowth and their branching pattern, which could eventually translate into alteration of neuronal circuitry. Since the changes in dopamine system appear to cause alteration in the brain architecture of regions rich in dopaminoceptive neurons (regions also implicated in the pathophysiology of neuropsychiatric disorders), the dopamine system becomes an obvious candidate to study the developmental etiology of neuropsychiatric disorders. Further studies that target both the expression pattern and the pharmacology of these receptors need to be conducted to define the effects of modulation of dopamine receptors on neuronal morphology.

REFERENCES

1. Levitt P (2003). Structural and functional maturation of the developing primate brain. *J Pediatr.* **143**: S35-45.
2. Stanwood GD & Levitt P (2004). Drug exposure early in life: functional repercussions of changing neuropharmacology during sensitive periods of brain development. *Current Opinion in Pharmacology.* **4**: 65-71.
3. McGlashan TH and Hoffman RE (2000). Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Arch Gen Psychiatry.* **57**: 637-48.
4. Sun D, Stuart GW, Jenkinson M, Wood SJ, McGorry PD, Velakoulis D, van Erp TG, Thompson PM, Toga AW, Smith DJ, Cannon TD and Pantelis C (2008). Brain surface contraction mapped in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. *Mol Psychiatry.* **14** (10): 976-986.
5. Weinberger DR (1995). From neuropathology to neurodevelopment. *Lancet* **346**: 552-557.
6. Lewis DA and Levitt P (2002). Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci.* **25**: 409-32.
7. Buznikov GA, Shmukler YuB and Lauder JM (1999). Changes in the physiological roles of neurotransmitters during individual development. *Neurosci Behav Physiol.* **29**: 11-21.
8. Rice D and Barone S (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect.* **108** Suppl 3: 511-533.
9. Koechlin E, Ody C and Kouneiher F (2003). The architecture of cognitive control in the human prefrontal cortex. *Science.* **302**: 1181-1185.
10. Rubia K, Smith AB, Brammer MJ, Toone B and Taylor E (2005). Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *Am J Psychiatry.* **162**: 1067-1075.
11. Drevets WC, Ongur D and Price JL (1998). Neuroimaging abnormalities in the subgenual prefrontal cortex: implications for the pathophysiology of familial mood disorders. *Mol Psychiatry.* **3**: 220-226, 190-191.
12. Stanwood GD (2008). Protein-protein interactions and dopamine D2 receptor signaling: a calcium connection. *Mol Pharmacol.* **74**: 317-319.
13. Sibley DR and Monsma FJ (1992). Molecular biology of dopamine receptors. *Trends in Pharmacological Sciences.* **13**: 61-68.
14. Greengard P, Allen PB and Nairn AC (1999). Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. *Neuron.* **23**: 435-447.
15. Ingham CA, Hood SH, Mijnster MJ, Baldock RA and Arbuthnott GW (1997). Plasticity of striatopallidal terminals following unilateral lesion of the dopaminergic nigrostriatal pathway: a morphological study. *Exp Brain Res.* **116**: 39-49.
16. Rashid AJ, O'Dowd BF, Verma V and George SR (2007). Neuronal Gq/11-coupled dopamine receptors: an uncharted role for dopamine. *Trends Pharmacol Sci.* **28**: 551-5.
17. Lee SP, So CH, Rashid AJ, Varghese G, Cheng R, Lança AJ, O'Dowd BF and George SR (2004). Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *J Biol Chem.* **279**: 35671-8.
18. Le Moine C and Bloch B (1995). D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J Comp Neurol.* **355**: 418-426.
19. Aizman O, Brismar H, Uhlén P, Zettergren E, Levey AI, Forssberg H, Greengard P and Aperia A (2000). Anatomical and physiological evidence for D1 and D2

dopamine receptor colocalization in neostriatal neurons. *Nat Neurosci.* **3**: 226-30.

20. Le Moine C & Gaspar P (1998). Subpopulations of cortical GABAergic interneurons differ by their expression of D1 and D2 dopamine receptor subtypes. *Brain Res Mol Brain Res.* **58**: 231-236.
21. Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A and Kitai ST (1992). Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proc Natl Acad Sci U S A.* **89**: 10178-10182.
22. Rashid AJ, So CH, Kong MM, Furtak T, El-Ghundi M, Cheng R, O'Dowd BF and George SR (2007). D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. *Proc Natl Acad Sci U S A.* **104**: 654-659.
    This article was chosen because it provides evidence for the existence of functional D1/D2 heterodimers that are associated with Gαq/11 protein. Using both in vitro and in vivo models they show that these receptors possess unique pharmacology. They go on to show that stimulation of these receptors results in triggering signaling cascades that cause activation of CamKII.
23. Missale C, Nash SR, Robinson SW, Jaber M and Caron MG (1998). Dopamine receptors: from structure to function. *Physiological Reviews.* **78**: 189-225.
24. Strange PG (2008). Antipsychotic drug action: antagonism, inverse agonism or partial agonism. *Trends Pharmacol Sci.* **29**: 314-21.
25. Puelles L and Verney C (1998). Early neuromeric distribution of tyrosine-hydroxylase-immunoreactive neurons in human embryos. *J Comp Neurol.* **394**: 283-308.
26. Levitt P, Harvey JA, Friedman E, Simansky K and Murphy EH (1997). New evidence for neurotransmitter influences on brain development. *Trends in Neurosciences.* **20**: 269-274.
27. Levitt P and Rakic P (1982). The time of genesis, embryonic origin and differentiation of the brain stem monoamine neurons in the rhesus monkey. *Brain Research.* **256**: 35-57.
28. Bhide PG (2009). Dopamine, cocaine and the development of cerebral cortical cytoarchitecture: a review of current concepts. *Semin Cell Dev Biol.* **20**: 395-402.
29. Araki KY, Sims JR and Bhide PG (2007). Dopamine receptor mRNA and protein expression in the mouse corpus striatum and cerebral cortex during pre- and postnatal development. *Brain Res.* **1156**: 31-45.
    This paper provides a detailed analysis of spatiotemporal expression of all the five dopamine receptors by quantifying their mRNA expression. The technique used, laser capture microdissection, to collect tissue samples from specific brain areas appear to be a reliable approach for the purpose of these experiments. The estimated mRNA levels also appeared to co-relate with the receptor protein expression patterns.
30. Lidow MS and Rakic P (1992). Scheduling of monoaminergic neurotransmitter receptor expression in the primate neocortex during postnatal development. *Cereb Cortex*. **2**: 401-16.
31. Jung AB and Bennett JP (1996). Development of striatal dopaminergic function. I. Pre- and postnatal development of mRNAs and binding sites for striatal D1 (D1a) and D2 (D2a) receptors. *Brain Res Dev Brain Res*. **94**: 109-20.
32. Ohtani N, Goto T, Waeber C and Bhide PG (2003). Dopamine modulates cell cycle in the lateral ganglionic eminence. *J Neurosci*. **23**: 2840-2850.
33. Van den Heuvel DM and Pasterkamp RJ (2008). Getting connected in the dopamine system. *Prog Neurobiol*. **85**: 75-93.
34. Kastenhuber E, Kern U, Bonkowsky JL, Chien CB, Driever W and Schweitzer J (2009). Netrin-DCC, Robo-Slit, and heparan sulfate proteoglycans coordinate lateral positioning of longitudinal dopaminergic diencephalospinal axons. *J Neurosci*. **29**: 8914-26.
35. Lin L, Rao Y and Isacson O (2005). Netrin-1 and slit-2 regulate and direct neurite growth of ventral midbrain dopaminergic neurons. *Mol Cell Neurosci*. **28**: 547-555.
36. Flores C, Manitt C, Rodaros D, Thompson KM, Rajabi H, Luk KC, Tritsch NX, Sadikot AF, Stewart J, Kennedy TE (2005). Netrin receptor deficient mice exhibit functional reorganization of dopaminergic systems and do not sensitize to amphetamine. *Mol Psychiatry*. **10**: 606-612.
37. Crandall JE, McCarthy DM, Araki KY, Sims JR, Ren JQ, Bhide PG (2007). Dopamine receptor activation modulates GABA neuron migration from the basal forebrain to the cerebral cortex. *J Neurosci*. **27**: 3813-22.
38. Stanwood GD, Washington RA, Shumsky JS and Levitt P (2001). Prenatal cocaine exposure produces consistent developmental alterations in dopamine-rich regions of the cerebral cortex. *Neuroscience*. **106**: 5-14.
39. Stanwood GD and Levitt P (2003). Repeated i.v. cocaine exposure produces long-lasting behavioral sensitization in pregnant adults, but behavioral tolerance in their offspring. *Neuroscience*. **122**: 579-83.
40. Lewis SJ and Barker RA (2009). Understanding the dopaminergic deficits in Parkinson's disease: insights into disease heterogeneity. *J Clin Neurosci*. **16**: 620-625.
41. Reinoso BS, Undie AS and Levitt P (1996). Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. *Journal of Neuroscience Research*. **43**: 439-453.
42. Stanwood GD and Levitt P (2007). Prenatal exposure to cocaine produces unique developmental and long-term adaptive changes in dopamine D1 receptor activity and subcellular distribution. *J Neurosci*. **27**: 152-157.
    This article was chosen because it provides evidence for the modulation of neuronal morphology due to altered dopamine receptor trafficking and uncoupling to G protein as a consequence of prenatal cocaine exposure. They also examine the effects of SKF 38393 (a D1 receptor agonist) on neuronal morphology and provide yet another evidence of how altering the physiology of dopamine receptors can result in permanent alterations in neuronal morphology.
43. Schmidt U, Beyer C, Oestreicher AB, Reisert I, Schilling K and Pilgrim C (1996). Activation of dopaminergic D1 receptors promotes morphogenesis of developing striatal neurons. *Neuroscience*. **74**:

453-460.
44. Reinoso BS, Undie AS and Levitt P (1996). Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. *J Neurosci Res*. **43**: 439-453.
45. Todd RD (1992). Neural development is regulated by classical neurotransmitters: dopamine D2 receptor stimulation enhances neurite outgrowth. *Biol Psychiatry*. **31**: 794-807.
46. Swarzenski BC, Tang L, Oh YJ, O'Malley KL and Todd RD (1994). Morphogenic potentials of D2, D3, and D4 dopamine receptors revealed in transfected neuronal cell lines. *Proc Natl Acad Sci U S A*. **91**: 649-653.
47. Dai R, Ali MK, Lezcano N and Bergson C (2008). A crucial role for cAMP and protein kinase A in D1 dopamine receptor regulated intracellular calcium transients. *Neurosignals*. **16**: 112-123.
48. Stanwood GD, Parlaman JP and Levitt P (2005). Anatomical abnormalities in dopaminoceptive regions of the cerebral cortex of dopamine D(1) receptor mutant mice. *J Comp Neurol*. **487**: 270-282.
49. Ritz MC, Cone EJ and Kuhar MJ (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. *Life Sci*. **46**: 635-645.
50. Jones LB, Stanwood GD, Reinoso BS, Washington RA, Wang HY, Friedman E and Levitt P (2000). In utero cocaine-induced dysfunction of dopamine D1 receptor signaling and abnormal differentiation of cerebral cortical neurons. *J Neurosci*. **20**: 4606-4614.
51. Sanchez C, Diaz-Nido J and Avila J (2000). Phosphorylation of microtubule-associated protein 2 (MAP2) and its relevance for the regulation of the neuronal cytoskeleton function. *Prog Neurobiol*. **61**: 133-168.
52. Goldenring JR, Vallano ML and DeLorenzo RJ (1985). Phosphorylation of microtubule-associated protein 2 at distinct sites by calmodulin-dependent and cyclic-AMP-dependent kinases. *J Neurochem*. **45**: 900-905.
53. Gong CX, Wegiel J, Lidsky T, Zuck L, Avila J, Wisniewski HM, Grundke-Iqbal I and Iqbal K (2000). Regulation of phosphorylation of neuronal microtubule-associated proteins MAP1b and MAP2 by protein phosphatase-2A and -2B in rat brain. *Brain Res*. **853**: 299-309.
54. Song ZM, Undie AS, Koh PO, Fang YY, Zhang L, Dracheva S, Sealfon SC and Lidow MS (2002). D1 dopamine receptor regulation of microtubule-associated protein-2 phosphorylation in developing cerebral cortical neurons. *J Neurosci*. **22**: 6092-6105. **This paper describes the modulation of the phosphorylation state of MAP2 following in vitro exposure to D1 receptor agonists and how it translates into altered neuronal morphology. The authors also report studies conducted on a D1 receptor overexpressing mouse model, that exhibit decreases in apical dendritic length of cortical pyramidal neurons and increases in phosphorylation state of MAP2 protein.**
55. Harada A, Teng J, Takei Y, Oguchi K and Hirokawa N (2002). MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction. *J Cell Biol*. **158**: 541-549.
56. Zheng JQ and Poo MM (2007). Calcium signaling in neuronal motility. *Annu Rev Cell Dev Biol*. **23**: 375-404.
57. McAllister AK (2000). Cellular and molecular mechanisms of dendrite growth. *Cereb Cortex*. **10**: 963-973.
58. Wayman GA, Impey S, Marks D, Saneyoshi T, Grant WF, Derkach V and Soderling TR (2006). Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. *Neuron*. **50**: 897-909.

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