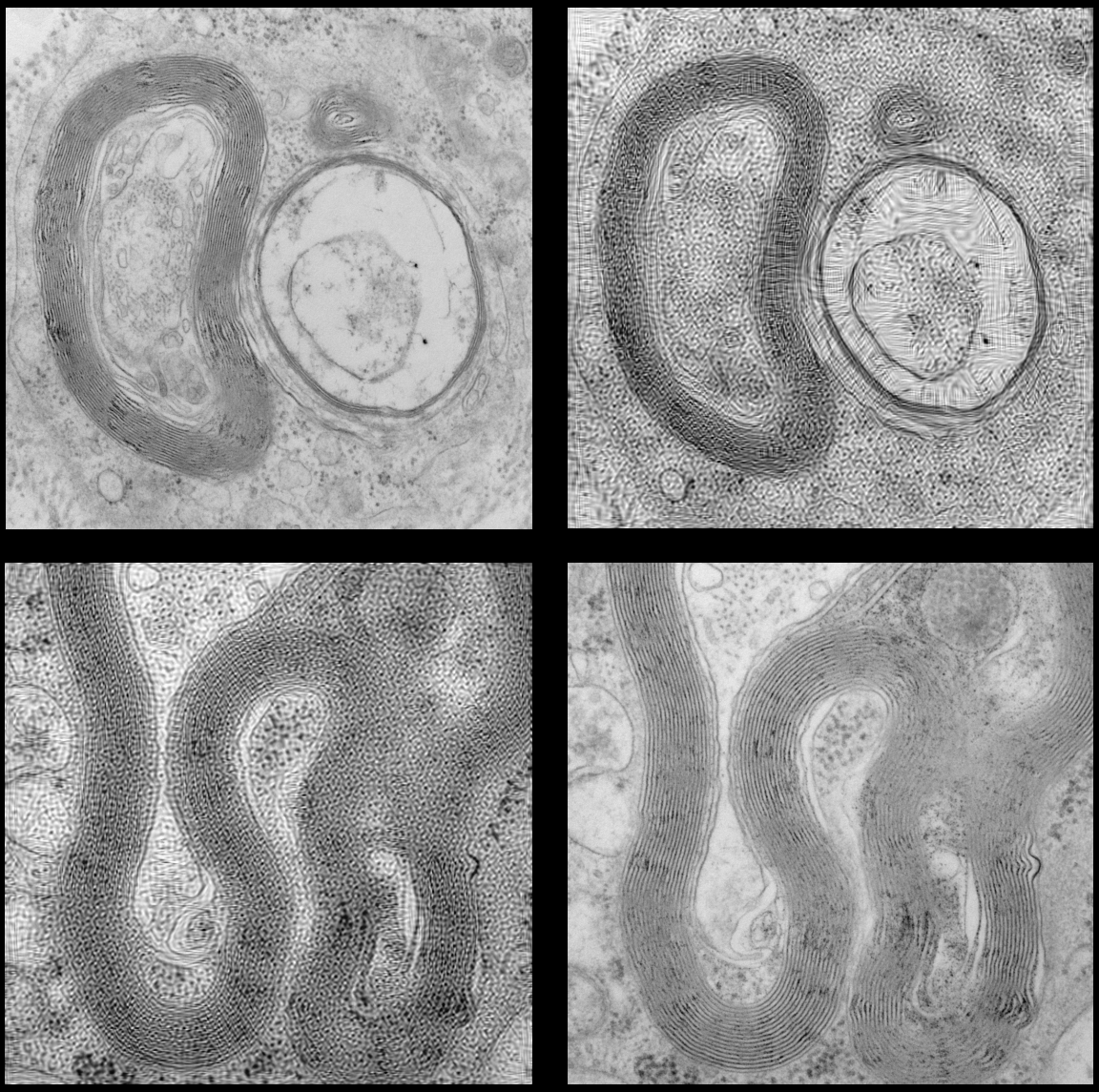
# Vanderbilt *Reviews Neuroscience*



Vanderbilt Brain Institute

Volume **11** | 2019

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(**indicates published manuscripts*)

# LETTER FROM THE EDITORS

Dear friends and colleagues of the Vanderbilt Brain Institute,

It is with great enthusiasm that we present to you the 11th Volume of the *Vanderbilt Reviews Neuroscience* (**VRN**), a journal showcasing the work of the newly-minted class of Ph.D. candidates in the *Neuroscience Graduate Program* (**NGP**). Over the past decade of its existence, the VRN has evolved to reflect the changing needs and wants of our neuroscience community, while still preserving many foundational traditions. Importantly, at its core, the VRN remains trainee-centric, with the contributions and content coming predominantly from current graduate students. Last year [Volume 10, 2018], the VRN welcomed an excellent and inaugural team of *Editor-in-Chief* and *Associate Editors* to compose its superb publishing process. This year, two *Editors* from respective Systems/Cognitive and Cellular/Molecular tracks of the NGP join efforts and assemble interdisciplinary insights into review articles of the current volume, highlighting the strength and accolades of the VBI.

First, we are honored to share with you warm messages and welcoming notes from Dr. Lisa Monteggia (*Director*) and Dr. Bruce Carter (*Graduate Studies*), as well as updates and ongoing efforts provided by the officials of the Neuroscience Student *Organization* (**NSO**). Also, we are privileged to work alongside with an outstanding administrative team, to whom we would like to dedicate our special appreciation and gratitude.

In Volume 11, you will find reviews from the brilliant cohort of doctoral candidates – composing rising scientists entering through the *Interdisciplinary Graduate Program* (**IGP**) or directly from the NGP, as well as promising scholars on the M.D./Ph.D. track via the *Medical Scientist Training Program* (**MSTP**). The breadth of this year's topics is quite exceptional: learning and memory (Collins), network science (Conrad), anxiety and abstinence form alcohol (Flook), environmental adversity and emotional socialization (Nguyen), Huntington's disease (Wilcox), chronic stress exposure (Williford), and neuroscience of numerosity (Yeo).

Aside from capturing the remarkable and insightful lines of research budding among our rising scientists, we highlight the wealth of productivity and accolades from our colleagues, including a number of first-author manuscripts.

We are excited to enjoy the continued success and growth of the NGP and the VBI at large.

Your *Editors*,

Bridget E. **Collins** & Tin Q. **Nguyen**

# MASTHEAD

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*Vanderbilt Review Neuroscience* (**VRN**) is an open-access journal. VRN is the official journal of the Vanderbilt University's *Neuroscience Graduate Program* (**NGP**) and the *Vanderbilt Brain Institute* (**VBI**). VRN is a collection of reviews submitted by the NGP's trainees whilst qualifying for doctoral candidacy. The journal also offers highlights and commentary on neuroscientific research conducted in laboratories at Vanderbilt as well as around the world. VRN was founded in 2009 in an effort to consolidate and recognize the hard work by each class of Ph.D. qualifiers, and is published annually by the VBI.

***Review Process***
Reviews submitted for doctoral qualifications must be approved by a committee of at least four tenured or tenure-track faculty members. Approved reviews accepted by the VRN.



# 2019 Editorial Board

Bridget E. **Collins**
*Cell/Molecular*

Tin Q. **Nguyen**
*Systems/Cognitive*

# OUTREACH + EDUCATION

## A Message from Director of the *Vanderbilt Brain Institute*

We are facing a year of unprecedented crisis, one that is upending people's lives, killing people, causing economic uncertainty, and raising a much needed focus on societal issues. COVID-19 is characterized as a pandemic and is producing once in a lifetime effects on people throughout the world. In these rather extraordinary times we also find ourselves asking questions on systemic racial injustice in the US, racial equity, and diversity as individuals and as scientists. 2020 has been quite a year and it is during this time that the doctoral students in the Vanderbilt Neuroscience Graduate program move forward to candidacy in the program.

The transition of a graduate student from the classroom to the dissertation phase represents the compilation of years of study, the fulfillment of numerous requirements including the publication of a review in the Vanderbilt Reviews Neuroscience (VRN), and the ability to successfully complete examinations by a candidacy committee. The preparation that goes into this process requires perseverance, critical thinking, passion for the field, and the ability to receive and act on constructive criticism. While this process is overall constructive and facilitates a student transition to an independent scientist, the current situation renders it somewhat stressful. Yet, the Vanderbilt graduate students met these challenges with hard work and determination and demonstrated why they are truly outstanding and promising scientists. These graduate students have brought great joy to our program in their scientific research endeavors and the manner in which they contributed to our program.

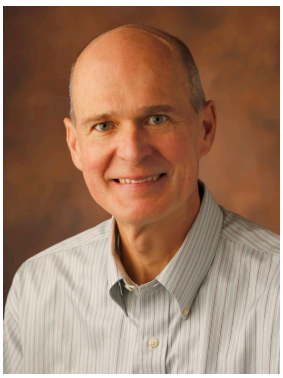
I offer my congratulations to our graduate students for their insight and scholarly aptitude as they present a review in their research area in the VRN. Seeing such talented young investigators thrive in the time of such uncertainty renews our hope about the future of scientific discovery.

Sincerely,

Lisa M. **Monteggia**, PhD
Barlow Family Director of the Vanderbilt Brain Institute

# OUTREACH + EDUCATION

## A Note from Director of the *Neuroscience Graduate Program*



With Lisa Monteggia starting this fall as our new Brain Institute Director, this is a particularly exciting year for the Neuroscience Graduate Program! Lisa brings new ideas and enthusiasm to our program and we look forward to how she will reshape and grow the VBI, including the Neuroscience Graduate Program! We also are very grateful to Ron Emeson for serving as Interim Director. Ron ensured that the program continued to flourish and worked to create a more equitable and balanced system for everyone. Ron consistently served as a champion of equal treatment for all students and we are all very grateful for the battles he's fought on behalf of the program.

It has been another successful year of recruiting new students. We admitted 6 students through the direct admit route and accepted 10 from the IGP and 2 MSTPs. As usual, they represent the cream of the crop and are from a wide variety backgrounds and locations.

As always, our curriculum continues to evolve, with substantial input from the students. Our Fundamentals of Neuroscience course, 8340, was significantly revamped this year under the direction of its new director, Thilo Womelsdorf. He plans for interesting new topics, fewer lecturers and a much more student-engaged approach. We look forward to seeing how this innovative revision develops! The Neuroscience Discussions course is now focusing on statistics, aiming at improving our training on rigor and reproducibility.

The remarkable scientific achievements, the bold leadership and the commitment to service by our students never ceases to amaze me. I am always impressed by the scholarly reviews written by our students for their Qualifying Exam and published in the *VRN*. These reviews serve as a springboard for further high quality publications based on their thesis research. Our students also continue to organize our annual retreat, the Brain Blast outreach program, as well as other activities and events, including running this unique publication (thank you to Tin Nguyen and Bridget Collins for this edition), which they founded. It is a privilege to serve as the Director of Graduate Studies for such a fantastic group of students!

Sincerely,

Bruce **Carter**, PhD

# OUTREACH + EDUCATION

## A Letter from President of the *Neuroscience Student Organization*

It was a prestigious honor to serve as the President of the *NSO* for the 2018-2019 academic year. The goal of the NSO is to uphold long-standing values that entail promoting diversity and inclusion, professional, and academic success of neuroscience graduate students through curricular support, community engagement, and public outreach. None of which, could have been achieved without the collective work of the NSO officers, VBI administration, and Faculty.

I would like to recognize and congratulate this year's neuroscience graduate students for passing the rigorous qualifying exam, and successfully becoming doctoral candidates. Their reviews featured in this year's *VRN* issue, reflects the impressive range of research taking place in the VBI. Many thanks to Bridget Collins and Tin Nguyen for this year's VRN. I would like to extend my gratitude to the *Academic Committee* (Bridget Collins, Elizabeth Flook, Jordyn Wilcox) for preparing students for their qualifying exam, by leading and review sessions and mock exams. And, thank you to the *Curriculum Committee* Resh Gupta and Sierra Palumbos for ensuring the didactic curriculum meets the satisfaction of neuroscience graduate students.

The VBI and NSO achieved exceptional accomplishments. The VBI's commitment to outreach efforts has been made possible thanks to the *Outreach Committee* led by Jacob Ruden, Rachana Nitin, and Kellie Williford. Several successful outreach engagements include: the annual Brain Blast, Neuroscience lectures (VBI, Osher Lifelong Learning Institute), sheep brain dissections at Metro Nashville Public Schools, and the Camp Vandy. I would also like to thank the incredible support by faculty members Rebecca Ihrie, Suzanna Herculano, and Ron Emeson. Tin Nguyen organized and steered this year's VBI Retreat at the Nashville Public Library, which featured Dr. Miguel Nicolelis as the keynote speaker, and new faculty talks by Alan Lewis, Kate Humphreys, Catie Chang, and Ege Kavalali.

Finally, I would like to thank the Barlow Family Director of the VBI, Lisa Monteggia on ensuring a smooth transition of leadership and supporting training efforts in strengthening the VBI. I am thankful to have been a part of the NSO leadership, and to be surrounded by an incredibly talented team of individuals.

Salma **Omer**

# OUTREACH + EDUCATION

## Community Outreach

*Second Harvest Food Bank*


*Campy Vandy*

# OUTREACH + EDUCATION

### A Report from the *Outreach Committee*

Community outreach is a key component of the **VBI**, with the NSO's *Outreach Committee* and the VBI's *Faculty Outreach Committee* forming the core. These committees are dedicated to planning outreach events designed to engage the Nashville community, both adults and children. These events include, but are not limited to, learning activities for children, seminars for adults, and invited talks.

The VBI's biggest outreach event of the year is *Brain Blast* and is held during the annual *Brain Awareness Month* celebration in March. Brain Blast targets children in elementary and middle school and seeks to raise awareness about brain health and disease. This year, the VBI partnered with the *Nashville Public Library* (Downtown) to host Brain Blast. Over **25** VBI-affiliated laboratories sponsored interactive booths that showcased their research and helped children learn about brain function. Over **400** visitors participated in hands-on activities, such as extracting DNA from strawberries, visualizing brain waves using portable EEG machines, dissecting brains, and learning about neurons using animal models.

Throughout the year, the NSO outreach committee actively conducted classroom-based neuroscience series in *Nashville Metro Public Schools*. VBI's faculty, post-doctoral fellows, and graduate trainees volunteered to visit schools during class hours, and led guided, hands-on brain dissections with middle school and high school students through basic neuroanatomy. We also teamed up with *Camp Vandy* (an annual summer camp for kids on Vanderbilt's campus), where we led sheep brain dissections for kids ages 8 and up, and helped the younger campers assemble real-life sheep brain puzzles. Moreover, faculty, post-doctoral fellows, and senior graduate trainees were involved in organizing educational seminars as part of the *Osher Lifelong Learning Institutes*' lecture series. Topics covered included neurophysiology, addiction, interface of technology and the brain, and the neuroscience of mindfulness.

The VBI hopes to continue sponsoring and leading such events and demonstrating the unwavering commitment that its trainees and faculty committees have to Neuroscience outreach. We are dedicated to making science more accessible and fun as an innovative way to gather interests, disseminate knowledge, and engage the community.

Rachana **Nitin**, Kellie **Williford**, and Jacob **Ruden**

# HIGHLIGHTS + BRIEFS

*Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors*

**DiCarlo**, G. E., Aguilar, J. I., Matthies, H. J. G., Harrison, F. E., Bundschuh, K. E., West, A., Hashemi, P., Herborg, F., Rickhag, M., Chen, H., Gether, U., Wallace, M. T., & Galli, A.

Dopaminergic neurotransmission and the components underlying its regulation are instructive to motor activity, motivation, attention, and reward processing. Dopaminergic dysregulation has been linked to a variety of neuropsychiatric disorders including substance use disorder, attention deficit hyperactivity disorder, bipolar disorder, and autism spectrum disorder (**ASD**). Mechanistic insights into how dopaminergic dysregulation leads to associated behaviors could direct development and refinement of therapeutic approaches for these disorders. Gabby DiCarlo, a neuroscience graduate from Dr. Mark Wallace's lab, and her colleagues employed chronoamperometry, voltammetry, and murine behavior to investigate the impact of an ASD-associated dopamine transporter mutation (*DAT T356M*) on striatal dopamine signaling and ASD-associated behaviors.

The DAT T356M mutation, a threonine-to-methionine substitution, was reported in an individual with ASD and is positioned in a transmembrane domain near the transporter's ion binding site. DiCarlo and colleagues modelled DAT T356M by studying mice homozygous for the mutation. Employing chronoamperometry and voltammetry studies in striatal slices, authors were able to examine transporter function and measure tissue-level dopamine metabolism. While the T356M mutant DAT is able to traffic to the membrane and is expressed at normal levels, dopamine reuptake from the extracellular space is reduced, leading to high levels of synaptic dopamine. As a result of this reduced clearance, the DAT T356M mutation drives increased dopamine metabolism and decreased dopamine synthesis in the striatum, which the authors suggest is due to dopamine receptor desensitization.

Following these neurophysiological studies, DiCarlo and colleagues turned to murine behavior to elucidate the behavioral correlates of the DAT T356M mutation. Homozygous mice display a range of abnormal behaviors, some of which correspond to ASD-associated behaviors in humans. Prominent among these is a persistent spontaneous hyperlocomotion; however, repetitive rearing behavior, reduced marble burying, and altered social behaviors are also seen. Interestingly, the increased spontaneous locomotor activity phenotype can be attenuated with administration of a dopamine antagonist, suggesting that this phenotype results from anomalous dopamine efflux rather than reduced dopamine uptake.

In linking the neurophysiological effects of a dopamine transporter mutation with ASD-associated behaviors in a murine model, this work both furthers a molecular understanding of dopaminergic dysfunction and provides clinical insights for neuropsychiatric disorders.

***Read more***:

DiCarlo, G. E. *et al.* (2019). Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. *Journal of Clinical Investigation*, 129(8), 3407-3419.

# HIGHLIGHTS + BRIEFS

*Network Topology of Symbolic and Nonsymbolic Number Comparison.*
**Conrad**, B. N., Wilkey, E. D., Yeo, D. J., & Price, G. R.

Mathematics, and the manipulation of numerical information, draws on individuals' ability to process both symbolic (e.g., Arabic digits; "1, 2, 3") and nonsymbolic (e.g., dots) formats of number representation. More refined understanding of the shared versus unique neural patterns of activity that underlie these formats of number representation may enable more targeted intervention strategies for, e.g., learning difficulties.

To unpack the link between number representation and neural activity, Ben Conrad, a neuroscience graduate, and his colleagues from Dr. Gavin Price's laboratory applied cutting-edge strategies and leveraged functional brain imaging (e.g., ***f*MRI**) and network theory. Functional brain imaging approaches such as *f*MRI allowed Conrad and colleagues to examine the extent to which different formats of number representation (symbolic, nonsymbolic) elicit neural activity across different regions. For instance, central to both symbolic and nonsymbolic number processing is the involvement of the intraparietal sulcus (**IPS**), a region within the parietal cortex implicated in quantity encoding.

Conrad and colleagues extended this notion by applying network theory, and asked as to whether brain regions operate together as systems to underlie, and differentiate, number processing. Notably, the IPS is among the regions within the broader fronto-parietal network, which is thought to support attentional control and cognitive flexibility. It is not altogether surprising, then, that Conrad and colleagues found support for the relations between the fronto-parietal network and both symbolic and nonsymbolic formats of number processing. Though, what distinguishes the two is their additional involvement of the auditory network for symbolic processing, versus the salience network (or cingulo-opercular network) for nonsymbolic processing.

- The engagement of the auditory network, which includes the left superior and middle temporal gyri, during symbolic processing is implicated in the involvement of phonological processing and verbal retrieval of arithmetic fact.
- The salience network, or cingulo-opercular network that includes the cingulate cortex, may operate in parallel with the fronto-parietal network to adaptively meet the cognitive demand during nonsymbolic processing.

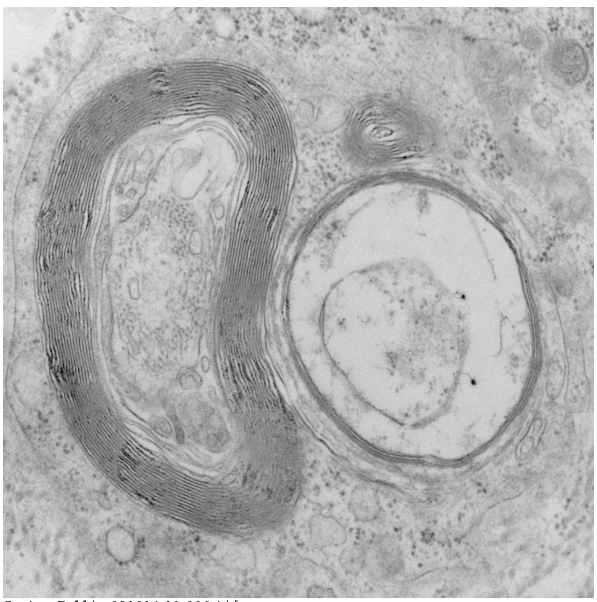
Overall, this study by Conrad and his colleagues elucidates the intricate link between number representation and neural activity, and at the same time, provides nuanced insights for future research and intervention strategies for individuals with learning difficulties.

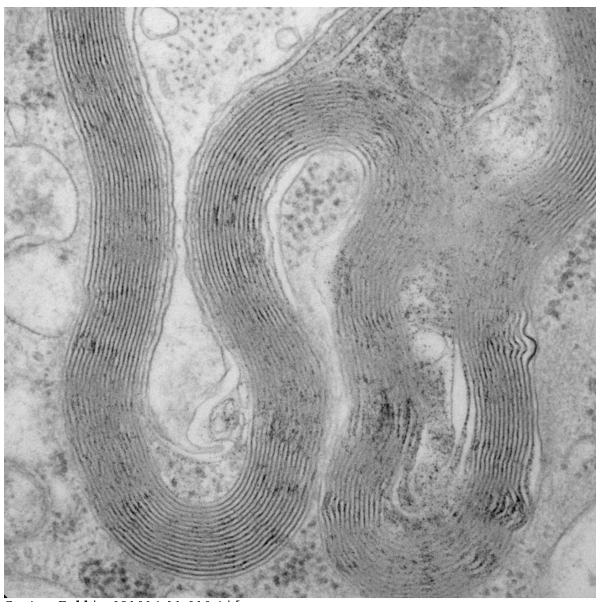
***Read more***:
Conrad, B. N., Wilkey, E. D., Yeo, D. J., & Price, G. R. (2020) Network topology of symbolic and nonsymbolic number comparison. *Network Neuroscience*. doi: 10.1162/netn_a_00144

# ON THE COVER

During myelination, it is estimated that Schwann cell plasma membranes expand 6500-fold during myelination (Webster 1971, *The Journal of Cell Biology*). This number may seem impossible to imagine but using high magnification Electron Microscopy on Sciatic nerve tissues allows us a glimpse at the amazing complexity of the myelin membrane. In the upper left corner, we see an axon surrounded by a perfect ring of compacted myelin enmeshed in the dots and speckles of a no less complex extracellular matrix. However, much as in life, myelination does not always go quite as planned, and in the lower right we can an esthetically pleasing but much less conductively practical example. Encouragingly, all nerves will occasionally have these errors in organization, which may result in a bit of shakiness and blurred focus, but unless the errors overwhelm the successes the signal will be carried on in the nerve regardless.

Rose **Follis**
*Carter Lab*



**CANDIDATE REVIEWS**

# Histone H3 lysine K4 methylation and its role in learning and memory

*Bridget E.* ***Collins***

## Abstract

Epigenetic modifications such as histone methylation permit change in chromatin structure without accompanying change in the underlying genomic sequence. A number of studies in animal models have shown that dysregulation of various components of the epigenetic machinery causes cognitive deficits at the behavioral level, suggesting that proper epigenetic control is necessary for the fundamental processes of learning and memory. Histone H3 lysine K4 (H3K4) methylation comprises one component of such epigenetic control, and global levels of this mark are increased in the hippocampus during memory formation. Modifiers of H3K4 methylation are needed for memory formation, shown through animal studies, and many of the same modifiers are mutated in human cognitive diseases. Indeed, all of the known H3K4 methyltransferases and four of the known six H3K4 demethylases have been associated with impaired cognition in a neurologic or psychiatric disorder. Cognitive impairment in such patients often manifests as intellectual disability, consistent with a role for H3K4 methylation in learning and memory. As a modification quintessentially, but not exclusively, associated with transcriptional activity, H3K4 methylation provides unique insights into the regulatory complexity of writing, reading, and erasing chromatin marks within an activated neuron. The following review will discuss H3K4 methylation and connect it to transcriptional events required for learning and memory within the developed nervous system. This will include an initial discussion of the most recent advances in the developing methodology to analyze H3K4 methylation, namely mass spectrometry and deep sequencing, as well as how these methods can be applied to more deeply understand the biology of this mark in the brain. We will then introduce the core enzymatic machinery mediating addition and removal of H3K4 methylation marks and the resulting epigenetic signatures of these marks throughout the neuronal genome. We next foray into the brain, discussing changes in H3K4 methylation marks within the hippocampus during memory formation and retrieval, as well as the behavioral correlates of H3K4 methyltransferase deficiency in this region. Finally, we discuss the human cognitive diseases connected to each H3K4 methylation modulator and summarize advances in developing drugs to target them.

**Keywords:** *Learning, Memory, Epigenetics, Neuroepigenetics, Histone methylation, H3K4*

# CANDIDATE REVIEWS

***Read more***:

**Collins B.E.**, Greer C.B., Coleman B.C., & Sweatt J.D. Histone H3 lysine K4 methylation and its role in learning and memory. *Epigenetics Chromatin*, **12**:7 (2019).

# Network neuroscience of numerical cognition: A new horizon

*Benjamin N.* ***Conrad***

## Abstract

It has long been presumed that the function of neurobiological systems is a product of distributed, parallel processes, occurring among complex networks of neural tissue[1,2]. Until recently however, the study of network organization in the nervous system has been limited. Advances in the acquisition and modeling of neural data have provided increasingly rich datasets which present new opportunities for understanding neural organization across many levels, from protein and cellular interactions to functional circuits and large-scale brain dynamics. Researchers have increasingly turned to the mathematical framework of graph theory to characterize these data, which allows for the quantification of topological properties in complex networks based on a representation of the connections among constituent units in the system[3]. Graph theoretical measures have been applied in the study of not only biological systems, but other real-world networks including social affiliations among individuals and links between computers over the internet[4]. The application of these tools in neuroscience, dubbed "network neuroscience," holds the promise of unifying our understanding of the relationships between brain structure and function, and information integration and segregation, as well as bridging descriptions of neural data across multiple spatial and temporal scales[5,6]. In this paper we discuss the prospect for application of these techniques in cognitive neuroscience (e.g. see[7] for review), and focus on the particular domain of numerical processing in the brain as a model system. We suggest a new horizon for the field of numerical cognition and argue that network approaches not only complement prior work but offer a novel framework for investigating cognitive mechanisms.

**Keywords:** *Network, Numerical Cognition, Connectivity, Brain Organization, MRI*

# Number Sense

The capacity for the representation and manipulation of numbers in the brain has long been a fundamental topic of interest in cognitive psychology and philosophy. While early theories suggested that the human capacity for number and arithmetic operations was solely a product of human linguistic faculties, a large body of work has demonstrated that number processing is present in many species, including in fish, birds, and other mammals[8]. While these species do not perform complex arithmetic, they do demonstrate a basic concept of number and set size. Furthermore, preverbal human infants have been shown to process numerical quantities[9].

Studies have also shown that many non-industrialized cultures demonstrate no system for exact quantification, but do retain a basic capacity for quantity discrimination in line with many other animals[10]. It has thus become clear that numerical approximation and quantification is, on some level, a primitive and innate faculty of the nervous system. In contrast, the ability for humans to perform higher-level mathematics is not fully explained by these inherited capacities for magnitude processing. Instead, the learning and efficient processing of number symbols (e.g. Arabic digits) is an integral feature of human mathematical cognition, which provides, for instance, an exact reference and notation system by which numbers can be flexibly represented and manipulated. The acquisition of basic numerical literacy thus involves the linking of visual symbols of Arabic numerals to physical quantities and abstract numerosity[11]. Additionally, the human language system may provide a structure for mathematical cognition via verbal representation and associations, such as through counting, rule-based procedures, and learning of arithmetic facts such as multiplication tables. The mapping of numerical quantities and number symbols to spoken number words is also important for early numerical literacy. It is thought that the uniquely human capacity for both numeral processing and reading represent a "recycling" of core brain circuits (such as magnitude and language networks), given the evolutionarily recent appearance of these systems in human cultures[12]. A final note is that domain-general capacities including working memory, fluid intelligence, and processing speed, support numerical and mathematical abilities[13,14]. Neurobiological accounts should strive towards a more unified understanding of how numerical cognition overlaps with and engages associated systems and mechanisms in the brain.

# The Triple-Code Model and Beyond

A prevailing framework for understanding number processing in the primate brain is that of the *Triple-Code Model* (**TCM**), and later refined in the "three parietal circuits" model[15,16]. Put forth by Dehaene and colleagues and motivated by earlier work by McCloskey, this theory postulates that number forms are first processed by early sensory mechanisms through which they are translated into an abstract representation independent of modality[17].

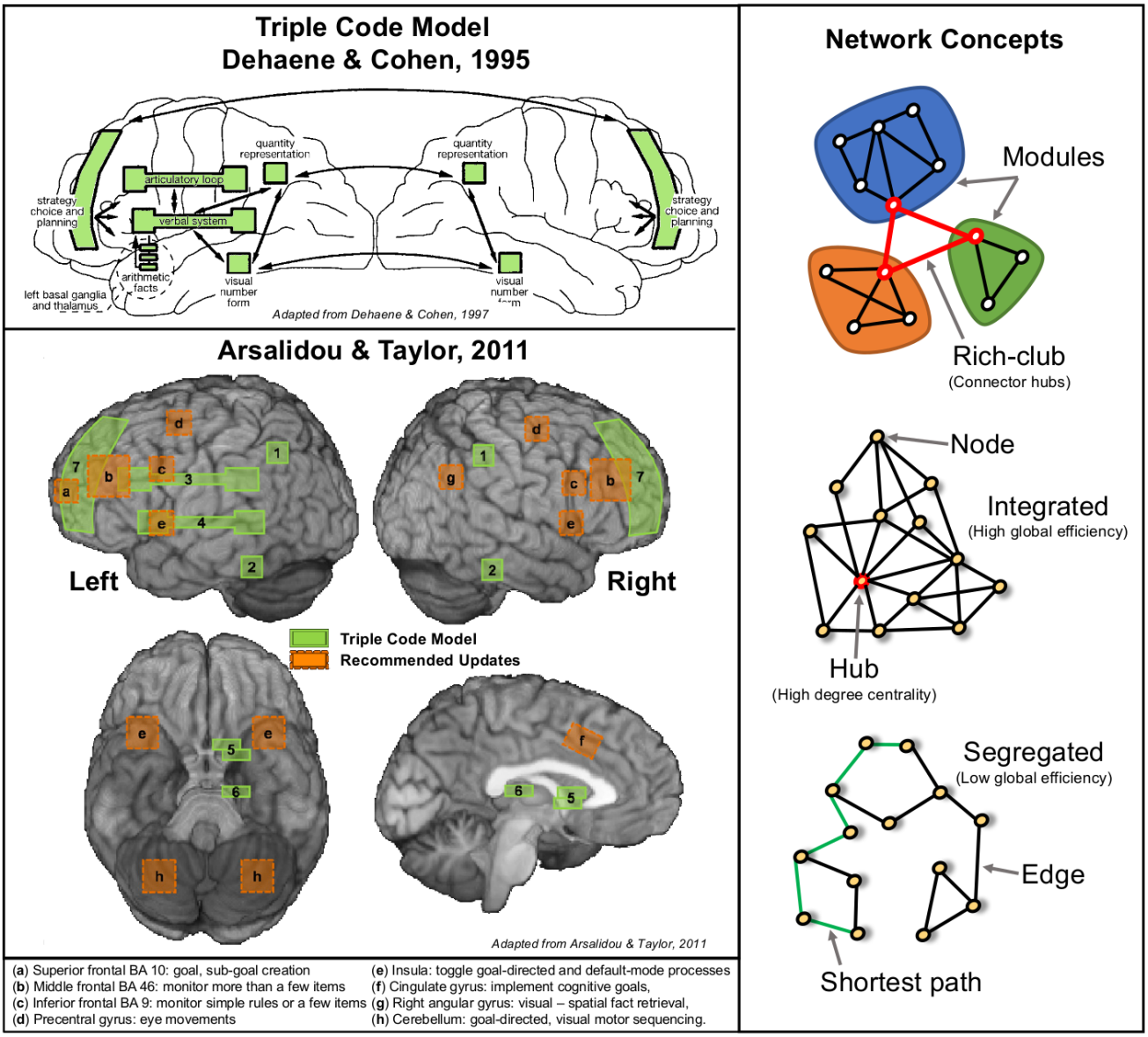



**Figure 1.** The Triple Code Model (**TCM**) of human number processing and calculation; The updated TCM (i.e. TCM+) from Arsalidou & Taylor, based on meta-analysis; Graph theoretical concepts for brain network analysis.

Dehaene hypothesized that three general systems subserve number processing in humans: 1) a quantity code providing an analog magnitude representation involving bilateral parietal areas, 2) a visual code for processing Arabic numerals housed in inferior occipito-temporal regions of the visual ventral pathway, and 3) a verbal code for auditory/linguistic representations of number and operands and utilization of verbal working memory/articulatory loops, housed in the left-lateralized language areas such as superior/middle temporal gyrus and inferior frontal gyrus[16,18]. While originally based on observations from lesion studies, subsequent neuroimaging studies have largely supported the primary components of the model.

Under the TCM framework (**Fig. 1**), the quantity code supports processes such as approximation, estimation, and numerical comparisons and engages the so-called "approximate number system". The intraparietal sulcus (**IPS**) has been particularly implicated as a region involved in quantity processing and semantic representation of numerical magnitude, responding to a variety of numerical stimuli both in the context of explicit number tasks and in passive viewing of number forms[19,20]. Neurophysiological work in macaques has corroborated these findings by demonstrating that a small proportion of IPS neurons are tuned to a particular numerosity, independent of presentation modality[8,21]. Topographically organized maps of numerosity preference have also been described in the parietal lobes, suggesting parietal areas contribute to representations of numerical magnitude[22]. In the ventral occipito-temporal cortex (**vOTC**) proposed to house the visual code, selective responses to number symbols have been shown in both neuroimaging and electrophysiological studies in humans[23,24], with a recent meta-analysis demonstrating at least some convergence across fMRI studies and providing evidence of functional specialization in right vOTC for number symbols[25]. Finally, angular gyrus (**AG**) and left perisylvian language areas have been shown to be involved during symbolic number processing and arithmetic, particularly in the case of addition and multiplication which are thought to tap into rote verbal memory, supporting the TCM's account of a verbal code for number[16,26–29]. The TCM rests on the assumption that the areas involved in the processing of each numerical code have reciprocal functional connections which facilitate the transfer of information as required for a given task. Whether these areas do indeed integrate their information into large-scale functional networks during numerical cognition is an open question which is particularly amenable to functional network analysis.

The TCM focuses on representational encoding of numerical information in higher-order sensory/perceptual and posterior association areas. Dehaene's original formulations did acknowledge that other regions play important roles in numerical cognition, including frontal cortex supporting domain-general "strategy choice and planning" processes, left inferior frontal regions supporting verbal-based "articulatory loops", and left hemisphere cortico-striatal loops supporting arithmetic fact-retrieval[18]. However, the TCM is lacking in its explanation of how numerical "codes" are incorporated and manipulated across

distributed cognitive systems. One consistent finding largely unaccounted for in the TCM is activation in both the lateral and medial prefrontal cortices (**PFC**) during numerical tasks, including from basic magnitude processing to higher-order mathematical thinking. Recent meta-analyses suggest revisions to the TCM regarding the fundamental role of PFC and its various subdivisions in numerical cognition[30–32]. The function of the (lateral) PFC has traditionally been attributed to working memory processes, including short-term storage and manipulation of information. Across many variants of the "*n*-back" working memory task, for instance, where subjects compare a present stimulus to one n-steps back in a sequence of stimuli, lPFC activity increases with increasing working memory load[33,34]. Understandings of the role of lPFC and its subdivisions (e.g. ventral versus dorsal) in cognition have become more nuanced, with lPFC areas now thought to support more than just working memory *per se*, but multiple aspects of cognitive control including response inhibition, top-down attentional control, and rule implementation[35]. The mPFC is also functionally heterogeneous and thought to be involved in goal setting and task maintenance[36]. Findings in the numerical cognition literature suggest PFC neuronal populations subserve a diverse set of domain-general functions, including maintenance and monitoring of multiple items, response selection, and procedural processes, with increasing involvement related to increasing task difficulty[30]. Interestingly, electrophysiological recordings of both lPFC and mPFC neurons in monkeys have revealed selective tuning for particular numerosities with properties similar to those in IPS neurons, suggesting at least some activity in PFC is domain-specific[21,37,38]. This indicates PFC may play an important role not only in manipulation and monitoring, but also representation, of numerical information. In accordance with this account, a recent meta-analysis of number comparison, involving simple judgments of magnitude, and passive viewing tasks, involving no explicit number processing, found consistent activation of mPFC across studies. The authors conclude their results "offer no reason to think that the parietal cortex is more specialized for number than the frontal cortex"[39]. Taken together, findings of diverse PFC involvement expand the framework put forth in the TCM and suggest the existence of a distributed network fundamental to numerical cognition involving both posterior and frontal brain regions.

# Towards a Unification of Functional Segregation and Integration

Since the advent of PET and fMRI several decades ago, cognitive neuroscience has largely followed a localization agenda in which hypotheses are tested regarding neural activity levels[40]. Massively univariate statistical models are used to assess functional specialization, asking at each voxel the same question, is the underlying tissue significantly "active" during the process of interest[41]? Note that this rests on neural activity *level* as the primary dependent variable, i.e. does local activity *increase* or *decrease* within an experimental

context. Experiments are designed to probe activity levels using task manipulations and condition contrasts, for instance to control for shared processing mechanisms via "cognitive subtraction"[42]. Subject activation maps are often carried on to group-level statistical tests to look for common effects across subjects (e.g. at each location, is the relative activity level difference between an experimental and control condition significantly greater than zero across the group?). The results of this endeavor have revolutionized neuroscience by providing an unprecedented mapping of cognitive functions and their spatial segregation across the brain[43]. The vast majority of neuroimaging studies of numerical cognition fall within this framework, purported to reveal "where" numerical information is locally processed in the brain. Importantly, these findings appear to corroborate observations from lesion studies, highlighting the utility of localization information in clinical settings[44]. For instance, a case study showed that a restricted infarct to the left IPS induced deficits in tasks which required processing of numerical quantities[45]. Lesion-deficit findings have been considered to indicate regions necessary for a behavior (i.e. if region *X* is lost then behavior *Y* is lost) and complement functional localization findings which indicate the regions sufficient for a behavior (i.e. if regions *X* and *Y* are active during behavior *Z*, they are sufficient for *Z*). While this framework has been useful, there are problems with both assumptions which highlight fundamental issues in the localization agenda[46,47]. For instance, localization findings are subject to concerns of sensitivity, such that some necessary regions may not reach the significance threshold for activation. And, lesion studies have been particularly challenged by findings of inter-subject variability, degeneracy, and plasticity, where the same cognitive function can be achieved via multiple brain regions or pathways[48].

Another central issue arising from localization work in numerical cognition is the finding of strikingly high overlap of activity maps during numerical tasks compared to maps from other domains. Indeed, IPS and PFC regions are consistently activated across many cognitive tasks that require attentional control (i.e. composing what is generally referred to as the frontoparietal network)[49], calling into question the specificity of these areas for number processing. The IPS, for instance, long held as region housing semantic representations of numerical quantity, is now understood in the numerical cognition field to be involved in a generalized magnitude processing system, including during judgments of physical size, temporal duration, or luminance, rather than selectively tuned to numerical quantity *per se*[39,50,51]. Other work has shown that IPS regions encode abstract representations of behaviorally relevant stimuli unassociated with magnitude information, such as the identity of faces presented at different viewing angles (e.g. Matt Damon versus George Clooney)[52]. To complicate matters further, IPS activity is consistently observed in working memory tasks[33] and has additionally been associated with spatial cueing, visual orienting, saccadic eye movements, shifts in attention, and guiding selection between competing stimuli[49,53–55]. It thus appears that areas most strongly activated during

numerical processing are multi-functional, at least at the spatial scale of fMRI (i.e. a voxel is typically 2-3mm$^3$, conceivably representing a summation of information from 600,000+ neurons)[56,57]. Note that while stimulus-selective neurons have been demonstrated via electrophysiological recordings, they are likely intermixed among neuronal populations tuned for different purposes within a sampled voxel. From this perspective it may be rather unsurprising to observe co-localization of activity across domains, highlighting a fundamental limitation in the interpretation of the voxel-level fMRI signal. However, compared to other noninvasive imaging techniques, the spatial resolution of fMRI affords a relatively high level of specificity in terms of cytoarchitectonic and anatomical location. We may have some confidence, for instance, that a patch of cortex sampled within a voxel has a relatively homogenous profile of afferent and efferent projection targets. Also, a standard fMRI voxel may capture on the order of several cortical columns and hundreds of minicolumns, with the latter considered to act as a fundamental functional unit[58,59], further reducing the potential complexity of a voxel's response profile. Despite limitations in the spatial resolution of fMRI, it is currently the most useful methodology for noninvasively examining both local and distributed cortical function. Thus, the question remains, how can we reconcile the multi-functionality of brain areas?

While there has been a tendency in the neuroimaging literature to attribute a particular cognitive mechanism to a singular brain region, this notion is increasingly becoming outdated[60]. Findings of regional flexibility, such as in the IPS, suggest that a cognitive construct (e.g. the abstract representation of numerosity) is not likely to take place in any one region, but is rather a distributed process involving multiple regions[2]. A view developed by Price and Friston more than a decade ago, the function of a region may be more appropriately conceptualized by considering its diverse set of interactions and patterns of coactivity across many cognitive states[47]. In this view, a brain region serves as a computational unit that performs an operation contributing to a given function but, should not be defined by the function itself[60]. While there are surely biological constraints on a region's so-called "operation-function", it is abstract in the sense that its relation to behavior is a product of context, i.e. the inputs and outputs to/from the region as defined by the dynamic and distributed state of the system. This perspective combines the notions of segregation and integration in the brain[61], and highlights the futility of focusing on localized processing without respect to inter-areal interactions. In the case of numerosity representation, for example, recent work by Harvey et al. demonstrated the existence of multiple topographic maps of numerosity preference in parietal, temporal, and occipital areas[62], suggesting a distributed encoding of quantity which may be differentially engaged depending on particular behavioral demands. We propose that investigating distributed patterns of regional activity and communication, rather than simply levels of local activity, is a critical step forward to understanding the apparent flexibility of brain regions and how they subserve complex cognitive function[63].

These insights provide motivation for a new "information" agenda in cognitive neuroscience which employs alternative methodologies to assess distributed and integrated brain function[40,64]. Methods such as multivariate pattern analysis (**MVPA**), which employ learning algorithms to "decode" cognitive states from fMRI, have been important in driving this agenda forward. MVPA results reveal there is more predictive information in the patterns of activity across many voxels than in the level of activity at a singular location[65]. Recent applications of these techniques, for instance, in studies of language and reading, suggest that both semantic and syntactic processes are more distributed than previous localization results have indicated[66,67]. We suggest that measurements of functional connectivity (**FC**) and in particular, the application of network models to these data, are a part of the same information agenda by providing complementary insights into the functional integration of distributed information.

FC measures examine the statistical relationships between activity in two or more locations in the brain[68]. FC is typically calculated as the correlation or coherence between two vectors of neural data (e.g. voxel-wise fMRI time series, though similar analyses can be performed with ECoG, EEG, or MEG data), with higher FC indicating greater coupling, communication, or information transfer between regions. Statistical independence, i.e. low FC, is interpreted as an absence of functional interaction. While correlated fMRI signals may not indicate direct neural communication, e.g. due to possibility of two regions being driven by a third source[69], FC may be more appropriately considered as a composite of the functional relationships along all anatomical paths between two regions[70]. Importantly, evidence suggests a strong coupling between connectivity patterns measured via fMRI and as measured from direct electrophysiological recordings[71,72]. In the context of numerical cognition, a recent study of epilepsy patients implanted with intracranial electrodes showed strong electrical coupling of neural population in vOTC and IPS regions specifically during simple arithmetic compared to control tasks[73]. These findings provide support for information transfer between components of the TCM, suggesting task-dependent coupling may be observable via connectivity measures derived from fMRI during numerical processing.

The application of FC measures in cognitive neuroscience has largely involved investigation of connectivity between *a priori* regions of interest or from "active" voxel clusters to the rest of the brain, e.g. as defined in univariate contrasts. This approach is crucial for bridging the gap between localization findings and FC information, providing results which may be more readily interpretable within current neurocognitive models such as the TCM. Studies of FC during numerical cognition have followed this approach, providing some significant insights which we review in subsequent sections. Importantly, however, restricting FC analyses to/from singular regions is unnecessarily biased towards the misconceptions of localized function outlined above. A more holistic approach is to

consider FC across many regions within network models and describe these networks in the language of graph theory[5,48]. Graph theory unifies concepts of functional segregation and integration in the brain and has the potential to provide novel, more parsimonious accounts of cognitive function[7]. We now outline this methodology and its recent contributions to cognitive neuroscience, and later consider its application in the field of numerical cognition.

# Network Theory for Cognitive Neuroscience: Concepts and Contributions

The mathematical study of networks, termed graph theory, has a long history spanning back to at least the 18th century. The mathematician Euler is credited with the first graph theoretical proof where he described the impossibility of traversing a path through the city of Konigsberg which crossed each of its seven bridges once and only once[74]. The key contribution was his abstract representation of the problem in terms of a mathematical structure. In today's terms, he had conceived of a *graph* in which the city's land masses served as fundamental units, or *nodes*, and the bridges as connections between units, or *edges* (**Fig. 1**). The number of edges between nodes, i.e. the *degree* of the nodes, was key to his solution and is a feature of a graph's *topology*, i.e. its particular arrangement of connections or organization. In the last several decades, new interests in describing complex systems, from economies and societies to telecommunication links to protein-protein interactions, have driven the development of graph theory-based methods for characterizing such systems and spawned the field of network science[74]. Applications of network theory in neuroscience have only recently become feasible thanks to methodological advancements in measuring the nervous system and its connectivity. In particular, MRI provides an unprecedented ability to non-invasively measure the structure and function of the living brain with high anatomical specificity. MRI-based connectivity measurements can be considered in the context of a larger endeavor in neuroscience to create comprehensive diagrams of brains across multiple scales and modalities, i.e. to characterize *connectomes*[75]. In this regard, network science holds particular promise as a parsimonious framework through which brain networks of different spatiotemporal scales may be unified and, will undoubtedly advance our understanding of nervous system complexity[76].

## *Functional Connectivity in Task Versus "Rest"*

Network approaches for understanding the functional topology of the brain during cognitive tasks have received relatively little attention compared to their application in resting-state FC data. Resting-state FC refers to correlations in spontaneous fluctuations in fMRI signals in the absence of a specific task, i.e. while subjects are simply laying in a scanner. The resting-state *connectome* is thought to reveal the intrinsic functional architecture of the

brain and, strong overlap has been noted between connectivity maps observed during rest (e.g. as defined via independent component analysis) and activation patterns derived from task data, such as among motor, auditory, visual, and frontoparietal networks[77–79]. On the other hand, topologies supporting higher-order cognitive functions may be seldom engaged at rest. For example, the putative visual word form area (**pVWFA**) is a region near the vOTC which is engaged during reading, forming a network with left SMG, AG, ITG, and IFG regions[80]. During resting-state, however, pVWFA was shown to have almost no connectivity to reading-related regions and instead demonstrated strong connectivity with the dorsal attention network which includes IPS areas[81]. This suggests functional networks dynamically reorganize to support cognition and highlights the necessity of studying task-based FC[70]. A further limitation of resting-state fMRI is that ongoing activity during rest reflects a potential myriad of cognitive states (e.g. mind-wandering, planning one's day, sleep, etc.) which are not controlled between subjects. These states, as revealed by self-reports, have been shown to systematically alter FC patterns and thereby confound interpretation of FC network properties and comparisons across individuals[82]. In other words, while resting FC patterns may reveal generalized functional organization, researchers should be cautious in interpreting individual differences from resting data. A recent network-based analysis showed that differential connectivity patterns could be observed between a sensorimotor task, movie-watching, and rest, and that these differences interacted with age, suggesting that inducing the cognitive state of interest may be particularly important for accurately characterizing functional network development[83]. Another study found changes in global functional organization scaled with complexity in a reasoning task, with significant alterations compared to rest[84]. In light of such findings, the utility of resting-state fMRI for questions in cognitive neuroscience has been intensely criticized[85,86].

Methods for assessing FC during cognitive tasks are available and should instead be preferred for modeling functional networks in cognitive neuroscience. One simple approach is to look at connectivity during active periods of a traditional block paradigm[87]. It has been pointed out that removal of coactivations effects may be desirable, since similar activation profiles may not indicate interaction *per se*, and thereby suggest correlation over residual time series after removing modeled task effects[88–90]. Other principled approaches for estimating task-modulated FC, which can also be applied in event-related data, include generalized psychophysiological interaction analysis[91] and beta-series regression[63,92]. Though originally developed for looking at task-related FC from pre-defined regions of interest, these methods have recently been extended for whole-brain network analyses[93,94]. These variants in measuring task-related FC may require subtle differences in interpretation of the resulting networks, highlighting the importance of careful consideration in regards to network construction[95]. Described in detail elsewhere, the choice of region parcellation and treatment of negative FC values are also important methodological considerations for

functional network analysis[3,7,96]. We now outline several examples of network metrics which have been applied in cognitive neuroscience.

## *Global Efficiency*

As described previously, the dichotomy of functional integration and segregation in the brain has been a central topic of debate in cognitive neuroscience. In network theory, the degree of integration across a network can be explicitly defined using the concept of shortest path length (**Fig. 1**), which describes the minimum number of steps, or edges, that occur between two nodes[3]. Global efficiency refers to the average inverse shortest path across all pairs of nodes in the network, and has been suggested to describe the overall capacity of information transfer between regions[97]. The interpretation that global efficiency in FC networks measures information flow *per se* is complicated by the potential for indirect anatomical connection between regions (see above). Nevertheless, this measure has been applied in a growing number of studies and has revealed important insights into cognitive function. It has been shown, for instance, that global efficiency in both functional[98] and white-matter structural networks correlate positively with IQ[99] and these findings have been taken to support an information efficiency hypothesis of intelligence[100]. Work using task-based FC has shown efficiency within a large group of frontoparietal, visual, salience, and subcortical regions increased with increasing reasoning complexity, and efficiency positively correlated with accuracy on the task[84]. Another study looking at emotional and motivational processing found increased efficiency in task-related networks in response to threat and reward compared to safe and control trials, respectively[101]. Furthermore, MEG studies employing n-back tasks have shown increased global efficiency with increasing cognitive load and in higher performers, as well as impairment in schizophrenics[102,103]. Taken together, global efficiency measures appear to track a large-scale, dynamic property of cognition which relates to individual differences in behavior. A final point to note is that global network properties are nonspecific in the sense that the same result may arise from different underlying topologies. Combination with local metrics is therefore necessary for more mechanistic interpretations[7].

## *Modular Organization and Hubs*

One principle of brain organization which has received considerable attention is modularity. In the context of network theory, a module refers to a highly connected community of nodes which demonstrate relatively weaker connectivity to the rest of the network[104]. Modular structure (**Fig. 1**) is observed in nearly all complex systems. In the brain, this feature is thought to have evolved to support resilience (i.e. since local perturbations are less likely to disrupt the system) and to minimize the biological costs associated with wiring/maintaining electrical conductions over long distances[105]. Modularity algorithms attempt to cluster or

partition networks into non-overlapping communities, providing a data driven methodology for assessing functional subnetworks in the brain. This information can be used to look for organizational differences in modular structure across cognitive states as well as within/between module interactions[106]. A recent study showed that, during an *n*-back task, greater flexibility and integration among several frontal cortex-based modules related to greater working memory performance and neuropsychological test scores[107], suggesting a potential domain-general mechanism for cognitive flexibility and executive control. Similar results have demonstrated reorganization of frontoparietal communities across tasks[108] and suggested fluid intelligence is related to higher global connectivity of lPFC[109]. An interesting application by Bassett et al. looked at longitudinal changes in modular organization as subjects gradually learned simple visual-motor tapping sequences over six weeks. Results demonstrated progressive segregation of the visual and motor modules over learning, and that release of connectivity from regions involved in top-down cognitive control predicted faster learning[110]. Other examples of modularity-based analyses have shown decreased segregation between modules during remembered versus forgotten trials in an episodic memory task[111] and, during conscious versus unconscious awareness of a visual target[94].

A defining feature of real-world, modular networks is the existence of hub nodes. These nodes share many more connections than other nodes in the network, with edge counts approximately following a power-law distribution[112]. Centrality measures are used to describe a node's importance in the network. For instance, degree centrality is a simple count or sum of weights of a node's edges. Other centrality measures are employed to capture the overall importance of a node in the network, such as betweenness centrality which quantifies the number of shortest paths involving a node[3]. In brain networks, centrality metrics have identified a small group of highly connected hub regions, referred to as the "rich-club," which are thought to facilitate integration across modular communities (Fig.1)[113]. Lesions to these connector hubs are particularly detrimental and result in widespread deficits across multiple cognitive domains[114]. Some have suggested that interactions among rich-club regions support a "global workspace" necessary for higher-order cognition[115]. Centrality metrics have also recently been applied to study visual search mechanisms. Higher centrality of regions within the frontoparietal network and lower centrality of subcortical regions during task processing associated with higher performance[93]. A study by Tomasi et al. employed a visual tracking task and found strong deactivation of the precuneus, a rich-club hub and region commonly deactivated in task paradigms[116]. Interestingly, the authors showed this was accompanied by widespread reductions in global connectivity from visual, language, and prefrontal areas irrelevant to the task, and that these reductions correlated with better task performance[116]. The authors suggest hub node deactivations may have distributed effects on information transfer among distant areas, and perhaps are crucial for reducing interference during specialized modular processing. This study provides a salient example of how (de)activation and connectivity

metrics are both dissociated and potentially complementary indices of brain function.

In summary, preliminary applications of complex network analyses in task-based neuroimaging studies suggest that large-scale, dynamic interactions support cognitive function and are observable at the global level as well as among regional communities. The existence of hub nodes and rich-club organization provides a novel framework for understanding regional roles within brain networks and may provide new insights for interpreting localization results with respect to distributed processing. Importantly, individual differences in network topologies are both functionally and behaviorally relevant. Network theory thus presents new challenges and opportunities for cognitive neuroscience. In the following section we outline some potential applications of these methods in numerical cognition.

# A Few Prospects for Network Analyses in Numerical Cognition

## *Complex Arithmetic and Functional Integration*

The TCM details a core network of brain areas supporting number processing and arithmetic in humans. Arsalidou et al. recently expanded this model (which we refer to as the TCM+) to include additional regions, particularly multiple PFC areas, based on findings of a meta-analysis of activation foci from studies involving number and calculation tasks (Fig.1)[30,32]. This model suggests that complex mental calculation such as the performance of multi-step arithmetic problems requires recruitment of nearly all regions of this system, e.g. visual symbol processing, quantity representation, goal/subgoal creation and implementation, monitoring of multiple items, etc. Recent findings suggest that increased functional integration (i.e. higher global efficiency) is observed with increasing complexity during reasoning tasks, particularly among frontoparietal and cingulo-opercular networks such as are included in the TCM+[84,117]. Higher levels of integration among these systems were shown to relate to performance. We speculate that complex arithmetic may also demonstrate this property, such that higher global efficiency across TCM+ regions relates to calculation ability. Interestingly, other findings may provide support for this hypothesis. For instance, one study showed math-gifted students demonstrate stronger, more bilateral activation of frontoparietal regions compared to controls while performing a fluid reasoning task (Raven Progressive Matrices)[118]. Another demonstrated increased frontoparietal connectivity during mental rotation in math-gifted adolescents compared to controls[119]. And finally, a recent study found that professional mathematicians have stronger activation across a widespread network largely corresponding to the TCM+ while listening to meaningful (versus meaningless) math statements compared to control subjects of equal academic standing[120].

## *Differential Pathways for Basic Operations*

It is thought that simple arithmetic operations engage different functional systems, such that addition and multiplication are more verbal-retrieval based whereas subtraction engages quantity processing[28]. Recent work suggest comparisons of network topologies during basic arithmetic tasks may provide further mechanistic insight into these differences. For instance, a study by Park et al. found stronger connectivity from a right parietal seed to left and right IPS during subtraction versus addition, with stronger connectivity relating to faster reaction times[121]. Importantly, univariate activation levels were not predictive of performance in this study. In a recent paper by Yang et al., dynamic causal modeling was used to assess connectivity during subtraction and addition in a small set of regions including bilateral IPS, bilateral caudate, and several regions in bilateral PFC[122]. Results showed that subtraction involved increased connectivity across this system, particularly among bilateral IPS, whereas addition was left-lateralized with weaker connectivity overall. These findings suggest differential connectivity patterns underlie arithmetic operations. Based on these results we may predict retrieval of rote-arithmetic facts involves a more segregated network architecture compared to engagement of the IPS-mediated quantity processing system. Simple assessment of degree distributions may reveal more bilateral connections during subtraction and left-lateralization for addition, as well as verbal-fact retrieval in general.

## *Number Processing Network Development*

A driving motivation for studying numerical cognition is the fact that numeracy, or one's ability to access and apply basic numerical and mathematical concepts, is critically important for functioning in modern life[123]. A significant proportion of individuals (i.e. as much as a fifth of adults) fail to achieve an adequate level of numeracy and there is growing appreciation that poor numerical skills represent a significant burden on society[124–126]. Despite a long tradition of empirical research in educational and cognitive psychology looking at numeracy development, the persistent achievement gap in numerical skills among the general population warrants investigation from new perspectives. In particular, development of numeracy skills in early childhood is strongly related to future mathematical abilities[127–130]. A mechanistic, neuroscientific understanding of numerical cognition and its development has the potential to help characterize individual differences in achievement and inform remediation practices.

Network analyses of functional architecture over development have revealed a transition from strong short-range connections towards increased long-distance connections over childhood[131,132]. This pattern is associated with enhanced segregation and local clustering of regional communities still observable at 5 years old, with a shift to a more distributed and integrated topology by late adolescence[133,134]. Additionally, while rich-club organization is

observed in structural networks in young children, functional connectivity between rich-club hubs undergoes a more protracted development[135]. These observations come from resting-state FC studies, likely reflect general trends in brain maturation, but may say little about functional topologies engaged during higher-order cognition. As an example, Vogel et al. found no differences in the resting-state modular organization of reading-related regions between children, adolescents, and adults[136]. Instead these regions are functionally segregated into distinct networks early on and remain so over development.

Studies of functional activation during numerical processing have revealed that PFC activity levels undergo significant change over development, involving a trajectory of decreasing engagement of PFC from childhood to early adulthood during basic magnitude and arithmetic processing, with concomitant increases in parietal activity[137–140]. This so-called "frontal-to-parietal shift" is thought to reflect a decreasing reliance on domain-general processing as parietal representations of number become more specialized and efficient[141]. A subsequent meta-analysis of this literature found no evidence of PFC involvement during number comparison in children, citing the fact that previously reported PFC locations were highly variable across studies[141], making the frontal-to-parietal shift a controversial topic in the field[142]. However, recent results from a longitudinal study report strong evidence for decreasing task-based connectivity between left lPFC and bilateral IPS during an arithmetic task in 8-14 year olds, along with increased connectivity among bilateral IPS and with vOTC[143]. Stronger task-based connectivity among bilateral IPS correlated with math ability at all ages, and importantly, activity levels were unrelated to the observed effects. This motivates revisiting the frontal-to-parietal shift in younger children during comparison tasks with a focus on task-based connectivity networks, such as to assess segregation of FPN or reductions in PFC centrality. Furthermore, these findings suggest task-evoked network topologies may be more dynamic and behaviorally relevant than those observed in resting-state data. Longitudinal studies employing task-based fMRI and network theory are thus particularly well suited to reveal novel mechanisms of cognitive development.

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**CANDIDATE REVIEWS**

# Anxiety during abstinence from alcohol: A systematic review of rodent and human evidence for the anterior insula's role in the abstinence network

*Elizabeth A.* ***Flook***

## Abstract

Alcohol Use Disorder (AUD) is a chronic, relapsing disease that impacts almost a third of Americans. Despite effective treatments for attaining sobriety, the majority of patients relapse within a year, making relapse a substantial barrier to long-term treatment success. A major factor contributing to relapse is heightened negative affect that results from the combination of abstinence-related increases in stress-reactivity and decreases in reward sensitivity. Substantial research has contributed to the understanding of reward-related changes in AUD. However, less is known about anxiety during abstinence, a critical component of understanding addiction as anxiety during abstinence can trigger relapse. Most of what we know about abstinence-related negative affect comes from rodent studies which have identified key brain regions responsible for abstinence-related behaviors. This abstinence network is composed of brain regions that make up the extended amygdala: the nucleus accumbens (NAcc), the central nucleus of the amygdala (CeA), and the bed nucleus of the stria terminalis (BNST). More recently, emerging evidence from rodent and human studies suggests a fourth brain region, the anterior insula, might be part of the abstinence network. Here, we review current rodent and human literature on the extended amygdala's role in alcohol abstinence and anxiety, present evidence for the anterior insula's role in the abstinence network, and provide future directions for research to further elucidate the neural underpinnings of abstinence in humans. A better understanding of the abstinence network is critical toward understanding and possibly preventing relapse in AUD.

**Keywords:** *Abstinence, Addition, Insula, Anxiety, Amygdala*

***Read more:***

**Flook E.A.**, Luchsinger J.R., Silveri M.M., Winder D.G., Benningfield M.M., & Blackford J.U. Anxiety during abstinence from alcohol: A systematic review of rodent and human evidence for the anterior insula's role in the abstinence network. *Addict Biol*. e12861 (2020).

# Commentary: Dimensionality in environmental adversity, mechanisms of emotional socialization, and children's characteristics and cognitive growth – a reflection on Miller at al. (2020)

*Tin Q.* ***Nguyen***

## Abstract

Disentangling the dimensionality in environmental adversity offers nuanced insights at both theoretical and practical levels, such as the ways that disadvantaged socioeconomic circumstances during childhood development may contribute to adolescent psychopathology. Miller and colleagues (2020) provide evidence into how early *deprivation* and *threat* may exacerbate later psychopathology. Yet, how certain factors in this early environment differentially facilitate children's cognitive and socioemotional growth may modulate the severity of later psychopathology. In this commentary, we reflect on the promising evidence offered by Miller and colleagues and extend additional considerations regarding academic growth, cognitive abilities, and protective environmental factors.

**Keywords:** *Development, Environment, Cognition, Psychopathology, Academic Growth*

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**Nguyen, T. Q.**, & Cutting, L. E. (2020). Commentary: Dimensionality in environmental adversity, mechanisms of emotional socialization, and children's characteristics and cognitive growth – a reflection on Miller et al. (2020). *Journal of Child Psychology and Psychiatry*, https://doi.org/10.11111/jcpp.13260.

# Manganese deficiency in Huntington's Disease

*Jordyn* ***Wilcox***

## Abstract

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease resulting from an expanded CAG repeat in the *Huntingtin (HTT)* gene translating to an expanded poly-glutamine (polyQ) tract in the huntingtin protein (HTT). The hallmark neuropathological sign is dysfunction and eventual death of striatal medium spiny neurons. Symptoms typically develop mid-life and manifest as hyperkinetic involuntary movements, psychiatric disturbances, and cognitive decline. An inverse correlation exists between age of symptom onset and number of CAG repeats, but the high variability in this trend supports a compelling role for additional genetic and environmental disease modifiers. A gene-environment interaction between HD and the essential micronutrient manganese (Mn) has been recently identified, such that mutant HTT confers a selective resistance to Mn toxicity. Though toxic in excess, Mn is crucial for development and serves as an essential co-factor for several enzymes regulating urea cycle metabolism, neurotransmitter synthesis, and antioxidant status. There is accumulating evidence supporting a deficiency in bioavailable Mn in HD. Certain HD phenotypes have also been rescued by Mn supplementation. The mechanisms that underlie this HD-Mn interaction have not been fully elucidated. This review discusses the current evidence for, and against, a role for Mn deficiency in the presentation of HD pathophysiology. Research that aims to further understand the mechanism of this gene-environment interaction will be a necessary and valuable tool for modifying age at onset (AO) and disease progression in this currently incurable disease.

**Keywords:** *Huntington's Disease, HTT, Manganese, Deficiency, Age at Onset*

# Introduction

Huntington's Disease (**HD**) is an autosomal dominant neurodegenerative disease resulting from an expanded cytosine-adenine-guanine (**CAG**) repeat in the *Huntingtin (**HTT**)* gene. The disease is fully penetrant when ≥40 CAG repeats are present in at least one allele of the *HTT* gene, which translates to an expanded poly-glutamine (**polyQ**) tract near the N-terminus of the mutant Huntingtin protein (**mHTT**)[1,2]. Changes in mood, cognitive decline, and chorea are hallmark symptoms that typically manifest mid-life (< 50 years of age) with a median survival of 18 years following symptom onset. There is no cure and current treatment, such as tetrabenazine, focuses on alleviating symptoms with no effect on progression of the disease[3,4]. *HTT* is ubiquitously expressed throughout all tissues and life stages, but expression of mutant *HTT* (***mHTT***) primarily causes dysfunction and atrophy of GABAergic medium spiny neurons (**MSN**) of the striatum through currently unknown mechanisms[5–7].

The precise function of wild type (**WT**) huntingtin protein (**HTT**) is uncertain. It is essential for development, demonstrated by embryonic lethality observed in mice homozygous null for *Htt*[8], and broadly necessary for neural maintenance[9,10]. Its large size (348 kDa) and the presence of several HEAT (Huntingtin, Elongation factor 3, protein phosphatase 2A, and TOR1) repeats have deemed it a general scaffolding protein and a "protein-protein interaction hub"[9,11–13]. Through its interactions with nearly 200 proteins12,14, HTT plays a role in vesicle trafficking and axonal transport[15–17], transcriptional regulation[18–21], autophagy[22–24], and cell survival[8,21,25]. Many of these functions and protein-protein interactions, particularly those related to transcription regulation and cell signaling, depend on the non-expanded polyQ tract (< 35 repeats) present in WT HTT[3,26,27]. When mutated, the elongated polyQ tract not only interferes with normal HTT function but also leads to the formation of toxic cytosolic and nuclear protein aggregates[13,17,28–30].

Though somewhat controversial, there is evidence to suggest that HD pathology is caused by the combined loss of WT HTT function and toxic gain of function exerted by mHTT aggregates[2,7,12,31,32]. This could, in part, explain why HD manifests as a neurodegenerative disease with mid-life onset despite continuous HTT expression throughout development. Reduced WT HTT function results in abnormal neuron development, and could subsequently render these cells more vulnerable to mHTT aggregate toxicity; neurodevelopment and neurodegeneration are not mutually exclusive[33]. For example, striatal MSNs rely on brain-derived neurotrophic factor (**BDNF**) produced by cortical neurons to be delivered via axonal transport, a process impaired by mHTT[16]. Throughout the aging process, mHTT aggregates accumulate in all cell types but particularly vulnerable striatal cells atrophy, contributing to signs and symptoms of the disease[6,33,34]. The clinical symptoms of HD include cognitive, psychiatric, and motor impairments. Cognitive

deficits present as executive dysfunction, difficulty multi-tasking, memory loss, and difficulty learning. Psychiatric disturbances include depression, apathy, suicidal ideation, anxiety, irritability, and agitation. Motor impairments present differently depending on the stage of the disease. Early on, chorea is typical, while in late-stage HD rigidity, dystonia, and dyskinesia are prevalent. Cognitive and psychiatric symptoms often precede motor symptoms, though this is often identified in hindsight. Manifest HD is defined by the point in time when characteristic motor symptoms develop[35].

# Age at Onset: Genetic and Environmental Contributions

Age at onset (**AO**) is largely determined by the length of *HTT* CAG repeats, such that a longer repeat length is associated with younger AO[36–39]. Interestingly, CAG repeat length is also negatively correlated with AO in other polyQ diseases, including the spinocerebellar ataxias[40]. Innate toxicity of expanded polyQ proteins has been demonstrated by the development of neurodegenerative phenotypes from the insertion of a large CAG repeat into an arbitrary mouse gene[41]. However, CAG repeat length only accounts for 70% of the total variance in HD AO[42]. In patients with repeat lengths in the 40-50 range, AO can vary by several decades between two individuals with the same number of CAG repeats. The remarkable variability in AO and the anatomical selectivity of neuronal dysfunction despite ubiquitous *HTT* expression suggest a pivotal role for environmental and genetic modifiers in the manifestation and progression of HD[43–45].

After accounting for expanded CAG repeat length, the remaining variance in AO is attributable to other genes (~40%) and environmental factors (~60%)[46]. Variants of genes coding for proteins involved in glutamatergic neurotransmission, energy metabolism, and autophagy have been shown to delay or accelerate AO[42,47–49]. Interestingly, CAG repeat length of the non-expanded allele has no influence on AO[36,42]. Environmental factors such as cognitive and sensorimotor stimulation, physical exercise, and caloric restriction have been identified as positive modulators that may delay AO[50–53]. Conversely, high caffeine intake has been associated with earlier AO[54].

Environmental exposures to transition metals also influence disease progression and possibly AO. In mouse models of HD, neonatal (but not adult) iron supplementation potentiates HD phenotypes[55,56]. Dysregulation of iron homeostasis in HD is well-documented although not well-understood[57]. In addition to increased iron, elevations in copper and zinc have been detected in post-mortem HD brains, but it is unknown if these exposures influence AO[58,59]. In a screen for gene-environment interactions between HD and neurotoxic metal exposures, a specific neuroprotective effect of *mHTT* against manganese (**Mn**) toxicity was revealed[60].

Following this discovery, evidence from *in vitro* and *in vivo* HD models as well as patient studies has accumulated in support of a bioavailable Mn deficiency in HD. *This review discusses the evidence for, and against, a role for Mn deficiency in the presentation of HD pathophysiology*. Of particular interest is the role that Mn deficiency may play during early development in the progression of disease later in life, reinforcing the idea that neurodevelopment and neurodegeneration are closely linked. Further understanding the mechanisms of the HD-Mn interaction beyond what is examined in the present review will be pivotal for modifying disease manifestation and progression, impacting the lives of those afflicted by this disease.

# Mn is an Essential Micronutrient

Mn is particularly crucial for nervous system health as a cofactor for enzymes regulating neurotransmitter metabolism, urea cycle metabolism, and antioxidant status. Brain Mn levels exhibit regional enrichment in human caudate, putamen, and globus pallidus suggesting these cells may have a greater requirement for Mn to function optimally[61]. Adequate Mn is obtained from the diet, absorbed in the gastrointestinal tract, and excreted via the hepatobiliary system. Whole grains, nuts, leafy greens, and legumes are excellent sources of Mn[62,63]. Though essential, excessive Mn is a potent neurotoxin and causes a Parkinsonian-like motor condition known as manganism. Mn intoxication usually occurs by inhalation of Mn-containing dust and is more common among welders and miners[64]. It is nevertheless crucial for cells to maintain proper Mn homeostasis using highly regulated mechanisms to balance its essential role as a co-factor but also minimize toxicity, especially during development[61,62,64–66].

## *Mn in Development*

Developing infants and children require more Mn than adults[65]. During the rodent neonatal period, brain Mn is regulated in a temporal and region-specific manner, suggesting a distinct requirement throughout this developmental stage[67]. This critical period has been referred to as the "brain growth spurt" and an especially vulnerable period to nutritional manipulations[68]. Under normal dietary conditions, lifetime striatal Mn accumulation is highest at postnatal day (**P**) 5 in rodents. Overall Mn concentrations continue to rise until P17, and then sharply decline. Neonatal mice do not excrete Mn until P17, explaining the high accumulation up to this point. Under conditions of excess Mn exposure, neonatal rats are most sensitive to overall brain Mn accumulation from P5 to P22. Therefore Mn toxicity among neonates can be a concern[69]. The temporal and regional regulation of Mn concentrations during the early postnatal period imply a critical and sensitive role for Mn during neural development. If Mn homeostasis is not properly maintained during this period, there could be developmental consequences that impact disease onset later in life.

Experimental Mn deficiency during development has not been implemented in humans. However, epidemiological studies have observed that blood Mn levels in children show a biphasic dose-response relationship with neurodevelopment. Low blood Mn concentrations, which may or may not be indicative of brain Mn concentrations, were correlated with diminished mental development[70]. At 12 months-of-age, children in the lowest and highest quintile of blood Mn concentrations obtained the lowest scores on the Mental Development Index[71]. This relationship between blood Mn levels and mental development did not persist once the children were 24 months of age. It is unknown if low Mn levels detected in these children at 12 months yielded long-lasting effects as they matured into adults. Nevertheless, appropriate Mn homeostasis during the developmental period is essential to prevent negative outcomes in adulthood. The potential for Mn dyshomeostasis during development to contribute to HD phenotypes in adulthood is discussed in a later section of this review.

The genetic mutation in HD confers specific resistance to Mn toxicity[60]. This trait could be beneficial under neurotoxic exposure scenarios; however, it concomitantly results in decreased accumulation, potentially during a critical developmental period. It is currently unknown if mHTT decreases brain Mn levels during neonatal development *in vivo* but postmortem human brains as well as *in vitro* and adult *in vivo* models of HD exhibit distinct alterations in Mn biology.

# Mn and Huntington's Disease

## *Mn Transport and Homeostasis*

Several lines of evidence point towards a defect in Mn handling in HD, however, the mechanisms are not well understood. This gap in knowledge can partially be attributed to the fact that Mn transport and homeostasis are an active area of research. Few selective Mn transporters have been identified, and how the body maintains appropriate Mn concentrations without impacting levels of other divalent metals is not clear[61]. Upon ingestion, Mn is absorbed through the gastrointestinal tract and enters the bloodstream through an unknown mechanism. Mn crosses the blood brain barrier via active transport by a variety of proteins. The major Mn uptake transporters are divalent metal cation transporter 1 (**DMT1**) and transferrin (**Tf**), which transports trivalent Mn (**Mn3+**)[62]. DMT1 preferentially transports Mn, but also transports cadmium, iron, lead, cobalt, nickel, and zinc[72]. Interestingly, there are no differences in either DMT1 mRNA expression in the blood of HD patients compared to age-matched controls nor alterations in DMT1 protein levels in HD cell models[73,74]. Cellular uptake of Mn3+ occurs through transferrin receptor (**TfR**)-mediated endocytosis of Tf-bound Mn3+, which is subsequently reduced to Mn2+ by ferrireductase in the endosome. Tf effectively transports Mn3+ but has a higher affinity for trivalent iron (**Fe3+**)[75]. Decreased Tf levels have been reported in cell and mouse models of HD, perhaps as a compensatory response to the increased iron accumulation that is

observed in HD[58,73,76]. Despite the shared transporter systems between Mn and Fe, one study has shown hat a defect in Mn homeostasis was not due to alterations in the iron transport system[73].

Mn export is mediated by the selective efflux transporter SLC30A10[77]. This transporter is highly expressed in brain and liver. In liver, it plays a role in the hepatobiliary excretion of Mn. Mutations in SLC30A10 are associated with decreased Mn excretion and consequently hypermanganesemia[78,79]. There are currently no published data reporting *SLC30A10* expression in HD patients or HD models, but this would be interesting to measure given the role of this efflux transporter in maintaining optimal cellular Mn levels. *SLC30A10* may be upregulated in brain or liver, resulting in decreased brain accumulation and increased biliary excretion. While DMT1, Tf, and SLC30A10 are some of the major Mn transporters, dozens of other proteins are involved in the complex regulation of cellular Mn homeostasis[61].

## *Mn Dyshomeostasis in HD*

There is substantial evidence that Mn homeostasis is disrupted in HD. An *in vitro* model of HD (immortalized mouse striatal line ST*Hdh*Q111/Q111 with 111 CAG repeats) shows significantly decreased sensitivity to Mn toxicity measured by cell survival assays compared to wild type (**WT**) cells of the same striatal origin (ST*Hdh*Q7/Q7)[60]. Resistance to toxicity can likely be accounted for by an impairment in Mn accumulation following exposure in these cells. Consistent with decreased accumulation, *STHdh*Q111/Q111 cells show a basal deficit in Mn. An *in vivo* model of HD [FVB-Tg(YAC128Q) mice expressing full-length human *mHTT* with 128 CAG repeats], also displays a striatal specific defect in Mn accumulation following exposure compared to WT mice at 12 weeks of age[60]. YAC128Q mice also exhibit a deficit in striatal bioavailable Mn, demonstrated by decreased *ex vivo* basal activity of the Mn-dependent enzyme arginase II (Arg2) that was corrected under *ex vivo* Mn-exposed conditions[73,80]. Post-mortem data indicate cortical Mn concentrations are also reduced in HD patients[58]. Further, HD models show blunted responses to Mn exposure compared to WT. *In vivo* (YAC128Q mice), *mHTT* suppresses Mn-induced decreases in dopamine (DA) concentration and arginase II (*Arg2*) mRNA levels[80,81]. Mn-dependent increases in S473-phosphorylated Akt (p-Akt), T308-pAkt, phospho-ATM(S1981), and phospho-p53(S15) are significantly reduced *in vitro* (ST*Hdh*Q111/Q111 cells and HD human neuroprogenitors[60,82]. These perturbations in Mn homeostasis impact many cellular processes and likely contribute to HD pathophysiology.

# Mn-Dependent and Mn-Responsive Processes are Disrupted in HD

Mn is a necessary cofactor for many cellular processes impaired in HD, such as urea cycle homeostasis, regulation and recycling of glutamate, redox status, and energy metabolism. At least one specific Mn-dependent metalloprotein from each of these cellular functions is altered in HD including arginase, glutamine synthetase (**GS**), Mn superoxide dismutase (**MnSOD**), and pyruvate carboxylase, respectively[62,83]. Arginase and GS activities are significantly altered in HD while reported changes in MnSOD or pyruvate carboxylase are varied[80,84,85]. HD-related changes and the role of Mn in urea cycle homeostasis, glutamate clearance and recycling, redox status, and energy metabolism will be discussed below.

## *Urea Cycle Homeostasis*

The urea cycle is an important physiological process for removing toxic nitrogenous waste that is generated from amino acid catabolism. Two of the enzymes in the partial urea cycle found in the brain are Mn-dependent enzymes: arginase and agmatinase. Alternations in arginase have been identified in HD. Arginase hydrolyzes arginine to ornithine and urea with a specific catalytic requirement for Mn[86,87]. Enzymatic activity of arginase II (**Arg2**), the mitochondrial specific isoform expressed in all tissues, is reduced with concomitant elevation of select urea cycle metabolites (citrulline, arginine and ornithine) in the striatum of prodromal YAC128Q mice; total striatal Arg2 protein levels become significantly reduced in aged YAC128Q mice[80,88–90]. Urea cycle perturbations have also been documented in postmortem human brain tissue and a prodromal HD sheep model (OVT73), presumably due to changes in Arg2 activity but this has not been directly addressed in these studies[91–95]. Urea cycle pathology has been directly linked to bioavailable Mn deficiency in YAC128Q mice and a Mn deficient diet in WT rats was shown to reduce arginase activity and alter levels of urea cycle metabolites[80,96]. A recent study demonstrated that three high dose subcutaneous injections of Manganese II Chloride (**MnCl2**) over one week can reduce the elevated levels of citrulline, arginine, and ornithine in 12-week-old YAC128Q mice to match levels of WT vehicle-treated mice. This Mn supplementation paradigm also attenuated the reduction in striatal Arg2 activity levels in HD mice with no negative impact observed in WT mice[80]. These data suggest that a Mn-dependent HD phenotype at 12 weeks of age may be rescued with Mn supplementation, supporting the theory that Mn deficiency is an integral component of HD pathophysiology.

## *Regulation and Recycling of Glutamate*

Under physiological concentrations (i.e., not in excess), Mn contributes to protection against excitotoxicity by maintaining glutamate-glutamine homeostasis. Glutamine synthetase (**GS**) is a predominantly astrocytic enzyme that preferentially requires Mn over magnesium (**Mg**) to produce glutamine via a condensation reaction of glutamate and ammonia[97–100]. GS activity is significantly reduced in the caudate, putamen, frontal and temporal cortices, and

cerebellum of postmortem HD brains compared to control brains[84,101,102]. GS activity has not yet been investigated in animal models of HD, but there are substantial disruptions in the glutamate-glutamine cycle with an increase in glutamate toxicity in R6/2 HD model mice (expressing exon 1 of human *mHTT* with 150 CAG repeats)[103–105]. The direct effect of Mn exposure on GS expression and activity in WT or HD models has not been elucidated, but the enzyme's preferred requirement for Mn implies a bioavailable Mn deficit could lower overall GS activity leading to glutamate toxicity phenotypes observed in HD.

Astrocytic glutamate transporter 1 (**GLT-1**; excitatory amino acid transporter 2, **EAAT2**) also plays a major role in glutamate recycling by clearing glutamate from the synapse. GLT-1 is not Mn dependent, but is Mn-responsive. Glutamate uptake by both GLT-1 and glutamate aspartate transporter (**GLAST**) is inhibited by high concentrations of Mn[106]. The proposed mechanism for this effect is through a yin-yang repressor 1 (**YY1**) mediated decrease in GLT-1 mRNA and protein[107]. Mn therefore exhibits contradictory roles on glutamate regulation and recycling, as it is required for GS function but itself can induce neurotoxicity from increased extracellular glutamate concentrations[108,109].

Interestingly, reduced GLT-1 mRNA and protein has been reported in both HD models and postmortem brain tissue, likely contributing to decreased glutamate buffering and excitotoxicity[110]. Decreased GLT-1 expression is the opposite of the expected phenotype based on the proposed role of Mn deficiency in HD, as high Mn levels lead to downregulation of GLT-1. However, the precise interactions between Mn and GLT-1 in an HD model have yet to be studied. Further, while GLT-1 is primarily astrocytic, approximately 10% is neuronal. A recent study demonstrated that a neuronal GLT-1 knockout independent of the *Htt* mutation produced an HD pattern of transcriptional dysregulation in mice[111], suggesting that the mechanism of neuronal dysfunction in HD is closely linked with glutamate regulation and Mn may produce differential responses in the disease state.

## *Redox Status*

Maintaining redox homeostasis, or the balance between oxidants and antioxidants, is crucial to the health of a cell. Increased oxidative stress has been implicated in HD, although it is not clear if this is a causative factor in the disease or the consequence of other dysfunctional processes[112,113]. MnSOD, as the name implies, is a Mn-dependent enzyme that detoxifies superoxide anions into hydrogen peroxide which is further reduced by catalase. MnSOD knockdown ($^{+}/_{-}$) mice exhibit increased oxidative stress[114]. In mouse models of HD, MnSOD activity is elevated in young mice compared to WT and significantly decreased in older mice85. Further, Mn exposure can increase MnSOD activity[115]. Yet, the effect of Mn exposure on MnSOD in the context of an HD model has not been explored. Mn itself can increase oxidative stress[116], but may benefit redox status in a Mn-deficient model e.g. HD.

### *Energy Metabolism*

Pyruvate carboxylase (**PC**) is a Mn-dependent mitochondrial enzyme that forms oxaloacetate by carboxylation of pyruvate. This is an important step for entrance into the Krebs cycle and subsequent energy production. Reports of alterations in PC activity in HD are inconsistent[84]. However, there is substantial evidence for overall perturbations in energy and glucose metabolism in HD. In 1985, before the *HTT* gene had even been identified, it was known that there is a greater prevalence of diabetes mellitus (type II) in HD patients than age-matched controls[117]. Reduced glucose metabolism was subsequently reported in the caudate of pre-symptomatic individuals at risk for HD in 1987[118]. Mouse models of HD also develop type II diabetes at higher rates than WT counterparts[119]. Genes related to glycolysis, the Krebs cycle, and glucose transport are differentially expressed in HD cell models120. Even if a diagnosis for diabetes is not met, a defect in insulin secretion was found in one group of HD patients compared to controls[121].

Impairment in insulin production goes beyond simple glucose metabolism, as alterations in the insulin-like growth factor 1 (**IGF-1**)/Akt pathway have been identified in both HD animal models and HD patients[122]. The net result is a decrease in activated Akt in HD, which normally serves a neuroprotective role via phosphorylation of HTT itself to inhibit HTT-induced cell death[123]. Treatment with insulin or IGF-1 attenuate disease phenotypes in cell and animal models of HD by rescuing the neuroprotective effects of the Akt pathway and restoring regular energy metabolism[124–126].

Interestingly, the IGF-1/Akt pathway is Mn-responsive. Mn exposure upregulates IGF-1 expression and increases Akt signaling[127,128]. Mn itself has an insulin mimetic effect and protects against diet induced diabetes[115,129]. The interactions between HD, Akt signaling and Mn suggest that Mn supplementation could serve as a potential treatment for certain HD phenotypes.

## Dietary Mn Deficiency Recapitulates Select Molecular HD Phenotypes

Given that a variety of food sources contain plentiful Mn, deficiency in humans is rare[63,64]. However, Mn deficiency imposed in experimental conditions induces many of the same phenotypes characteristic of HD. It is not surprising that the Mn-dependent and Mn-responsive pathways altered in HD discussed above are also affected by a Mn-deficient diet. Rats placed on a Mn-deficient diet showed decreased liver arginase activity, although elevations in arginine were not detected and the effect on the neuronal urea cycle was not examined in this study[96]. Glutamate recycling and clearance has not been directly investigated in a Mn-deficient state, but low blood Mn levels have been detected in individuals with epilepsy compared to healthy controls, suggesting an association between

dysregulation of the glutamatergic system and low Mn concentrations[130]. Impaired antioxidant defenses have also been reported in Mn-deficient conditions[131]. A Mn-deficient diet also impairs insulin production and decreases IGF-1 signaling in rats[132]. Cholesterol metabolism, which is perturbed in HD, is also impacted by Mn-deficiency[131,133]. Finally, Mn deficiency in rats beginning *in utero* and continuing through adulthood produced changes in liver mitochondria structure at 9 months of age[134]; mitochondrial dynamics are altered in HD and contribute to the pathophysiology of the disease[135,136].

Despite the overlap between HD phenotypes and those of Mn-deficiency, additional consequences arise from inadequate Mn that are not observed phenotypes in HD. For example, skeletal bone growth abnormalities, osteoporosis, decreased fertility and irregular estrous cycles can occur without sufficient Mn[137]. However, the overall consistency between Mn deficiency and molecular HD phenotypes strongly support a role for Mn in the pathogenesis of HD. Insufficient Mn during development may also contribute to behavioral symptoms in adulthood.

# A Role for Developmental Mn Deficiency in the Manifestation of Behavioral HD Phenotypes?

Mn requirements during development exceed those in adulthood[65]. Nutrient deficiency during the critical developmental period known as the "brain growth spurt" can have profound consequences on behavioral outcomes later in life[68,70]. Severe *in utero* Mn deficiency can lead to ataxia following birth. However, this incoordination is primarily associated with an improperly developed otolith and vestibular system dysfunction[138]. Nevertheless, this is an example of how early Mn deficiency can negatively impact the development of a system associated with movement and coordination. Interestingly, mice placed on a Mn-deficient diet beginning at 4-5 weeks of age for 90 days did not develop changes in strength, motor activity and motor coordination, or irritability despite a significant decrease in brain Mn levels[139]. The lack of behavioral impairments found in this study suggests that inadequate Mn at younger ages (< 4 weeks in mice) may play a more critical role in the generation of motor phenotypes.

Conditional expression of *mHTT* in mice from embryogenesis up to P21 recapitulates analogous motor phenotypes to mice expressing *mHTT* throughout life. These conditional-*mHTT* mice were not as severely impaired on the RotaRod at 3 months of age, but by 9 months of age they displayed the same magnitude of motor coordination deficits as mice that expressed *mHTT* continuously[140]. These conditional-*mHTT* mice also exhibited striatal degeneration by 9 months of age. This study exemplifies and further supports the idea that neurodevelopment and neurodegeneration are not mutually exclusive.

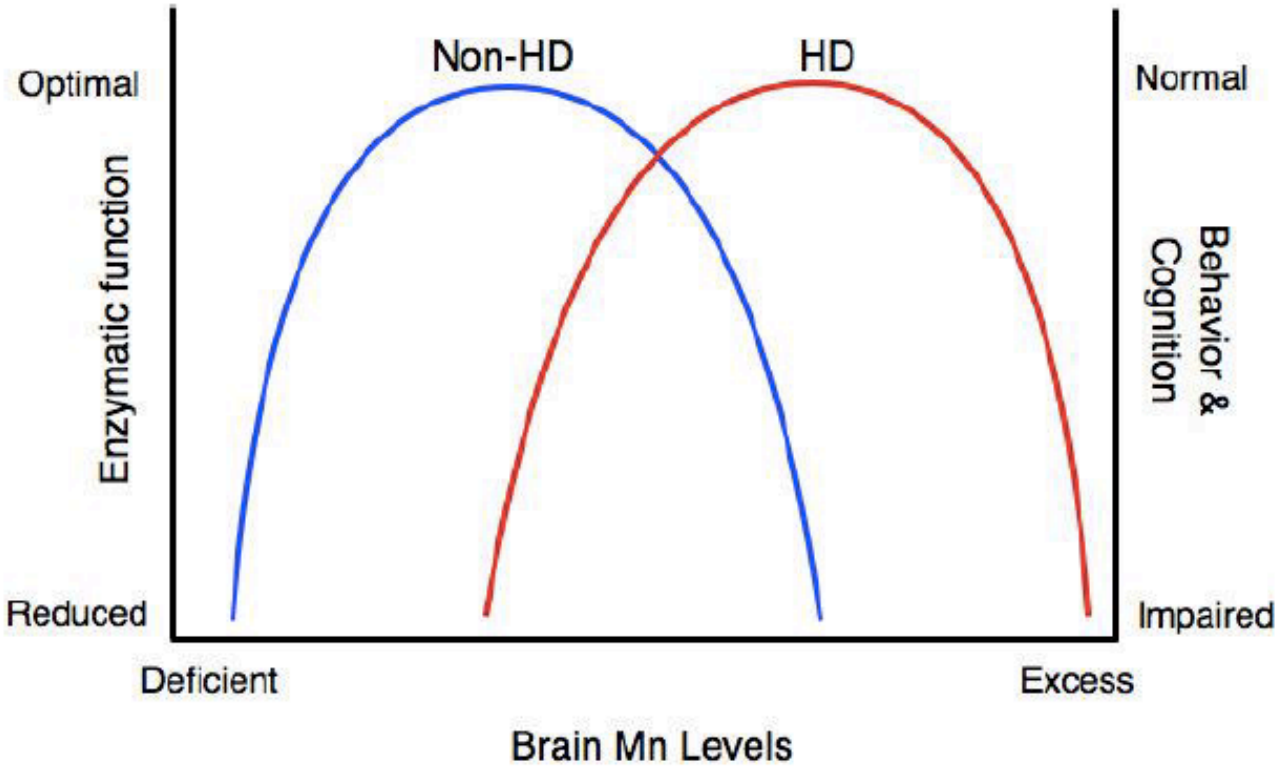


**Figure 1.** The relationship between Mn deficiency and toxicity can be represented as an inverted U-shape. The proposed effect of developmental brain Mn, from levels of deficiency to toxicity, on enzymatic function and behavior & cognition in individuals without (blueline) or with Huntington's Disease (HD; red line) is plotted. Brain Mn levels that are optimal in unaffected individuals are not sufficient for normal behavior and optimal enzymatic function in HD. Additionally, the detrimental effect of excess Mn is shifted in HD. Mn supplementation beginning in early development could increase available Mn stores in the brain to delay symptom onset and allow for appropriate enzymatic function and behavioral outcomes.

Due to the vital role that Mn plays in health and development, early Mn-deficiency may contribute to formation of molecular and behavioral HD phenotypes. Expression of mHTT in the mouse up to the first 3 weeks of life was sufficient to induce motor impairments in adulthood[140]. Perhaps mHTT impacts Mn homeostasis earlier than previously examined, and the changes in Mn during development allows for or exacerbates HD phenotypes that present later in life.

The relationship between optimal Mn levels, or the balance between sufficient and toxic levels, follows a biphasic inverted U-shape[70]. Too little Mn results in decreased enzymatic function and behavioral impairments, while excess Mn produces the same outcome. The

concentration of Mn that is optimal for a healthy individual may not be sufficient for an individual with HD (**Figure 1**). Furthermore, a higher level of Mn that may cause detrimental effects in a healthy individual may be the optimal Mn concentration in HD. Mn supplementation during the developmental period may delay symptom onset and act as an important disease modifier for HD. Additional studies that assess the effects of Mn supplementation on HD phenotypes will help advance our understanding of the interaction between Mn biology and HD pathophysiology.

# Conclusion

Huntingtin's Disease is a devastating neurodegenerative genetic disorder with midlife onset, despite continuous expression of the *mHTT* from embryogenesis until death. Several genetic and environmental modifiers have been previously identified that may either accelerate or delay AO and disease progression. Evidence is accumulating in recent years supporting the theory that Mn may be an important environmental modifier of HD. Particularly, a striatal bioavailable Mn deficit is observed in HD. Exposure to Mn has rescued some Mn-dependent phenotypes in HD, such as perturbations of the urea cycle. However, whether Mn has the ability to rescue or prevent additional phenotypes, particularly those related to behavioral symptoms, has yet to be explored.

The mechanistic relationships between Mn and HD are also not currently well defined. It is unknown if mHTT directly leads to Mn deficiency, or if mHTT acts on another pathway that as a consequence produces a Mn deficiency. Further research investigating these exact mechanisms is challenging as there are few specific Mn transporters identified. Progression in the Mn transport field will be necessary to better understand the interactions between Mn deficiency and HD pathology. Future studies that examine the efficacy of intervening in early postnatal development with Mn supplementation on delaying HD symptom onset and conversely the extent to which severe Mn-deficiency during development exacerbates HD symptoms will benefit the field greatly.

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# Cell-type heterogeneity of the rodent BNST

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## Abstract

Chronic stress exposure is associated with a number of maladaptive psychological disorders. One major region responsible for mediating stress- and anxiety-related disorders is the Bed Nucleus of the Stria Terminalis (**BNST**). The BNST is known to have extensive heterogeneity among cell types, and several approaches have been used in order to classify these cells into functionally relevant categories. Here, I will review two of the major categories used to classify these neurons (peptide- and electrophysiologically-based), and discuss their merits and shortcomings in determining functionally distinct classes of cells in mediating stress- and anxiety-like behaviors.

**Keywords:** *BNST, Stress, Anxiety, Electrophysiology, Amygdala*

# Introduction

Chronic stress exposure can lead to numerous psychological disorders such as anxiety, depression, and PTSD[1–3]. It is also heavily implicated in addiction, with stress being one of the most cited causes of drug relapse[4,5]. The extended amygdala, including the Central Nucleus of the Amygdala (**CeA**) and its primary output region, the Bed Nucleus of the Stria Terminalis (**BNST**), play a central role in mediating stress and anxiety-like behaviors[6–10]. Specifically, the CeA and the BNST are thought to mediate short-term fear-like responses and longer-term anxiety-like responses, respectively[7,8,11,12]. The BNST contains a large amount of cell-type heterogeneity[13–16]. Evidence suggests that these various cell-types have distinct roles in mediating anxiety-like behaviors, and as such, there is much interest in systematically identifying and classifying these neurons into functional categories.

Initial attempts to classify cell-types focused on anatomical subdivisions within the region. There is some inconsistency regarding the exact number of subdivisions within the BNST, with some identifying as many as fifteen distinct subdivisions[13,17]. However, it is generally accepted that the region can be broadly divided into anterior and posterior BNST. The majority of work investigating the role of the BNST in anxiety-like responses has been conducted on cells within the anterior BNST. This region can be further divided into anteroventral (**BNST-AV**) and dorsal (**dBNST**), the latter of which contains medial and lateral subdivisions (**BNST-AM** and **BNST-AL**)[13,17] (**Figure 1**).

However, the functions of cells within even the smallest anatomical division are not homogeneous. Thus, other classification systems are necessary to better characterize the various functional groups within this complex region. A number of methods have been used to identify a characteristic that can reliably predict the function of a given cell. In this review, I will focus on two such methods that have been used, discussing the subtypes that have been identified and their respective functional relevance in the context of anxiety-like behavior.

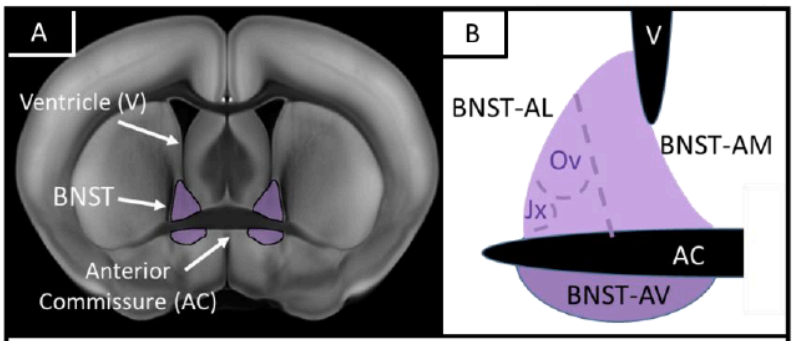


Figure 1. (A) Allen Brain Atlas Diagram of the Bed Nucleus of the Stria Terminalis (BNST) highlighted in purple. (B) Diagram of the BNST subregions discussed in this review. AC - Anterior Commissure. V – Ventricle. AV – Anteroventral. AL – Anterolateral. AM – Anteromedial. Ov – Oval Nucleus. Jx – Juxtacapsular nucleus.

anxiogenic output[10]. The advent of CRF-driven Cre lines has enabled the functional investigation of CRF cells specifically, and it has been shown that the expression of a Gi-coupled **DREADD**s (Designer Receptor Exclusively Activated by Designer Drugs) in BNSTCRF cells is also anxiolytic, further supporting the role of BNSTCRF cells in promoting anxiety-like behavior[29].

## *Pituitary Adenylate Cyclase-Activating Polypeptide*

It has long been known that there is a population of neurons within the BNST that express Pituitary Adenylate Cyclase-Activating Polypeptide (**PACAP**)[30,31]. These cells are concentrated in BNSTCRF-rich regions, but there is little to no overlap in the expression of these two peptides[31–34]. Early studies using global knockouts of PACAP and/or the PAC1 receptor found that, similarly to CRF, PACAP increases anxiety-like behaviors[35–38]. Within the BNST specifically, there is an increase in both terminal expression and BNSTPACAP cell number following a stressor, also paralleling the increase seen in CRF expression[39]. However, BNSTPACAP neurons may be more tuned to regulating responses to chronic stress specifically, as this increase is seen after 7 days of chronic restraint stress, but not after a single session of restraint[40].

There is evidence that actions of BNSTPACAP neurons are at least in part sex-dependent. Following chronic variable stress, subthreshold infusions of PACAP into the BNST were able to enhance anxiety-like startle responses and plasma corticosterone levels in male, but not female rats[41]. Interestingly, a polymorphism in the PAC1 receptor gene, ADCYAP1R1, is associated with increased susceptibility to PTSD and alcohol use disorder specifically in women[42,43]. Together, these studies suggest that BNSTPACAP neurons represent an additional pathway for anxiogenic output of the BNST that is distinct from that of BNSTCRF neurons.

## *Protein Kinase C Delta*

The majority of work regarding the role of Protein Kinase C delta (**PKCδ**) in the extended amygdala has been conducted in the CeA. Within this region, CeAPKCδ neurons and CeACRF neurons are distinct cell populations that work in opposition to decrease or increase fear-like responses, respectively[11]. Recent work from our lab has found cells expressing mRNA for CRF (BNST*Crh*) and cells expressing mRNA for PKCδ (BNST*Prkcd*) are also largely distinct populations[28]. It is possible that BNSTPKCδ cells act to counterbalance the anxiogenic output of the BNSTCRF cells, paralleling their opposing roles in the CeA, though further studies are necessary to test this hypothesis.

As with PACAP, the regulation of PKCδ expression in the BNST is also sex-dependent. Our lab has shown that, following acute restraint stress, there is an increase in the number

of cells that co-express CRF and PKCδ mRNA (**BNST*Crh/Prkcd***) specifically in female mice, and there is evidence that this is the emergence of *Prkcd* in BNST*Crh* cells, rather than *Crh* in BNST*Prkcd* cells[28]. The fact that it is only a subset of CRF cells which begin expressing PKCδ suggests that there may be functional heterogeneity within BNSTCRF cells – something that characterization based on the expression of a single peptide alone is insufficient to capture. The function of this co-expressing population remains unknown, but it is possible they play a role in the heightened anxiety-like responses seen in females[44,45].

## *Other Neuropeptides*

Work shows that there are a number of other peptide-expressing populations of cells within the BNST, including those that express Neuropeptide Y (**NPY**), Substance P (**SP**), Neurotensin (**NT**), Enkephalin (**ENK**), Galanin (**GAL**), Vasopressin (**VP**), and Cholecyctokinin (**CCK**)[13–15]. However, little work has investigated the function of these populations, with the majority of studies focusing on the signaling role of the peptide itself. For instance, in contrast to the actions of CRF, NPY has been shown to have anxiolytic effects[46,47], which are mediated at least in part through its actions at NPY receptors (**YR**s) in the BNST[19,48]. It has also been shown that BNSTNPY neurons and BNSTSP neurons are distinct populations, but the function of either cell type remains to be determined[49,50]. Studies on the expression of GAL and VP have found that these are largely overlapping populations[51]. Both are sexually dimorphic, with reduced expression in female rodents, and their expression levels can be regulated by the presence of testosterone[52,53]. A number of studies have implicated these populations in social- and reproduction-related behaviors, but any role BNSTGAL and BNSTVP neurons may play in stress- and anxiety-like behaviors remains largely unknown[54].

Finally, recent work found that blocking NT receptors in the Oval Nucleus of the BNST has anxiolytic effects. Postsynaptic depolarization of BNSTNT neurons induced release of NT and CRF, suggesting that at least a subset of BNSTNT neurons coexpress CRF, and that together these work to increase anxiogenic output of the BNST[55].

## *Protein Kinase C Delta*

Electrophysiological characterization is a common method that has been used to classify neurons in a number of brain regions including cortical areas, the striatum, and the amygdala[56–61]. Knowledge of the physiological properties of cell types can provide insight into their functional role, and characterized synaptic responses can enable cells to be identified in-vivo to understand activity in awake and behaving animals. However, relative to anatomical or chemogenic characterizations in the BNST, and relative to physiological characterization in other brain regions, few studies have investigated electrophysiological

categorization of cell types within the BNST, with a large concentration of work coming from a small handful of laboratories.

Early attempts at parsing physiological distinctions found subregion-specific differences between the dorsal and ventral delineations of the BNST (**dBNST** and **vBNST**)[62]. Specifically, Egli and Winder found that vBNST neurons on average had a faster τ value than those in the dBNST, reflecting a decrease in membrane resistance and/or capacitance. Additionally, the dBNST was under tonic GABAergic tone, which could be alleviated by the application of the GABAA receptor antagonist picrotoxin.

There were also differences found in the proportion of cells displaying particular characteristics. In the vBNST, 75% of cells displayed a low-threshold Ca-mediated spiking (**LTS**) following a depolarizing step, compared to 23% in the dBNST. The dBNST had the largest proportion of cells showing inward rectification (33%) and/or displaying a depolarizing sag after the injection of hyperpolarizing current (49%), compared to 18% and 16% in the vBNST, respectively. They also investigated cells displaying an intersection of these properties. In the vBNST and dBNST, respectively, 8% and 13% of cells showed both LTS and a depolarizing sag, and 4% and 20% showed inward rectification and a depolarizing sag. 8% showed LTS and inward rectification in the vBNST, with no cells found in the dBNST. In each region, one cell displayed all three characteristics (Findings of Egli and Winder 2003 summarized in **Table 1**)[62].

| **Table 1.** | LTS | Depolarizing Sag (DS) | Inward Rectification (IR) | LTS and DS | IR and DS | LTS and IR | All 3 |
|---|---|---|---|---|---|---|---|
| dBNST | 23% (16/70) | 49% (34/70) | 33% (23/70) | 13% (9/70) | 20% (14/70) | 0 | 1% (1/70) |
| vBNST | 75% (38/51) | 16% (8/51) | 18% (9/51) | 8% (4/51) | 4% (2/51) | 8% (4/51) | 2% (1/51) |

Data summarized from (62)

The majority of physiological classification of cell types within a subregion has been done within the anterolateral BNST (**BNST-AL**), which contains the oval nucleus, the juxtacapsular nucleus, and the anterolateral nucleus[17]. Rainnie and colleagues classified neurons within the rat BNST-AL into three categories: Type I, Type II, and Type III[63] (**Figure 2***a*). Since this initial characterization, these cell types have also been identified in the anteromedial and anteroventral BNST (**BNST-AM** and **BNST-AV**), as well as in the BNST-AL of mice, though their prevalence is region- and species-specific[21,64,65]. While the amount of physiological classification within the BNST is beginning to increase, much work is still needed to connect these cell types to their respective roles in stress- and anxiety-like behaviors.

## *Type I*

Type I neurons are typically characterized by a prominent depolarization sag following a hyperpolarizing step (which is likely mediated by Ih current) and the absence of LTS or burst firing[63,66–68]. Additionally, they show a regular firing rate (and as such, have also been termed Regular Spiking Cells) but show spike frequency adaptation after prolonged depolarization64.

Type I cells make up approximately one quarter of cells within the rat BNST-AL, -AM, and –AV, though this number in the BNST-AL has varied from 11% to 30% between studies[63–65,68,69]. They are ventrally concentrated in the BNST-AV, but evenly distributed within the other two regions. However, there are differences in the proportion of cells displaying a large depolarization sag, with the higher concentration in BNST-AL (~80%) followed by BNST-AM and –AV with ~60% and ~35%, respectively[64]. Though they make up a substantial portion of cells in the rat, Type I cells are much less prevalent in the mouse, making up approximately half of the proportion seen in rat BNST[21,65]. Additionally, mouse Type I neurons display a significantly smaller Ih compared to rat, making them more difficult to distinguish from Type III cells[65].

## *Type II*

Type II neurons are characterized by a more pronounced depolarization sag in response to hyperpolarization, which is followed by rebound spiking mediated by IT current, and generally show prevalent LTS[63]. The majority of these neurons also display burst firing, leading to the alternate name of Low-Threshold Bursting (**LTB**) cells[64]. However, the presence of bursting is highly variable within Type II neurons, with some displaying only single spikes at depolarizations larger than -70mV, and others exclusively responding with single spikes[64,65]. While most still include these neurons in the Type II classification, one group separated these cells into their own division termed Type O, which resemble Type II but show regular, oscillatory firing after hyperpolarization and single spikes after depolarization[64,68].

Type II neurons are the most prevalent cell type within the BNST-AL(40-66%), BNST-AM (68%), and BNST–AV (63%) of rats[63–65,68,69]. They are evenly distributed throughout these regions, but show several differences in characteristics between regions. As with Type I, Type II cells with a large depolarization sag are more prevalent in the BNST-AL (~90%) than BNST-AM (~62%) or BNST-AV (~50%)[64]. Consistent with variable bursting properties, analysis also reveals a significantly reduced bursting rate and number of spikes per burst in the BNST-AL, while the spikes per burst are significantly increased in the BNST-AV[64]. There are also significant species differences, with Type II cells again being less prevalent and less easily distinguished in mice than in their rat counterparts. They display less-pronounced LTS, thus resembling Type I cells, and despite being the most prevalent cell type in rats,

make up only 22% of the mouse BNST-AL[65]. It is possible that this cell type is even less prevalent in other subregions of the BNST, based on the finding by Egli and Winder that 13% of neurons in the dBNST displayed both LTS and a depolarization sag[62]. These characteristics are reminiscent of a Type II categorization, though a further characterization would be necessary to confirm their cell-type identity[63]. In the vBNST, on the other hand, only 8% of cells shared these properties, following the pattern of prevalence between subregions of the rat BNST[62,64].

## *Type III*

The defining feature of Type III neurons is the presence of fast inward rectification without rebound firing following hyperpolarizing current (indicating the presence of inwardly rectifying potassium channels (IK(IR))), and little to no depolarization sag[63]. Type III cells are less common than the first two cell types in the rat BNST, comprising 16% to 29%, 8%, and 6% of neurons in the BNST-AL, -AM, and –AV, respectively[63–65,68,69]. However, they are the most prevalent cell type in the mouse BNST, making up 54% of cells. Type III neurons show uniform (though low) distribution in the BNST-AM and –AV, but are concentrated in the juxtacapsular and oval regions of the rat BNST-AL[64].

## *Other Physiological Classifications*

Rodrigues-Sierra et. al. describe two other cell types in addition to the three described by Rainnie and colleagues. The first is a group of Late Firing (**LF**) cells that are characterized by a more negative resting potential, an extended latency to fire following depolarization, spike frequency acceleration, and a sharp increase in the rising phase of the voltage in response to increasing depolarizations[64]. These cells were only seen in the BNST-AL, and made up only 4% of the population. Due to the fact that these cells also display fast inward rectification, it is possible that they could be grouped in with Type III neurons[65]. Further studies will reveal whether LF cells hold up as a distinct classification. An additional cell-type that has been identified is the Spontaneous Activity (**SA**) group, which is located exclusively within the BNST-AV[64]. They are a minority of the cells, making up only 8% of the population. Yet, they are distinct from other BNST neurons in that they show high regular spontaneous activity at rest, and unlike the other types, show no Ih or IT mediated current.

Though these cell-type classifications can fully describe neurons seen within the rat BNST, there are a number of "other" cells within the mouse BNST that cannot be classified as such. Daniel et. al. found that 10% of neurons in the mouse BNST-AL did not fit within the above cell-types, and this number increases to 25% of neurons in primate BNST[65]. In fact, mouse and primate neurons were surprisingly found to share more in common in terms of physiological classification than either species did with rats (Summarized in **Figure 2***b* and **Table 2**).

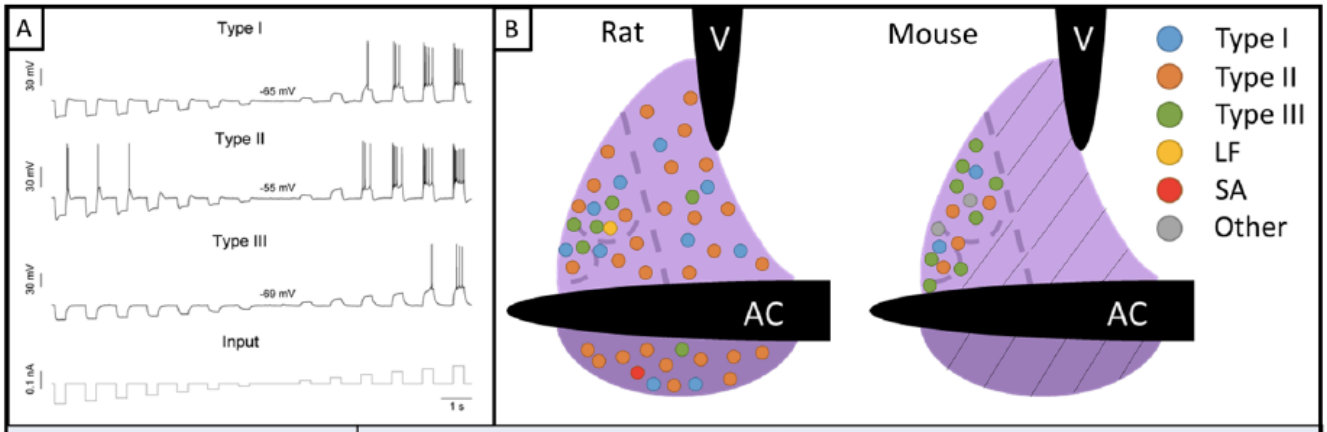


**Table 2**

| | | Rat / Mouse | | | | | |
|---|---|---|---|---|---|---|---|
| | | Type I | Type II | Type III | LF | SA | Other |
| BNST-AL | % | 24% / 13% | 54% / 23% | 21% / 54% | 4% / - | 0% / - | 0% / 10% |
| | Distribution | Even | Even | Concentrated in Oval and Jx | Even | - | - |
| BNST-AM | % | 24% / - | 68% / - | 8% / - | 0% / - | 0% / - | |
| | Distribution | Even | Even | Even | - | - | - |
| BNST-AV | % | 23% / - | 63% / - | 6% / - | 0% / - | 8% / - | - |
| | Distribution | Ventrally Concentrated | Even | Even | - | Even | - |

Figure 2. (A) Sample traces of Type I, Type II, and Type III neurons in the BNST (68). (B) Pictorial representation of the relative distribution of electrophysiologically classified cell types in the BNST of Rats and Mice. Dashes represent no data.
Table 2. Summary of electrophysiological cell type classifications in the BNST. Values are averaged from available data in (4, 63-65, 69).

## *Functional Relevance*

Though more work is beginning to emerge regarding physiological cell-type characterization within the BNST, there is still a striking lack of research into the functional differences that these cell types may hold. One recent study found that, in addition to non-cell-type specific effects, opiate withdrawal leads to exclusive changes in the properties of Type III cells[70]. For example, Type III cells have high rheobase and inward rectification at baseline, but both values are significantly reduced during withdrawal. They also showed an increase in membrane resistance, resting membrane potential, and excitability specifically in Type III cells. While it is still unclear how these cell-type specific alterations in opiate withdrawal related to behavioral output, this study shows that there may be some functional relevance to these physiological categories.

Part of the paucity of functional data is likely due to a lack of intersectional research bringing physiological characterizations together with other methods of classification such as peptide expression data. In rats, it has been shown that the majority of Type III neurons also express CRF[71]. However, in mice, the majority of CRF neurons do not fit into any of the three cell types, suggesting that there is likely significant heterogeneity within the established peptide classifications of cells[21,65]. Overall, lack of similarity in electrophysiological classifications between species, coupled with poor functional relevance of the cell types, casts doubt on the utility of the existing classification scheme.

# Conclusions

It is clear that there exists significant heterogeneity within the BNST, and that this heterogeneity contributes to the complex role of the BNST in mediating stress- and anxiety-like behaviors. However, developing a classification system that sufficiently captures and divides cells into functionally relevant groups will require considerably more research. Initial studies were able to sub-divide the BNST into several anatomical regions, but even within these regions, there are a number of functionally distinct cell types, as evidenced by the fact that BNSTCRF, BNSTPACAP, and BNSTPKCδ are all present in the Oval Nucleus, but each represent distinct populations.

Classification of BNST neurons based on the expression of peptides or proteins has shown promise in distinguishing functional classes. BNSTCRF and BNSTPACAP populations do not overlap, but both increase anxiety-like responses following a stressor. BNSTPKCδ neurons also show very little overlap with BNSTCRF neurons at baseline, but based on work in the CeA, may be anxiolytic in nature. Despite work done on the signaling role of other peptides in stress-related behaviors, and the presence of these peptide-expressing cell types in the BNST, little work has been done to determine the functional significance of these populations. Further, considerably more work is needed to understand the electrophysiological profile of BNST neurons, as current attempts are limited in their application across species and functional relevance based on type alone.

However, it is likely that there is still heterogeneity even within these peptide- and protein-based divisions. Only a subset of cells expressing *Crh* begin co-expressing *Prkcd*, and CRF cells have been found that can be classified into all three electrophysiological types, as well as many others that do not fit into any defined type at all. This suggests that characterization based on the expression of a single peptide or protein will be insufficient to capture the functional heterogeneity of cells within the BNST. Rather, it will likely be necessary to begin multiple types of classification systems. It is possible that electrophysiological profiling will prove useful in dissecting out subtypes of peptide-expressing neurons. Also, in addition to the peptides and proteins discussed here, there is a

large body of work grouping cells based on their expression of various receptors. Single-cell transcriptome analysis may help shed light on the complexity of expression profiles and enable a more holistic grouping of cells. Finally, drawing on work describing the connectivity of cells types based on input and output projections will be critical in understanding the function of these cells. By furthering our understanding of the complex cellular subtypes in the BNST, we will be better positioned to develop selective targets in order to improve our treatment of stress- and anxiety-related disorders.

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**CANDIDATE REVIEWS**

# ‘Mapping’ between symbolic and nonsymbolic representations of numerosity: A developmental cognitive neuroscience model

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## Abstract

How do we know that there are about 30 people in a room, or pick out a hundred buttons without counting? It has been suggested that numerals such as number words and Arabic numerals are ‘mapped’ onto a mental ‘number line’ comprising representations of approximate magnitudes. This ‘mapping’ enables us to estimate systematically. While developmental models focus on how children learn to associate numerals with exemplars of nonsymbolic quantities, neuroscientific models focus on whether the same neurons respond to both “5” and an array of 5 items as a product of associative learning. Cognitive models, however, focus on how we transcode between symbolic and nonsymbolic quantities during estimation. All of these processes and products are referred to as ‘mapping’. Besides the broad use of ‘mapping’, each disciplinary perspective alone is also inadequate for making a decisive evaluation of the ‘mapping’ hypothesis. Here, we propose a developmental cognitive neuroscience model that integrates extant ‘mapping’ models from different disciplines. The proposed model demonstrates that estimation tasks do not directly measure the mental number line or the shared neural mappings, but a composite of multiple processes and products. Importantly, the model provides more precise nomenclature for the processes and products related to estimation, and novel predictions for further investigations of the ‘mapping’ hypothesis.

**Keywords:** *Numerals, Transcoding, Learning, Mapping, Estimation*

# Introduction

In recent years, there has been an increase in attention given to how we comprehend numerals such as spoken number words and Arabic numerals, and how these skills related to math competence[1–3]. A dominant hypothesis for how we are able to comprehend and use numerals is that we possess innate mental representations of approximate numerical magnitudes on a continuum or 'number line' (e.g., an intuitive sense of 'sixness' as being distinct from 'fourness' and' eightness'), and that over the course of associative learning, numerals are 'mapped' onto this mental number line[4–10]. This mental number line thus provides the basis of numerical meanings for numerals.

'Mapping' in relation to numerals have often been mentioned, but it has remained a broadly abstract concept across disciplines. 'Mapping' is used in developmental models to refer to the process of *learning the associations* between numerals and nonsymbolic quantities (e.g., a visual array of items). In neuroscientific models, 'mapping' refers to the *same neuron* being tuned to nonsymbolic quantities and their corresponding numerals as a result of learning that they both represent an abstract number concept (e.g., 'threeness' of three dots and numeral "3"). In cognitive models, 'mapping' refers to the *transcoding* of nonsymbolic quantities or numerals to mental representations of numerosity, or transcoding of mental representations to symbolic or nonsymbolic estimates. Hence, it has been used both as a verb (process) and a noun (product) in empirical studies and reviews[4,11,12] investigating or evaluating the 'mapping' hypothesis. Despite each discipline having an incomplete understanding of what the 'mapping' hypothesis entails, researchers are already moving forward with investigating how 'mapping' may support math competence[13,14,23,24,15–22], often focusing on different models and tasks. In this review, we integrate extant 'mapping' models from different disciplines, and propose a unifying developmental cognitive neuroscience model (hereafter, the Transcoding-Learning-Mapping model). The goal of this model is to provide a foundational mechanistic framework with precise nomenclature for the processes and products related to 'mapping', so that we can have a shared understanding as we further investigate and evaluate the 'mapping' hypothesis as a whole, and its alternatives.

# Mental Representation of Numerosity

We represent numerosities mentally using two core distinct systems that we possess since infancy[25,26] – an object-tracking system (**OTS**)[27,28], and an approximate number system (**ANS**)[8,10]. The OTS and ANS are subserved by distinct neural mechanisms within the posterior parietal cortices[5,26,29,30]. The OTS allows us to represent up to 4 discrete items in parallel (also known as "subitizing", "object file", or "parallel individuation")[31–35]. It is suggested to be supported by a capacity-limited visuo-spatial working memory system that

allows us to hold a visuo-spatial mental representation of attended items (see **Figure 1**(a))[5,36,37]. By individuating the items in a set and binding item-specific features such as shape and color in working memory[38,39], the OTS suppresses any representation of the set size[26–28]. The ANS, on the other hand, represents the set size approximately, but does not represent individual items[26]. It can take over from the OTS to represent numerosities less than 4 as a set approximately when attentional demands impairs the OTS's ability to individuate (e.g., closely spaced)[26,40]. In this sense, the OTS and ANS are mutually exclusive. As shown in **Figure 1**(a), the ANS comprises analog mental representations of approximate numerosities on a logarithmically compressed continuum[26,27,41,42]. Regardless of whether numerical stimuli are symbolic or nonsymbolic, activation of the ANS representations manifests in behaviors that (1) obey Weber's law: more errors and slower responses in distinguishing a pair of numerosities that are numerically closer (e.g., 4 vs. 5 and 4 vs. 9) or have a ratio approaching 1 (e.g., 1 vs. 2 [ratio = .5] and 8 vs. 9 [ratio = .89]) (hereafter, distance and ratio effects)[43–45]; (2) show scalar variability during estimation: greater variability in estimates as numerosity increases[46,47].

The ANS mental representations are thought to arise from the population coding of "numerosity-selective neurons" primarily in the posterior parietal cortices, but can also found in the prefrontal cortices[6,8,41,48–50]. Numerosity-selective neurons are tuned approximately to a preferred numerosity[8,51]. For instance, a neuron selective for numerosity 3 will, on average (across stimulus presentations or trials), respond optimally to 3 objects, but less to 2 or 4, and even less to 1 or 5. Response on a single trial, however, relies on population coding rather than single-neuron coding[49]. Hence, 3 objects will excite most neurons selective for numerosity 3, and few that are selective for 2 and 4 (see **Figure 1**(b)). As proportionally more neurons that are selective for numerosity 3 are active, a mental representation of 'threeness' emerges[49]. Evidential support for the existence of such numerosity-selective neurons have been gathered with single-cell recordings in numerically trained[52–58] and numerically naïve monkeys[59], with neuronal and behavioral modeling in humans using functional magnetic resonance imaging (**fMRI**)[60,61,70,62–69], and with simulations using computational modeling[51,71]. These mental representations have also been shown to be logarithmically compressed using neurophysiological methods in monkeys[52,72]. Recently, using ultra-high field (7 Tesla) fMRI, numerosity-selective neuronal populations have been shown to be logarithmically compressed spatially in the posterior parietal cortices with more neurons coding for smaller numerosities than larger ones (see **Figure 1**(b))[61]. This avoids an exponential increase in neuronal resources as numerosity increases[73].

# The Transcoding-Learning-Mapping (TLM) Model

To behaviorally assess whether numerals are 'mapped' on a fuzzy mental number line via prior associations with exemplars of nonsymbolic quantities, we can ask if an individual can

systematically assign numerals to given sets of items (hereafter, numerosity perception task), or conversely, produce sets of items that correspond to given numerals (hereafter, numerosity production task). In this review, we will use extant cognitive models of numerosity estimation[6,74–77] to anchor our description of the proposed model. To guide our description, we employ Marr's (1982)[78] levels of analysis: Given the computational goal of transcoding between numerals and nonsymbolic quantities in an estimation task, what are the processing algorithms, and how can the algorithms be implemented neurobiologically?

The TLM model comprises five temporally ordered components at both developmental and task levels: (1) how nonsymbolic quantities are transcoded into mental representations of numerosity, (2) how some numerals can be associated with these mental representations, (3) what neural mappings result from learning, (4) how numerals are transcoded into mental representations after learning, and (5) how mental representations are transcoded into symbolic and nonsymbolic estimates.

# Nonsymbolic Stimulus-to-Representation Transcoding (Component 1)

Computational models suggest that an ANS mental representation is activated by nonsymbolic quantities through a hierarchy of three computational stages – object location coding, summation coding, and numerosity-selective coding (**Figure 1**(c))[6,51,71]. Firstly, visual input is segmented into discrete objects with a fixed number of active neurons allocated to create a shape- and size-independent code, resulting in an "object location map"[51,71,79,80]. Although this object location coding is not specific to numerosity, it is sensitive to numerosity as the number of locations increases with the number of objects. This map likely supports visuo-spatial working memory and the OTS[81–83]. Next, these maps provide input to "summation neurons" in the superior parietal cortex, which would show a monotonic increase in their activity as numerosity increases[51,57,71,84–87]. The summation neurons in turn provide input to numerosity-selective neurons. In humans, these three stages occur along an occipito-parietal processing gradient extending from the inferior occipital gyri to the superior parietal lobule[86].

While the processing stages described suffice for a *single* judgment of numerosity in real-world contexts, additional mechanisms come into play in laboratory studies in which participants have to make a *series* of numerosity judgments. When the numerosity of nonsymbolic stimuli is varied across experimental trials, some non-numerical continuous magnitudes, such as surface area, convex hull, density, or perimeter, would co-vary with numerosity, and it is unclear whether and when participants use non-numerical cues to make their judgments[88–90]. For instance, a common method to control for such confounds is to maintain a constant total surface area across some stimuli (such that numerosity is

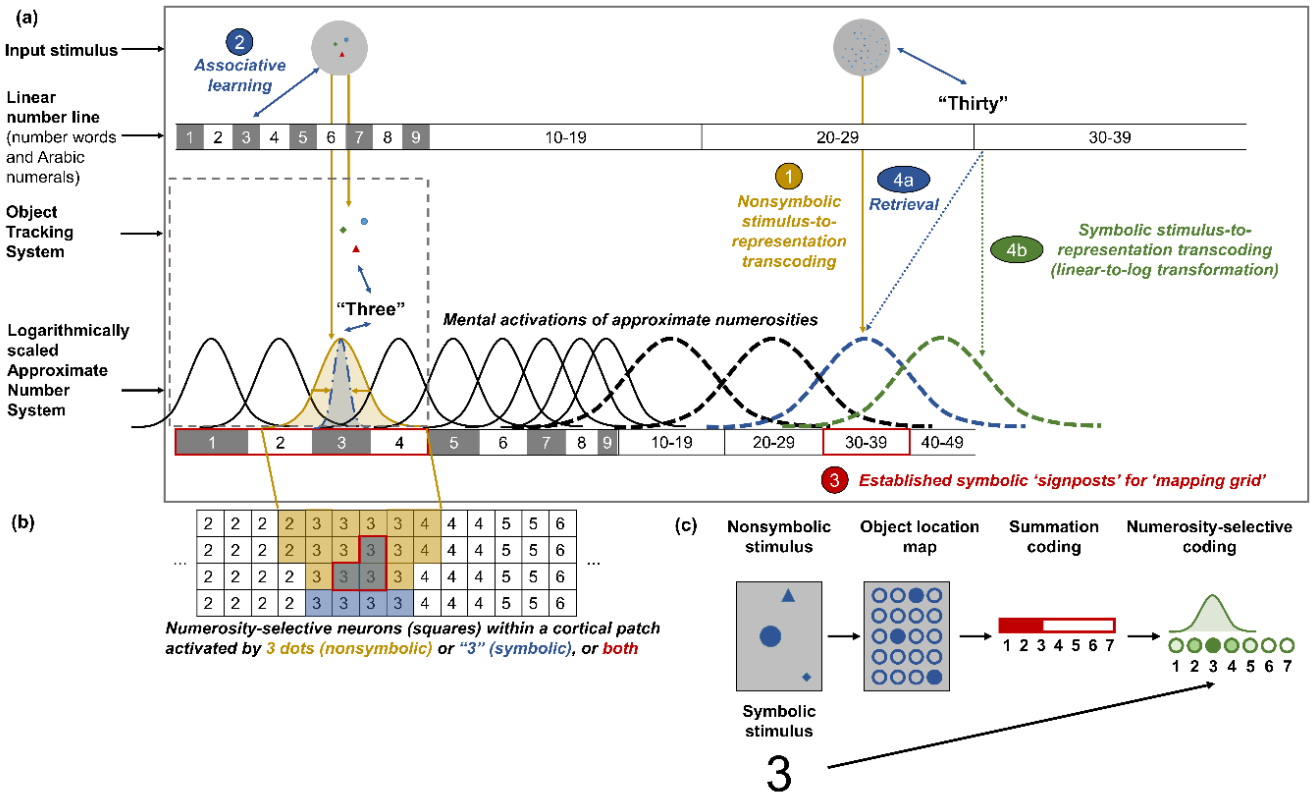


**Figure 1.** (a) Cognitive and neural bases of numerical meanings of nonsymbolic and symbolic numerical stimuli. In this schematic, mental activations (tuning functions) for larger numerosities are averaged within decade boundaries as shown by the wider distributions. (1) Nonsymbolic stimuli are transcoded to mental representations. (2) Some associations among numerals, nonsymbolic quantities, and mental representations are learned idiosyncratically. (3) Associative learning establishes symbolic signposts (red outlines). The tuning function for the learned numeral is also sharpened (yellow to blue tuning functions; see (b) for a neuronal-level depiction). The signposts in turn constrain the development of an idiosyncratic mapping grid to enable transcoding of numerals and numerosities that we do not have prior associative experience with. (4a) Numerals with established signposts may be transcoded directly to mental representations by retrieval. (4b) Numerals without established signposts undergo a linear-to-logarithmic transformation guided by the mapping grid during transcoding. (b) Squares depict neurons selective for a particular numerosity. Some neurons respond to nonsymbolic or symbolic input only, and some to both. (c) Computational stages involved in numerosity-selective encoding of nonsymbolic and symbolic stimuli: Sensory input is normalized for shape, size, and location rendering an object location map. Activity on the object location map is summed up. Summed activity is proportional to numerosity. Numerosity-selective neurons that are tuned to a preferred numerosity (e.g., 3) will be activated maximally. Activation of these neurons decreases with increasing numerical distance from its preferred numerosity. Symbolic input bypasses summation coding.

correlated with size of the items), and maintain the item size across some stimuli (such that numerosity is positively correlated with total surface area)[91]. Thus, on some trials, participants may use surface area as a cue, and on other trials, they may need to inhibit surface area. Leibovich and Ansari (2016)[11] argue that when children and adults learn to associate a series of numerals with nonsymbolic quantities (e.g., using number charts), they need to first disentangle the numerical and non-numerical magnitudes when attending to numerosity. Cognitive control is therefore a crucial factor to consider when nonsymbolic stimuli are used in real-world learning and experimental contexts[11]. The impact of confounding non-numerical magnitudes is sometimes observed during estimation tasks[17,21], but not always[75,92], depending on methodological considerations such as numerosity range, task, and the extent of control of the stimulus non-numerical properties. Nonetheless, the integrity of transcoding nonsymbolic quantities to mental representations is crucial for the 'mapping' hypothesis because laboratory tasks involving nonsymbolic stimuli are unable to provide a pure and direct measure of the mental representations of individual numerosities or the ANS as a whole[11,93].

In sum, transcoding of nonsymbolic stimuli to mental representations may have a numerosity-specific processing pathway. At the task level, complete independence from non-numerical magnitude processing is impossible, and inhibitory control is necessarily involved[11]. However, it is important to note that Leibovich and Ansari's (2016)[11] concern does not undermine the fact that in real-world contexts, children and adults *can* attend to numerosity, and *can* ultimately learn the associations between numerosities and numerals if they are motivated to. Hence, the nature of formed 'mappings' is orthogonal to how they are formed.

# Associative Learning (Component 2)

Children go through a protracted period of about a year from 2.5-3.5 years of age to learn the meaning of "one" (vs. "some"), followed by "two" ("one" and "two" vs. "some), then "three", and finally "four" before they understand that the last number word during counting represents the total number of items in a set for all other numbers within their counting range[94,95]. Contemporary models implicate both the OTS and ANS in children's acquisition of the meanings of "one" through "four"[41,42,96]. In Spelke's (2017)[42] model, whenever a child sees three items and hear the word "three", the word is associated with the OTS representation of three individual items held in visual working memory. The word "three" can then replace the active maintenance of the representation of three individual items in visual working memory, freeing up the OTS, which allows the ANS to come online to represent approximate 'threeness' of the set and be associated with "three"[42]. In other words, number words are crucial in linking the mutually exclusive OTS and ANS[42]. With repeated exposure, children then correlate the word "three" with both an exact

representation of three individual items and an approximate representation of 'threeness' (**Figure 1**; Component 2)[41,42].

Numerals beyond "four" can also be learned by association with nonsymbolic quantities, but given the attentional limits of the OTS, only the ANS can support the associative learning of large numerals (e.g., see **Figure 1** for "thirty")[6,41,42,96]. It is, of course, impossible for us to form associations between *every* number word and a nonsymbolic quantity. However, it is likely that we can learn *some* large numerals by associative learning[76,97]. Although associative learning for large numerals may be largely idiosyncratic[76,97–99], there are some universal regularities. For instance, certain large numerals are frequently used in spoken and written communication across languages and cultures[100]. Dehaene and Mehler (1992)[100] observed that the frequency use of numerals decreases with increasing numerosity, even for numerals 1 to 9, but the frequency of numerals such as 10, 12, 15, 20, 50, and 100 ('round' numbers) are significantly higher than their neighboring numerosities. The elevated frequency of 'round' numbers suggests that we may have more associative experience with them (e.g., eggs come in a dozen, small items are often sold in multiples of 10) such that we come to have an approximate grasp of the quantities the round numbers represent[100]. Alternatively, the ANS may provide psychological constraints that allow us to better grasp and use these round numbers as points of references[100]. Moreover, Izard and Dehaene (2008)[74] observed that when adults were asked to estimate arrays containing 9 to 100 dots, they tended to assign as many as 40% of their estimates to numbers below 10 and the decade numbers (10, 20, 30, etc.).

Large numerals learned by associations with approximate quantities have also been shown to be constrained by the ANS. Several studies that trained adults to associate large approximate quantities (10–90) with artificial symbols have observed canonical distance or ratio effects in a numeral comparison task with the learned symbols[101–105]. This suggests that the acquired symbols are possibly linked with the mental representations of the ANS, which in turn influences learners' usage of these artificial numerals.

Taken together, associative learning is a key mechanism for numerals 1 to 4, and may underlie the learning of some large numerals as well, especially round numbers. In the TLM model, numerals learned via associations are then linked with the mental representations of the ANS to establish symbolic 'signposts' or reference points along the mental number line.

# Established Symbolic 'Signposts' for 'Mapping Grid' (Component 3)

The symbolic signposts can be conceptualized as a common set of neurons tuned preferentially to a nonsymbolic quantity and its associated numeral as a result of Hebbian learning. As numerals are represented as exact rather than approximate numerosities

during associative learning, the tuning functions of numerosity-selective neuronal populations to numerals gradually become sharpened (see **Figure 1**(a); yellow and blue tuning functions for numerosity 3)[71]. This sharpening of the tuning functions may be supported by feedback from categorical-coding neuronal populations in the prefrontal cortex[106–109], and by local inhibitory interneurons that are responsible for crafting the numerosity-selectivity of the neurons even before numeral learning[49,108]. As a result, a subset of the initial pool of numerosity-selective neurons that respond more reliably to 3 objects would respond to "three" as well (i.e., a symbolic signpost or shared neural mapping; see Figure 1(b))[4,5,49,58]. Such sharpening of the tuning functions for numerals tend to be observed only in the left intraparietal sulcus, possibly due to the left-lateralization for exact and categorical representations[5,67,110], or to maturation and experience with symbolic knowledge such as language[70,111]. Notably, the acquisition of numeral knowledge and higher-order math skills may reciprocally sharpen the tuning functions for nonsymbolic input, resulting in better overall acuity of the ANS[4,5,21,63,112–116]. This possibility has been supported by the finding of neurons that code for both symbolic and nonsymbolic inputs after monkeys have been trained to associate Arabic digits 1-4 with their corresponding nonsymbolic quantities[58,117]. While there are format-independent coding neurons, human fMRI studies[110,118] and monkey single-cell recordings[58] have found distinct neuronal populations coding for one format or the other. Hence, our model proposes that there are also numerosity-selective neuronal populations that code for numerals only (see Figure 1(b)). It is possible that these numerosity-selective neurons specific to numerals may code for both spoken number words and Arabic numerals[69]. Some of these may be asemantic and may not respond to numerosity *per se*. This is because preschoolers first learn to associate spoken number words with nonsymbolic quantities, followed by number words with Arabic digits, and finally digits with nonsymbolic quantities[19,119–121]. This developmental trajectory with number words mediating the links between digits and nonsymbolic quantities[19,119] suggest that when the verbal labels for digits are first learned, children do not immediately associate digits with any numerical meaning. However, it is possible that there are separate neurons coding for either symbolic format[122,123].

Next, our model proposes that these symbolic signposts may *constrain* the location along the mental number line that a numeral activates (see **Figure 1**(a), Component 3; red sections). The ordinal structure of the mental number line allows for an idiosyncratic symbolic 'mapping grid' to be established[74]. This mapping grid, which retains the logarithmic scale of the ANS[74], then allows us to systematically transcode between numerals or nonsymbolic quantities even for those we do not have any prior associative experience with. Firstly, the existence of such an ordinally structured symbolic mapping grid is suggested by evidence that a single instance of calibration (e.g., showing 30 dots and labeling it as "30") tend to lead participants to modify their subsequent estimates not only locally for the calibrated numerosity (30), but for all other numerosities

tested[74,76,97–99,124,125]. Hence, this local-to-global calibration suggests that mappings between numerals and nonsymbolic quantities are highly interdependent. Using a similar calibration paradigm, Yeo and colleagues (submitted for publication)[98] found that while adults modify most of their estimates for large numerosities, some large numerosities appeared unaffected by calibration within each participant resulting in discontinuities in the effect of calibration in majority of the participants. The findings suggest the possibility of interspersed symbolic signposts for large numerosities that might have been established through associative learning. Secondly, estimates tend to show scalar variability, a behavioral signature of the ANS[21,74]. Nonetheless, calibration studies provide strong evidence that the established mapping grid is not fixed and is highly malleable in both children[97,99,126,127] and adults[74,76,98,124,125,128–130].

Taken together, symbolic signposts can be established along the mental number line, whose ordinal structure allows for the development of an idiosyncratic, but malleable, symbolic mapping grid. This mapping grid supports transcoding of numbers that we may not have prior experience with. Indeed, the role of ordinal relations between numerals has been argued as an alternative to the 'mapping' hypothesis[12]. We propose that such ordinal relations alone do not suffice in explaining the canonical behavioral signatures of the ANS often observed with numerals, or that the transcoding between numerals and nonsymbolic quantities is highly constrained (e.g., not estimating 100 items as "10,000"). We also hypothesize that some neuronal populations that are format-independent[58,131] may underlie the linking of the symbolic mapping grid and the ANS. Neuroimaging experiments investigating such a shared neural mapping between symbolic and nonsymbolic formats have presented mixed results[67,110,132–137]. The TLM model seeks to reconcile these mixed findings in the next few sections.

# Symbolic Stimulus-to-Representation Transcoding (Component 4)

Spoken number words are hypothesized to be first processed by left-hemispheric perisylvian language regions extending to the temporoparietal junction[77,138,139]. Arabic numerals, on the other hand, have recently been shown to be processed by a region in the inferior temporal gyrus (**ITG**) that is distinct from regions involved in processing other symbol categories such as letters (putative "number form area"[77])[140,141]. However, the specific computations that the number form area performs are still unknown[142,143]. For number phrases (e.g., "twenty-eight"), the left inferior frontal gyrus and inferior parietal lobule are additionally recruited for syntactic processing, particularly in merging the constituent elements into whole magnitudes[144]. It is likely that similar mechanisms may subserve the place-value processing of multi-digit numerals (e.g., "28")[145], which may not rely on verbal representations[146]. Subsequently, both spoken number words and Arabic numerals are

hypothesized to be transcoded to mental representations via numerosity-selective coding in the left intraparietal sulcus (IPS)[64,65,69,70,85], bypassing the summation coding stage necessary for nonsymbolic stimuli[85,147] (**Figure 1**(c)).

In the TLM model, the transcoding process from numeral to mental representation differs significantly depending on whether a presented numeral has an established signpost. It should be noted that a signpost can be established on an ad-hoc basis, even just after a single instance of learning[74] (e.g., calibrating participants to a reference numeral-nonsymbolic quantity association prior to a task). For numerals with such established signposts, the mental representations may be directly activated (**Figure 1**(a), Component 4(a)). For numerals that do not have established signposts, they are likely to undergo a linear-to-logarithmic transformation during transcoding (**Figure 1**(a), Component 4(b))[75,148]. This is because, in a standard laboratory task, multiple numerals are presented, and are first represented on an objective *linear* number line (e.g., 5, 6 and 7 are equally spaced), which differs from the logarithmic scale of the ANS.

As shown in **Figure 1**(a), the linear-to-logarithmic transformation (Component 4(b)) can account for a tendency to spontaneously overestimate in numerosity production tasks[75,148]. It is also likely that the implementation of this linear-to-logarithmic transformation between representations may explain why 2.5-year-olds typically take about a full year to learn the meanings of "one" through "four"[149]. Children and even highly numerate adults have been shown to represent symbolic numerosities approximately on both linear and logarithmic scales, depending on their familiarity with the number range[126,127,129,130,150–153]. This suggests that we can flexibly switch between scales depending on our numeral experience and task demands. How such transformations are implemented neurobiologically is yet unknown.

There is some neuroimaging evidence to support the retrieval pathway that is driven by prior associations (**Figure 1**(a); Component 4(a)). Using an fMRI-adaptation paradigm, Piazza and colleagues (2007)[67] sought to investigate whether numerals and nonsymbolic quantities activate a common population of numerosity-selective neurons in adults. The authors first had participants learn to associate 17 to 20 randomly arranged dots with "approximately 20" and 47 to 50 dots with "approximately 50". This calibration was done to account for participants' tendency to underestimate large numerosities, possibly due to the linear-to-logarithmic transformation. The authors then adapted participants' neural responses to either dot arrays or numerals, using small (17-19) and large (47-49) numerosities. Participants were told to "pay attention to the quantity conveyed by the stimuli" (p. 303). After adaptation, they presented a new or deviant numerosity (20 or 50), which could be in the same format (e.g., "17", "19", "18", …"50") or a different format (e.g., "18", "17", "19", …fifty dots). Numerosity-selective neuronal populations were predicted to be more sensitive to a large numerosity change than a small numerosity change, and would

then recover from adaptation to a large numerosity change with an increase in activation. If both adaptation and recovery from adaptation was irrespective of the deviant stimulus' format, that suggested the presence of some neurons coding for a format-independent representation of numerosity. Indeed, the authors found such format-independent numerosity-selective neuronal population in multiple brain regions, including the bilateral IPS and frontal regions. The critical aspect of this study in relation to the TLM model is that numerals and nonsymbolic quantities *can* activate a common population of numerosity-selective neurons, *especially when there is prior associative learning*. When there is no prior associative learning, however, the TLM model predicts that a linear-to-logarithmic transcoding of numerals would result in a mismatch in the locations of the mental number line activated by nonsymbolic quantities and their corresponding veridical numerals (see Figure 1(a); "Thirty" may activate a representation of a larger numerosity than an array of 30 dots would). There is indeed evidence that adaptation to nonsymbolic quantities such as that in the study by Piazza and colleagues (2007)[67] is due to the *perceived* numerosities rather than *veridical* numerosities[154]. Hence, it is crucial for future experiments investigating a shared neural mapping between stimulus formats to consider whether the veridical numerosity of nonsymbolic stimuli matches the perceived numerosity in each participant. In fact, Liu, Schunn, Fiez, and Libertus (2018)[137] recently used electroencephalography (**EEG**) to provide support for this methodological consideration. In their study, adult participants passively viewed during EEG recording a series of dot arrays superimposed with one- and two-digit Arabic numerals that were either matched in terms of numerosity ("36" and 36 dots) or mismatched ("36" and 24 dots). Importantly, they also had participants complete a separate numerosity perception task to obtain participants' idiosyncratic perceived numerosities. No significant differences in the event-related potentials (**ERP**s) between the matched and mismatch conditions were found when the *veridical* numerosities of the dot arrays were used. However, using the idiosyncratic *perceived* numerosities revealed a significant difference in the ERPs between the matched and mismatched conditions. Their findings suggest that future neuroimaging studies can minimize the discrepancy in the activated locations on the mental number line by carefully matching a perceived dot array (e.g., 50 dots) to a numeral (e.g., "40")[137]. Alternatively, future studies should calibrate participants to associate numerals with nonsymbolic quantities prior to a task[67].

In summary, numerals may undergo a linear-to-logarithmic transformation unless there is prior associative experience. The linear-to-logarithmic transformation may lead to a mismatch in activation locations on the mental number line by veridical and perceived numerosities, which could partly account for the absence of evidence of a shared neural mapping between numerals and nonsymbolic quantities.

## CANDIDATE REVIEWS

# Absence of Evidence for Shared Neural Mapping and Alternative Explanations

A series of fMRI[110,118,132–135] and magnetoencephalography (MEG)[136] studies have used multi-voxel pattern analytic approaches such as representational similarity analyses (**RSA**) and decoding to investigate a shared neural mapping between nonsymbolic quantities and numerals. In an RSA, significant correlations between the fine-scaled spatial activation patterns evoked by dot arrays and their corresponding digits are indicative of a shared neural mapping. In a decoding analysis, a classifier is trained to distinguish the activation patterns between different dot arrays and different digits. The classification accuracy of the trained classifier on an independent set of data is then measured. Successful generalization in classifying digits from information decoded from dot arrays, and vice versa, is indicative of a shared neural mapping. The evidence thus far has been mixed. Here, we put forth some methodological considerations that could account for an absence of evidence in these studies.

Firstly, all of these studies other than Piazza and colleagues' (2007)[67] have focused exclusively on numerosities 1-9 without much justification. One possibility is that there are presumed established mappings due to their more frequent use compared to multi-digit numerals[100]. However, there is ample evidence that estimation of numerosities 5-9 tend to be highly error-prone[32,33,155]. Hence, it may not be justified to expect participants to consistently associate 7 dots with "7", much less to have a shared neural mapping for 7 dots and "7" without prior learning. We hypothesize that calibration via associative learning may be necessary even for single digits. Alternatively, instead of random dot patterns that were used in these studies reporting an absence of evidence, canonical dice dot patterns could be used as they are easily recognizable[33,156]. Indeed, although fMRI studies have failed to decode or find significant representational similarity across formats using random dot patterns[110,132,133,135], Teichmann and colleagues (2018)[136] have recently found significant representational similarity and have successfully decoded across formats with *canonical* dice dot patterns using MEG. Nonetheless, it is important to note that canonical dice dot patterns may be perceived symbolically, as standing for a number (e.g., Roman numeral III). Future studies can test these hypotheses.

Secondly, what participants are told to do with the stimuli may be crucial. Bulthé and colleagues (2014, 2015)[132,133] had participants compare each digit or dot array (e.g., 1, 2, 4, 8) to a fixed reference quantity (e.g., 5). Using decoding, a shared neural mapping between formats were not observed. It is important to note that the mental representations measured with fMRI's temporal resolution may not be of the estimation stage *per se*, but also of the comparison stage[50]. It is likely that the estimation stage more directly reflects an access of the mental number line than the comparison stage[50]. To dissociate these stages in a numerosity comparison paradigm, Eger and colleagues (2009)[110] used a delayed

match-to-sample design, in which they introduce a delay between a sample stimulus (e.g., 8 dots) and the comparison of a match stimulus (e.g., "2") with the sample. Only the mental representations of the sample stimuli were analyzed. Also using decoding, the authors found partial support for a shared neural mapping. Specifically, while the classifier trained with digits successfully predicted the numerosity of a dot set, the classifier trained with dot sets was unsuccessful in predicting the numerical representation of digits. This asymmetric generalization across formats was also observed by Piazza and colleagues (2007)[67], and were interpreted to indicate a more precise tuning function for numerals than for nonsymbolic quantities[71]. This in turn leads to poorer generalization from numerals to nonsymbolic quantities than the converse. Interestingly, Lyons and colleagues (2015)[135] also used a delayed match-to-sample paradigm in which participants were told to indicate whether the sample and match stimuli were numerically equal or different, but failed to find significant representational similarity between formats. The null finding could be due to the use of the same format for both sample and match stimuli within each trial. This might influence participants' strategy particularly for digits, such as using verbal or shape matching, as the canonical behavioral distance effect was not found for digits[157].

# Representation-to-Estimate Transcoding (Component 5)

An activated mental representation may finally be transcoded to a nonsymbolic estimate. In numerosity production tasks, participants are typically required to select from a series of dot arrays (e.g., by rotating an analog dial) one array that corresponds to a given numeral. If numeral "thirty" has previously been associated with an array of 30 dots, and has an established signpost, the mental representation for 30s will be activated via retrieval, which can then be transcoded to a *calibrated* nonsymbolic estimate (**Figure 2**; Component 5(a) blue tuning function). If, however, no prior association has been established for "thirty", a mental representation of a larger numerosity will be activated due to the linear-to-logarithmic transformation. This is then transcoded to a *spontaneous* nonsymbolic estimate, leading to a typical overestimation (Figure 2; Component 5(a), green tuning function)[75,158]. Importantly, the response selection process inevitably involves iterative nonsymbolic stimulus-to-representation transcoding (i.e., Component 1), possibly until a nonsymbolic array activates a mental representation that matches the initial representation activated by the numeral[47,159,160]. Inhibitory control may thus play a critical role during this transcoding process[11,93].

When transcoding an activated mental representation to a symbolic estimate, an individual is confronted with two unique challenges (**Figure 2**; Component 5(b)). Firstly, the individual has to choose from multiple response bins from the mapping grid (e.g., 30-39 vs. 20-29 and 40-49) and sample an integer from the chosen bin. To overcome this,

inhibitory control may be crucial to suppress the noise from the signal. Secondly, a logarithmic-to-linear transformation of the established mapping grid is necessary during a task that requires multiple symbolic estimates to be made[74,75,159]. As proposed by Izard and Dehaene (2008)[74], the established mapping grid itself is not directly used to generate an estimate, but undergoes a spontaneous idiosyncratic affine transformation (i.e., compressed or stretched and/or shifted). This transformation is likely to be common in numerate participants as they may be motivated to ensure that their estimates would make sense linearly or proportionally with their prior estimates. For instance, if an array of 30 dots was assigned "20", an array of 90 dots would need to be around "60" and not "120". This may involve knowledge of analogical reasoning[76,97,99]. Addition and subtraction are also possible strategies. Nonetheless, numerosities 1-4 and other strongly established symbolic signposts for larger numerals may be less resistant[76,97–99]. The logarithmic-to-linear transformation of the remaining segment of the mapping grid results in a spontaneously rescaled mapping grid (see Figure 2). In the presence of an external calibration, a calibrated mapping grid results from another iteration of affine transformation[74]. This transformation from the spontaneous mapping grid to calibrated mapping grid has found to be moderated by calculation competence[98] and analogical reasoning[99]. The importance of such advanced skills in supporting the logarithmic-to-linear transformation is consistent with a developmental lag in which children tend to be less successful in transcoding approximate quantities to verbal number words (logarithmic-to-linear) than the reverse (linear-to-logarithmic)[17,159,161]. Finally, the hypothesis that the calibrated and spontaneous mapping grids are constrained versions of the established mapping grid has received support with evidence of a high reliability ($r > .7$) between participants' spontaneous and calibrated estimates across various estimation metrics[74,98].

In summary, transcoding from a mental representation to either nonsymbolic or symbolic estimate necessarily involves inhibitory control. Any numerosity perception or production task should thus statistically account for inhibitory control when individual differences are examined[20,162–164]. Moreover, transcoding to a symbolic estimate may involve advanced reasoning skills (e.g., analogical and mathematical) to better support the logarithmic-to-linear transformation. The ability to dissociate the spontaneous and calibrated mapping grids from the ANS is also consistent with the hypothesis that over development, numerals may become more estranged from the ANS as we become more reliant on the syntax of numerals (e.g., place value of the base-10 system) [12,131,165–167]. With this final section, it should be clear that estimation is not a trivial process, but a multifaceted process that we have only just begun to unravel.

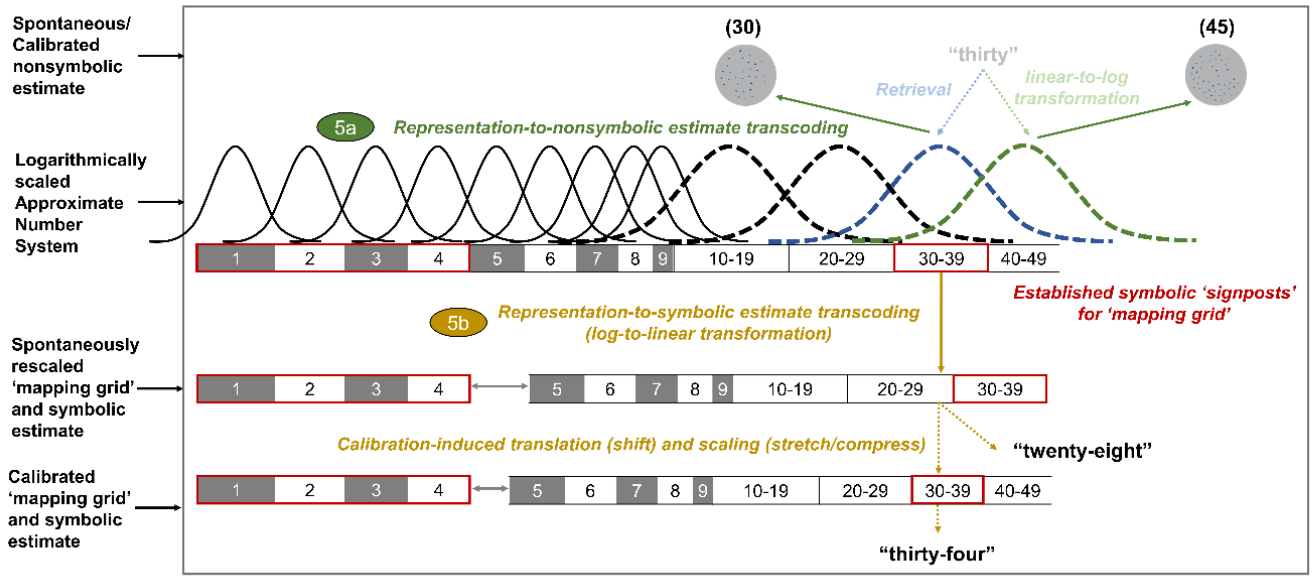


**Figure 2.** Transcoding of activated mental representations to nonsymbolic and symbolic estimates. (5a) Resulting nonsymbolic estimate depends on the activation location on the mental number line. (5b) The established mapping grid can be rescaled spontaneously or with an external calibration to generate linear-like estimates.

# Concluding Remarks

In this review, we draw from the developmental, cognitive, and neuroscience literature to integrate extant models of how meanings of numerals are learned and accessed. This unifying Translation-Learning-Mapping (**TLM**) model distinguishes transcoding, associative learning and shared neural mappings, so as to provide a more precise nomenclature of the processes and products related to the 'mapping' hypothesis. The model is certainly not without limitations. Firstly, it is limited to estimation. To comprehend that "98" is one more than "97" does not rely on approximate representations in the model, but on language[168]. The symbolic mapping grid alone may however aid computational estimation (e.g., estimating tip amounts). Secondly, as the ANS has an upper bound[169–171], the mapping grid ought to have one too, but what it might be has yet to be defined. Nonetheless, the TLM model clearly shows that estimation tasks do not directly measure the ANS or the access to the sparse shared neural mappings, but a composite of multiple domain-specific and domain-general (e.g., inhibitory control) processes. Hence, the jury is still out on whether the 'mapping' hypothesis is fully supported until we gather more evidence pertaining to the several hypotheses put forth by the TLM model.

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