BEHAVIORAL PHARMACOLOGY

Copper on the Brain

Jason Rihel

Understanding how metals contribute to brain function is a major health priority. A new study combining pharmacology and genetics implicates the accumulation of copper in a brain arousal center as a regulator of zebrafish activity.

The brain is a ravenous user of transition metals, especially copper, zinc, and iron. These metals serve as critical co-factors for enzymes that play fundamental roles in the regulation of DNA, cellular metabolism, redox regulation, neuronal firing, and synaptic plasticity. Little wonder then that the disruption of metal homeostasis in the brain has been linked to myriad neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer's, and Parkinson's disease[1]. A better understanding of how metals impact normal brain function will therefore likely provide insights into disease, but methodological challenges of observing and manipulating metals *in vivo* have hindered progress. Xiao et al. leverage the optical translucency and facile genetics of the zebrafish to demonstrate how deficiency in one metal, copper, alters the behavioral output of an arousal-regulating neuronal circuit[2].

Copper is an essential trace element that strongly accumulates in the brain, where it participates in maintaining the redox balance of our most energetic organ. Copper is also an essential co-factor for many enzymatic reactions involved in critical brain functions[3]. One such enzyme is dopamine-beta hydroxylase (DBH), which serves in the final biosynthetic step in the production of the neurotransmitter norepinephrine (NE). NE is predominantly made by neurons in a small brainstem area called the locus coeruleus (LC), incidentally the site of the highest copper concentrations in the human brain[4]. NE-expressing neurons in the LC send widespread processes throughout the brain and are a major positive regulator of arousal and wakefulness in vertebrates[5]. Although some patients with genetic disruption of copper metabolism (e.g. Wilson's disease) report daytime sleepiness[6], whether copper deficiency alters NE production in the LC to impact sleep-wake behavior had not been tested.

To investigate a copper-LC-behavior connection, Xiao et al. turned to the larval zebrafish, an excellent system in which to forge these links because of the ability to image deep into the brain and to readily manipulate behavior with genetics and pharmacology. The authors first developed a novel molecular sensor of copper, Copper Fluor-4 (CF4), which, together with the copper insensitive dye, Control Copper Fluor-4 Sulfur 2 (Ctrl-CF4-S2), can penetrate into the live zebrafish brain and sensitively visualize copper levels with high selectivity over zinc and iron. Using these dyes together with direct measurements of total copper distribution in the brain by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), they observed dynamic changes in labile copper accumulation during brain development that was restricted to neuronal processes and the brain ventricles. In contrast, zinc accumulated in the neuronal cell bodies, demonstrating that the distribution of these metals is tightly and differentially regulated. As predicted, copper deficient *calamity* mutants, which harbor mutations in the copper transporter ATP7A[7], showed a large loss of brain copper levels, especially in neuronal processes. This loss was fairly specific to the brain, as total body levels of copper in *calamity* mutants was similar to wild type larvae.

Previous work had demonstrated that NE signaling from the LC in zebrafish larvae is critical for arousal, as in mammals[5]. To investigate whether copper deficiency impacts zebrafish arousal behaviors that are mediated through the LC, Xiao et al. examined by videography the impact of copper depletion on larval behavioral activity during a 14 hour:10 hour light:dark cycle and on startle responses to an acoustic stimulus. They found that both genetic (*calamity*) and pharmacological (copper chelation) reduction of brain copper altered larval behavior in a similar manner to laser-guided ablation of the LC, suggesting that copper loss affects behavior via the LC. Consistent with this interpretation, larval LC neurons strongly expressed the copper transporters CTR1 and ATP7A, the production of NE is reduced in the LC of the copper deficient *calamity* mutant, and drugs that boost NE signaling can partially rescue *calamity* behavior. Intriguingly, they also found that the switch to relatively high production of NE over dopamine emerged in vertebrate evolution at the time when the ATP7 copper transporter gene family expanded.

What emerges is a model in which copper is loaded into the LC and then shuttled by ATP7A to the DBH enzyme for the production of the neurotransmitter NE, which is then involved in modulating behavioral arousal (Fig. 1). However, this is only one copper-dependent enzyme/circuit/behavior, and there are likely to be many others, as humans with disorders of copper metabolism display a variety of neurological phenotypes[8]. Zebrafish have a wide range of genetically tractable behaviors, from visual-guided behaviors to social preference, and neuronal activity can be non-invasively monitored throughout the brain at single cell resolution[9]. How the brain and behavior are affected in copper deficient mutants should uncover additional copper sensitive circuits. Finally, how other transition metals, such as manganese, impact the zebrafish and human brain have received recent experimental attention[10]. Xiao et al.'s work provides a template for using the fish model to probe the role of metal in development, behavior, and disease.

Figure 1. During wakefulness, the locus coeruleus (LC) delivers norepinephrine (NE) across the brain to maintain arousal. In the LC, the copper transporters CTR1 and ATP7A load copper onto dopamine-beta hydroxylase (DBH), which converts dopamine (DA) into NE. Loss of copper transport impacts NE production and reduces arousal.

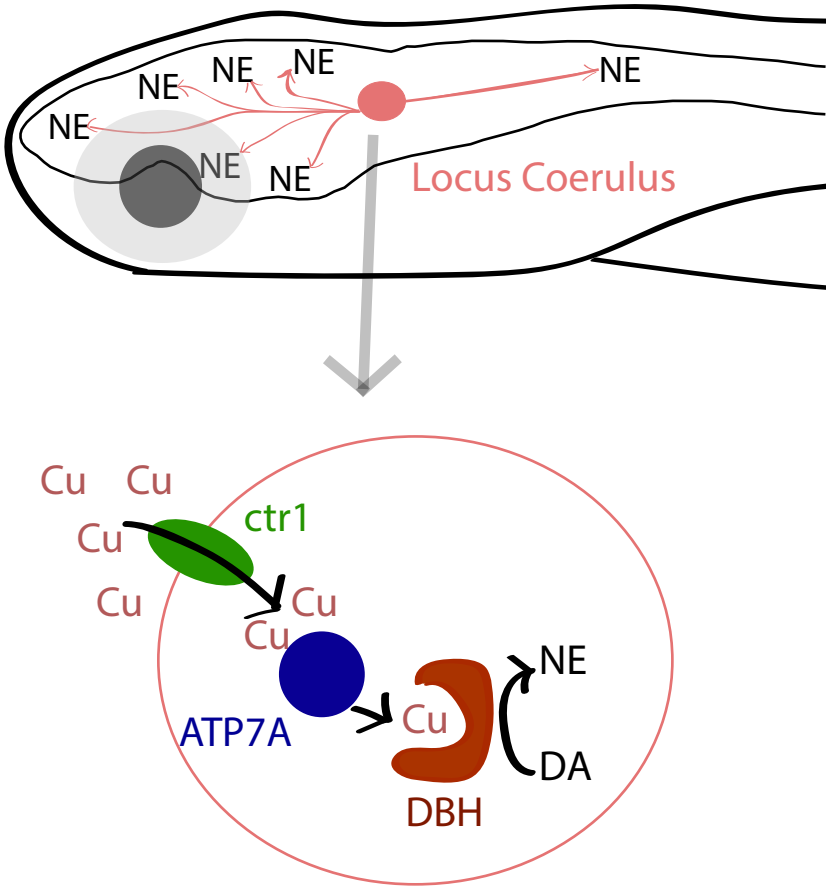
Jason Rihel is in the Department of Cell and Developmental Biology, University College London, London United Kingdom

e-mail: j.rihel@ucl.ac.uk

References:

1. Bush, A. I. *Curr. Opin. Chem. Biol.* **4,** 184–191 (2000).
2. Xiao, T. *et al. Nat. Chem. Biol.*
3. Ackerman, C. M. & Chang, C. J. *J. Biol. Chem.* **293,** 4628–4635 (2018).
4. Warren, P. J., Earl, C. J. & Thompson, R. H. *Brain J. Neurol.* **83,** 709–717 (1960).
5. Singh, C., Oikonomou, G. & Prober, D. A. *eLife* **4,** e07000 (2015).
6. Nevsimalova, S. *et al. Eur. J. Neurol.* **18,** 184–190 (2011).
7. Madsen, E. C. & Gitlin, J. D. *PLoS Genet.* **4,** e1000261 (2008).
8. Madsen, E. & Gitlin, J. D. *Annu. Rev. Neurosci.* **30,** 317–337 (2007).

9. Orger, M. B. & de Polavieja, G. G. *Annu. Rev. Neurosci.* **40,** 125–147 (2017).
10. Tuschl, K. *et al. Nat. Commun.* **7,** (2016).


NE
NE
NE
NE
NE
NE
NE
Locus Coerulus
Cu
Cu
Cu
Cu
ctr1
Cu
Cu
ATP7A
Cu
NE
DA
DBH