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Research Summary

There is one common theme in my Behavioral Genetics Lab. Ultimately, we want to understand and predict fundamental behavioral processes using simple and well-defined mechanisms at the level of information processing in specific pathways, circuit functions, cellular properties, synaptic plasticity or molecular events. We often use disease models to give us a unique angle, an abnormal state, to help us understand normal mechanisms at molecular, cellular, circuits and systems levels. In return, these studies often open new opportunities for the understanding and treatment of those diseases. We are especially interested in the basal ganglia function and disease. Our approaches include mouse genetics, fly genetics, optogenetics, animal learning paradigms, pharmacology, electrophysiology, biochemistry and RNA-seq.

Research Projects

1. The role of dopamine in reward and reward-dependent behavioral modification

Animal behaviors can be largely modified by reward/punishment histories. Understanding the neurobiological basis of reward learning, motivation and response selection is a critical step in understanding many mental disorders such as addiction and depression. We are especially interested in dopamine, the corresponding postsynaptic signaling pathways and corticostriatal plasticity in the above processes.

Our earlier findings indicate the role of distinct dopamine signaling in reinforcement learning and exploration-exploitation choice bias. As an extension of the above research, how do reward learning and economic decision making ultimately affect fitness? In a natural environment, these behaviors are critical for maximizing rewards/gains and minimizing risks/losses and for survival. We are investigating these more complex behaviors (e.g foraging) in a semi-natural environment and how genetic variations may affect fitness in this context. We

take advantage of microeconomic analysis of feeding behavior, combining mouse genetics and fly genetics. Fly genetics allows us to do gene discovery work while mouse genetics allows us to examine the neurobiological basis rigorously and provides the relevance to human conditions.

2. The role of dopamine in motor learning and motor performance

In parallel to studies on the role of mesolimbic dopamine in reward learning and response selection, another focus of the lab is on the role of nigrostriatal dopamine, the corresponding postsynaptic signaling pathways and corticostriatal plasticity in motor learning and motor performance, in particular, in the context of Parkinson's disease symptoms and therapies.

In the nigrostriatal pathway, dopamine modulates the intrinsic excitability of striatal neurons. However, it also modulates corticostriatal plasticity, potentially producing cumulative and long-lasting changes in motor performance. Our findings indicate that loss of dopamine leads to both direct motor performance impairments as well as D2 receptor-dependent and task-dependent inhibitory motor learning that gradually and cumulatively deteriorates motor performance. We hypothesize that such inhibitory learning is accompanied by increased LTP in the indirect pathway corticostriatal synapses. We are using a number of approaches to reduce such LTP as a novel therapeutic strategy for Parkinson's disease.

3. The biochemical basis of dopamine neuron degeneration in Parkinson's disease

Parkinson's disease is caused by progressive loss of dopamine neurons. Its biochemical basis is poorly understood. Our earlier studies using transgenic mice indicate that dopamine itself can cause oxidative stress. We hypothesize that under normal conditions, dopamine neurons are able to handle such cellular

stress. However, in aged animals or in animals with genetic defects, dopamine neurons may die when protective mechanisms are impaired (e.g. defects in protein folding and/or protein degradation pathways). We have recently developed a novel positive feedback gene amplification system to overexpress genes specifically in dopamine neurons. Such an approach has allowed us to mimic human genetic mutations with dominant inheritance and develop Parkinson's disease models with severe mitochondria pathology and progressive dopamine neuron degeneration. We are using these models to test the above hypotheses.

4. Genetic and neural mechanisms of PNPO deficiency in Vitamin B6-dependent epilepsy.

Pyridoxine 5'-phosphate oxidase (PNPO) converts inactive forms of vitamin B6 (VB6) in a diet, to the biologically active form, pyridoxal 5'-phosphate (PLP). PLP is a cofactor for enzymes required for the syntheses of dopamine, serotonin and GABA. Mutations in *PNPO* have been increasingly reported in neonatal epileptic encephalopathy and early-onset epilepsy patients. *PNPO* is one of the sixteen main epilepsy genes involved in the common epilepsies as well. However, our understanding of the neurobiological mechanisms of PNPO deficiency is limited. We have identified a *Drosophila PNPO* gene, established the first animal model of PNPO deficiency and found that PNPO deficiency leads to seizures. We have since generated fly knock-in lines in which the endogenous *Drosophila PNPO* gene was replaced by disease-causing human *PNPO* cDNAs. In these models, severe PNPO deficiency leads to lethality in early development, intermediate PNPO deficiency results in conditional lethality and seizures, whereas mild PNPO deficiency shortens lifespan. We are now characterizing mouse knock-in lines with disease-causing *PNPO* mutations and performing human genetic studies.

5. Functions of mRNA methylation (m6A) in adult neurons

N6-methyladenosine (m6A), the most abundant internal eukaryotic mRNA modification, affects almost every phase of mRNA metabolism and function by regulating the splicing, nuclear export, transport, localization, stability and translational efficiency of mRNAs. Although m6A is already known to be important in the nervous system, its unique significance in spatial temporal control of protein synthesis in neurons and synapses, and in learning and memory has not been systematically studied. We are taking genetic, behavioral, electrophysiological, anatomical, biochemical and live imaging approaches to study m6A function in neurons and in behaving mice. The causal effect of m6A will be studied in mutant mice with cell type specific m6A deficiency or mutant mice defective in m6A reader proteins (specific RNA binding proteins that recognize m6A to determine the fate of the modified RNA and their downstream effects).

Select Publications

Project 1.

Pecina S., Cagniard B., Berridge K.C., Aldridge J.W. & Zhuang X. (2003) Hyperdopaminergic mutant mice have higher 'wanting' but not 'liking' for sweet rewards. *J. Neurosci.* 23, 9395-9402.

Cagniard B., Beeler J.A., Britt J.P., McGehee D.S., Marinelli M. & Zhuang X. (2006) Dopamine scales performance in the absence of new learning. *Neuron* 51, 541-547.

Kheirbek M.A., Beeler J.A., Ishikawa Y. & Zhuang X. (2008) A cyclic AMP pathway underlying reward prediction in associative learning. *J. Neurosci* 28, 11401-11408

Beeler J.A., Daw N.D., Frazier C.R.M. & Zhuang X. (2010) Tonic dopamine modulates exploitation of reward learning. *Frontiers in Behavioral Neuroscience* 4, 170

Beeler J.A., McCutcheon J.E., Cao Z.F.H., Murakami M., Alexander, E. Roitman M.F. & Zhuang X. (2012) Taste uncoupled from nutrition fails to sustain the reinforcing properties of food. *Eur. J. Neurosci.* 36, 2533-2546

Beeler J.A., Frazier C.R. & Zhuang X. (2012) Putting desire on a budget: Dopamine and energy expenditure, reconciling reward and resources. *Frontiers in Integrative Neuroscience* 6, 49.

Beeler J.A., Faust R.P., Turkson S., Ye H. & Zhuang X. (2015) Low Dopamine D2 Receptor Increases Vulnerability to Obesity Via Reduced Physical Activity Not Increased Appetitive Motivation. *Biological Psychiatry* 79, 887-897

Lee G.Y.C., Yang Q., Chi W., Turkson SA, Du WA, Zheng Z., Long M. & Zhuang X. (2017) Genetic architecture of natural variation underlying adult foraging behavior that is essential for survival of *Drosophila melanogaster*. *Genome Biol Evol.* 9, 1357-1369.

Project 2.

Kheirbek M., Britt J.P., Beeler J.A., Ishikawa Y., McGehee D.S. & Zhuang X. (2009) Adenylyl cyclase type 5 contributes to corticostriatal plasticity and striatum-dependent learning. *J. Neurosci* 29, 12115-12124

Beeler J.A., Cao Z.F.H., Kheirbek M.A., Ding Y., Koranda J., Murakami M., Kang U.J. & Zhuang X. (2010) Dopamine-dependent motor learning: Insight into L-dopa's long-duration response. *Ann Neurol*, 67, 639-647

Beeler J.A., Frank M.J., McDaid J., Alexander, E., Turkson S., Sol Bernandez M., McGehee D.S., & Zhuang X. (2012) A role for dopamine-mediated learning in the

pathophysiology and treatment of Parkinson's disease. *Cell Reports* 2, 1747-1761.

Zhuang X, Mazzoni P, Kang UJ (2013) The role of neuroplasticity in dopaminergic therapy of Parkinson's disease. *Nature Reviews Neurology*, 9, 248-256.

Koranda J.L., Cone J.J., McGehee D.S., Roitman M.F. Beeler J.A. & Zhuang X. (2014) Nicotinic receptors regulate the dynamic range of dopamine release *in vivo*. *J Neurophysiol.* 111, 103-111.

Augustin S.M., Beeler J.A., McGehee D.S. & Zhuang X. (2014) Cyclic AMP and afferent activity govern bidirectional synaptic plasticity in striatopallidal neurons. *J. Neurosci.* 34, 6692– 6699

Koranda J.L., Krok A.C., Xu J., Contractor A., McGehee D., Beeler J.A. & Zhuang X. (2016) Chronic nicotine mitigates aberrant inhibitory motor learning induced by motor experience under dopamine deficiency *J. Neurosci.* 36, 5228–5240.

Project 3.

Zhuang X., Masson J., Gingrich J.A., Rayport S. & Hen R. (2005) Targeted gene expression in dopamine and serotonin neurons of the mouse brain. *J. Neurosci. Methods.* 143, 27-32.

Chen L., Cagniard B., Mathews T., Jones S., Koh H.C., Ding Y., Carvey P.M., Ling Z., Kang U.J. & Zhuang X. (2005) Age-dependent motor deficits and dopaminergic dysfunction in DJ-1 null mice. *J. Biol. Chem.* 280, 21418-21426.

Chen L., Ding Y., Cagniard B., Van Laar A.D., Mortimer A., Chi W., Hastings T.G., Kang U.J. & Zhuang X. (2008) Unregulated cytosolic dopamine causes

neurodegeneration associated with oxidative stress in mice. *J. Neurosci.* 28, 425-433

Chen L., Xie Z., Turkson S. & Zhuang X. (2015) A53T human α -synuclein overexpression in transgenic mice induces pervasive mitochondria macroautophagy defects preceding dopamine neuron degeneration. *J. Neurosci.* 35, 890–905

Project 4.

Chi W., Zhang L., Du W. & Zhuang X. (2014) A nutritional conditional lethal mutant due to pyridoxine 5'-phosphate oxidase deficiency in *Drosophila melanogaster*. *Genes, Genomes, Genetics.* 4,1147-1154

Chi W., Iyengar A., Albersen M., Bosma M., Verhoeven-Duif N. M., Wu C.-F. & Zhuang X. (2019) Pyridox(am)ine 5'-phosphate oxidase deficiency induces seizures in *Drosophila melanogaster*. *Hum Mol Genet.* 28, 3126-3136

Project 5.

Koranda J.L., Dore L., Shi H., Patel M., Vaasjo L.O., Rao M.N., Chen K., Lu Z., Yi Y., Chi W., He C. & Zhuang X. (2018) Mettl14 is essential for epitranscriptomic regulation of striatal function and learning. *Neuron.* 99, 283-292.