As the primary inhibitory neurotransmitter in the central nervous system, γ-aminobutyric acid (GABA) plays a critical role in controlling and shaping neuronal activity. Due to its importance during regular brain function, dysregulation of GABA neurotransmitter synthesis or neurotransmission has devastating pathological consequences. Epilepsy is a highly heterogeneous neurological condition believed to stem from disruptions in the excitation and inhibition balance of the brain. The most common causes of epilepsy are brain lesions stemming from injuries, metabolic disorders, and genetic mutations that affect nervous system development or function. Since discovery of the first epilepsy-associated gene in 1995, nearly 1000 contributing loci and mutations have been found. However, despite many decades of research and drug development, more than a third of epilepsy patients remain resistant or develop resistance over time to available anti-epilepsy drugs (AEDs). The generation of novel genetic animal models as vehicles for developing new AEDs and understanding neural mechanisms underlying epileptogenesis and ictal propagation is therefore critical for future epilepsy treatment research.

One group of refractory epilepsies occurs in human patients with mutations in the pyridox(am)ine-5'-phosphate oxidase (*PNPO*) gene. PNPO metabolizes dietary inactive forms of vitamin B6 into the active pyridoxal-5'-phosphate (PLP), which is a critical cofactor for synthesis of GABA. Interestingly, human *PNPO* mutant variants cover a spectrum of phenotypes with varying degrees of influence on seizure onset time, severity, and comorbid conditions. *PNPO* was also identified as 1 of 16 most significant epilepsy risk genes, suggesting that some common *PNPO* variants cannot cause epilepsy by themselves but may contribute to epilepsy susceptibility through interactions with other genes or the environment (i.e., diet). Therefore, *PNPO* is a fascinating genetic factor for investigating pathogenic GABAergic circuitry and inhibitory control of brain activity. We generated novel genetic knock-in mouse models containing 2 *PNPO* point mutations identified in human epilepsy patients: D33V and R116Q. Homozygous D33V mutants require supplemental PLP feeding for survival and exhibit spontaneous seizures around P15. Meanwhile, homozygous R116Q and heterozygous D33V do not have spontaneous seizures but exhibit decreased latency to chemically-induced seizures, increased hyperactivity, and impaired spatial learning and memory, especially carriers of the more severe D33V mutation. Using electroencephalography (EEG) and virally-expressed fluorescent GABA sensors, we found that heterozygous D33V mice exhibited increased theta rhythm power which could be rescued with PLP supplementation, increased ictal propagation speed across the frontal and occipital lobes, and decreased GABA neurotransmitter release compared to wild-type controls. The data suggests that *PNPO* mutant mice recapitulate many behavioral phenotypes exhibited by human epilepsy patients and could serve as a robust vehicle for future epilepsy treatment research, and that GABA deficiency in *PNPO* mutant mice contributes to a collapse of feedforward inhibitory control leading to increased seizure susceptibility and propagation.

One of the largest contributors to onset of seizures in humans is excessive alcohol use, and the variable behavioral and psychosocial consequences of alcohol consumption between individuals suggests a strong genetic component affecting alcohol response. In the central nervous system, one of the main targets of alcohol are $GABA_A$ receptors, where it acts mostly as a positive allosteric modulator. However, activation of GABAergic signaling via pharmacological manipulation in turn also causes increased alcohol consumption in

rodent models, suggesting a bi-directional relationship. Moreover, chronic alcohol consumption has been shown to cause deleterious effects on PLP content in humans. Despite these overlapping connections, the intricate inter-relationship between alcohol use, PLP content, and GABAergic transmission has not yet been systematically explored. We previously generated and characterized knock-in fly models in which we replaced the fly *PNPO* gene with mutant human *PNPO* from epilepsy patients across a range of mutation severity. Combining these genetic models of *PNPO* mutations with dietary PLP supplementation offer a unique approach to investigate how PLP and GABAergic signaling may contribute and respond to alcohol use. Our data demonstrates that *PNPO* mutations play a highly significant role in both acute and chronic alcohol use. We show that 1) alcohol consumption leads to PLP reduction; 2) PLP deficiency increases alcohol consumption; 3) *PNPO* mutations impair alcohol clearance; and 4) *PNPO* mutations have potentially lethal consequences which are worsened by alcohol consumption and rescued with PLP supplementation. We also discovered altered neurotransmitter levels and behavioral responses to alcohol in *PNPO* mutant flies. In summary, *PNPO* mutant flies exhibit an increase in alcohol consumption and decreased metabolic alcohol clearance, both of which lead to increased body alcohol content and further exacerbation of endogenous PLP deficiencies. These findings suggest 2 separate vicious cycles, both of which are fed by *PNPO* mutations and/or excessive alcohol consumption, leading to continual increase of anti-homeostatic alcohol consumption.

Understanding the neural mechanisms and GABAergic signaling contributing to neuropathological disease is critical for future development of long-lasting palliative and curative treatments. Our studies leverage the powerful genetic tools and robust life cycle available to *Drosophila melanogaster* with innovative *in-vivo* neuron imaging techniques and complex behavior assays in *Mus musculus* to investigate vitamin B6 deficiency-mediated effects on inhibitory signaling and behavior. These novel studies introduce a new clinically applicable genetic mouse model for epilepsy research and treatment development, investigate the role of *PNPO* mutations in weakening GABAergic control during seizures, and reveal the contribution of *PNPO* in development of alcohol addictive behaviors.