

Sophia B. Guillory
Tulane University

Dr. Rajani Maiya
LSUHSC Physiology Department

“Characterization of a Novel Marker for Neuronal Inhibition”

BACKGROUND: For many years, molecular markers such as Fos have been used to represent neuronal activation, and these markers have been instrumental in physiological research, specifically in the mapping of neuronal circuits. However, no equivalent marker existed for neuronal inhibition until 2023, when the phosphorylation of pyruvate dehydrogenase (pPDH) was identified as having an inverse correlation with action potential firing intensity in primary neurons. This relationship allows for the immunostaining of pPDH by monoclonal antibodies to act as a detection method for inhibition across the brain in *in vivo* mouse models. pPDH is a revolutionary tool in physiological research, allowing for the recognition of inhibitory pathways that were previously undetectable.

OBJECTIVE: This project explores this novel marker’s ability to detect neuronal inhibition caused by various modalities including exposure to drugs known to cause profound inhibition in the brain such as isoflurane and alcohol, agonists of Kappa Opioid Receptors (KORs) which are inhibitory G-Protein coupled receptors, and social defeat stress (SDS). Our lab is interested in understanding neural mechanisms by which repeated SDS leads to increased alcohol consumption. The current working hypothesis is that SDS leads to activation of Dynorphin (Dyn) release from dorsal raphe Dyn neurons into the bed nucleus of the stria terminalis. The released Dyn then binds to KORs located on basolateral amygdala terminals in the BNST leading to neuronal inhibition. Here, we attempt to visualize this inhibition in BLA neurons after SDS and after systemic injections of a selective KOR agonist, U50,488.

METHODS: Mice were exposed to one of four conditions: exposure to the general anesthetic isoflurane, intraperitoneal (i.p.) injections of alcohol (2 g/kg), U50,488 (a KOR agonist, 10mg/kg), and exposure to repeated (10 sessions) brief SDS. Mice were sacrificed 30 minutes post exposure to these stimuli. Mice were perfused and brains were postfixed in paraformaldehyde followed by sucrose and 40 μ m thick sections were prepared. Sections were then processed for IHC to detect pPDH and cFos.

RESULTS: Exposure to isoflurane as well as systemic administration of alcohol (2g/kg) led to robust pPDH activation in many brain regions including the basolateral amygdala and several hypothalamic nuclei. Both alcohol and isoflurane potently activate GABA receptors in the brain and lead to profound inhibition, which is consistent with our data. However, systemic administration of U50,488 and SDS did not lead to significant changes in pPDH staining throughout the brain. Both U50, 488 and SDS lead to transient inhibition which may be difficult to capture with this method. Future studies will examine the inhibitory landscape in the brains of mice subject to voluntary binge alcohol consumption. In summary, our results indicate that pPDH is a useful tool to visualize strong patterns of neuronal inhibition mediated by alcohol and anesthetic exposure.