**Summary of previous paper**

Locus coeruleus neurons that release noradrenaline (LCNA) have been implicated in sensory gain, action, and learning. However, how these cells encode stimuli and facilitate action during reinforcement learning is still unknown. To elucidate these questions the authors utilized a go/no-go task, single cell calcium imaging, optogenetics, in vivo electrophysiology, and retrograde tracers in transgenic mice.

Head-fixed mice trained in an go/no-go auditory tone discrimination task. Inhibition of LCNA during the trial lowered presses only when presented with low intensity tones. This suggests these cells facilitate action when presented with low-evidence stimuli. Following a punishment trial, mice showed increased hit rates. This was abolished with LCNA inhibition during the whole punishment trial or delivery phase. This suggests that LCNA have a role in the integration of reinforcement signals. Electrophysiology revealed LCNa responded before lever presses, and after reward and punishment delivery. This suggests LCNA are implicated in action and reinforcement. Calcium imaging and electrophysiology recordings revealed different clusters of LCNa: pre-press, post reward and post punishment. Using two-photon calcium imaging, they observed LCNa projections to efferent targets, namely the dorsal medial prefrontal cortex and the motor cortex (MC). The LCNa pre-press cluster showed heavy projections to the MC and silencing these projections reduced lever pressing. Taken together, these data reveal that modular targeting of LCNa outputs encode and enable learning functions.

Recent studies have revealed the importance of astrocytes in learning and synaptic strength. Norepinephrine has been heavily implicated in neuroplasticity and is known to act directly upon astrocytes. To continue this previous line of research during the summer I shall be working with my mentor to elucidate the effect of norepinephrine in astrocytes, specifically in the dorsomedial prefrontal cortex and the MC, during reinforcement learning.

**Background**

The locus coeruleus (LC) is a small neuromodulatory structure located in the brainstem that has low density noradrenergic projections to most cortical and subcortical structures. LC noradrenergic expressing (LCNA)neurons have been implicated in a wide variety of brain functions such as stress (Likhtik, E. 2019), learning (Breton-Provencher 2022), arousal (Sara, S. J. 2012), and wakefulness mediation (Carter M. 2010). As the primary source of noradrenaline (NA) in the brain (Breton-Provencher 2021), the LC is comprised of a dense core of noradrenergic neurons that establish far projections, as well as a shell that is made up of mostly glutamatergic and GABAergic neurons that receive various inputs and project locally to mediate LCNA. It has been previously shown that LCNA neurons participate in learning by reacting to behavior in learned tasks and to unexpected stimuli (Breton-Provencher 2019; Takeuchi 2016). Recent studies have focused on the role of LCNA neurons in reinforcement learning and have shown that noradrenaline release before task execution promotes reward-seeking behavior when stimulus evidence is low(Breton-Provencher 2022). Additionally, they revealed a system of LC neuronal subpopulations that differentially project to the dorsal medial prefrontal cortex (DMPFC) and the motor cortex (MC) to mediate learning (Breton-Provencher 2022).

Astrocytes are the most abundant glial cells found in the brain and have a variety of functions including neuron protection, maintaining the blood brain barrier, and maintaining the synaptic space between neurons. Seeing how astrocytes maintain the synaptic space, it makes sense that they are also known to modulate plasticity in cortical structures to aid memory formation (Henneberger C. et al 2010) and aid in the structuring of neuronal networks(Adamsky et al. 2018). For these reasons, in the last decade astrocytes have been the focus of research that aims to elucidate the mechanisms of learning. Astrocytes mediated plasticity is known to be dependent on NA. Interaction between NA and astrocytes “evokes robust calcium transients in astrocytes” (Holy & Wahis 2021)by activating apha1-adrenoreceptors(α1-AR). Activation of these receptors in astrocytes results in an increase of short-term synaptic plasticity through a co-operative mechanism with corticotropin-resealing factor that allows for enhanced glutamate release(Perez 2020). NA can also modulate synaptic strength in the prefrontal cortex by acting on presynaptic α1-ARs to enhance release of glutamate2, allowing for a stronger and faster excitatory post synaptic currents (EPSC). Projects previously conducted in the Sur lab have revealed the importance of astrocytic glutamate regulation in the Motor Cortex(M1) for learned behavior and movement coordination(Chloe Delepine et al., 2023).

Because the M1 receives projections from LCNA neurons and the importance of astrocytes in the motor cortex in learned behavior, this summer will be dedicated to further elucidating the role of LCNA neurons in reinforcement learning. Water restricted mice will be trained in a head-fixed go/no-go behavioral task. In this task mice will learn to press a lever in response to a “go” tone to receive a water reward and to not press in response to a distinct “no-go” tone to avoid an air puff punishment. Throughout the task the tones will vary in intensity ranging from 5 DB to 35 DB(Breton-Provencher, et al. 2022). Once the mice learn the task NA expression in the LC will be chemogenetically inhibited in every other trial and neuronal activity will be evaluated differently in two groups. The electrophysiological activity of the LC of one group will be monitored throughout the trial while the other group will have the activity in their DMPFC recorded with two-photon imaging.

**Research Characteristics and Timeline**

* Electrophysiology mice
  + Mouse line
    - Purchased from Jackson labs
    - Adult Dbh-cre line mice
  + Surgery
    - Bilateral viral injections were performed in anesthetized mice in a stereotaxic frame.
    - Virus
    - Glued a head plate parallel to the bregma–lambda axis of the skull.
    - Recovery was monitored for 72 hours.
* Two-photon imaging mice
  + Mouse line
    - Purchased from Jackson labs
    - Adult C57BL/6J wild type
    - Adult CaMKII;mTTA;GCAMP6s
  + Surgery
    - Performed under isoflurane in a stereotaxic frame.
    - Viruses
      * (AAV)8-GFAPhM3D(Gq)-mCherry
      * AAV5.GFAP.Cre.WPRE.hGH
      * Syn.GCaMP6s.WPRE.SV40
    - Cranial window glued on the skull allowing a view to the motor cortex.
    - Recovery was monitored for 72 hours.
* Training
  + After recovery mice will be put on a water restriction schedule only receiving 1.5mL of water per day
  + Mice will be trained to associate a lever press with reward and to detect a go tone until mouse made more than 80% of lever presses for 50 consecutive trials.
  + Then they were trained to not press the lever to avoid a punishment and to detect a no-go tone until they reached 85% hits and less than 30% false alarms
* Electrophysiology
  + Before recording a craniotomy will be preformed to insert the probe into the LC
  + Electrophysiological spikes will be recorded in trial with CNO or saline.
* Two-photon imaging
  + (Calcium activity) Imaging will be recorded with a Prairie Ultima IV two-photon microscopy system.
  + Two-photon excitation will occur at 920nm wavelength.
  + Will be recording GCaMP fluorescence.
* Histology
  + After perfusion and extraction brain will be sliced with a vibratome at 100µm
  + Slices will be washed with Phosphate buffer solution before application of antibodies.
  + Antibodies:
  + Slices will be mounted in mounting medium with DAPI.
* Confocal imaging
  + Utilized to capture fluorescence of cells.
* Two-photon imaging and electrophysiology spike data analysis
  + Two-photon data will be analyzed with software “Suite2p” and data run on IDE Spyder with an environment and code provided by post-doc Jennifer Shih.
  + Spike data will be preprocessed and analyzed in MATLAB with software developed and provided by Gabrielle Drummond.

I, as an undergraduate student, will be working directly with the training, imaging, data collection and data analysis. Training of mice will be done primarily in June while analysis of collected data will occur during July and my participation in the project should be completed in the first week of august.

**References**

1. Holt, M. & Wahis J. Astrocytes, Noradrenaline, α1-Adrenoreceptors, and Neuromodulation: Evidence and Unanswered Questions. Frontiers. 15 (2021)
2. Perez D. α1-Adrenergic Receptors in Neurotransmission, Synaptic Plasticity, and Cognition. Frontiers. 11(2020)
3. Durkee D. A & Araque A. Diversity and Specificity of Astrocyte–neuron Communication. Neuroscience. 396 73-78 (2019)
4. Breton-Provencher, V. & Sur, M. Active control of arousal by a locus coeruleus GABAergic circuit. Nat. Neurosci. 22, 218–228 (2019).
5. Breton-Provencher V. Drummond D. Feng J. Li Y. Sur M. Spatiotemporal dynamics of noradrenaline during learned behaviour. Nature. 606 (2022)
6. Breton-Provencher, V. Drummond, D. Sur M. Locus Coeruleus Norepinephrine in Learned Behavior: Anatomical Modularity and Spatiotemporal Integration in Targets. Frontiers. 15 (2021)
7. Delepine C. et al. Differential Effects of Astrocyte Manipulations on Learned Motor Behavior and Neuronal Ensembles in the Motor Cortex. J. Neurosci. 43 (2023)
8. Likhtik, E. & Johansen, J. P. Neuromodulation in circuits of aversive emotional learning. Nat. Neurosci. 22, 1586–1597 (2019)
9. Sara, S. J. & Bouret, S. Orienting and reorienting: the locus coeruleus mediates cognition through arousal. Neuron 76, 130–141 (2012).
10. Carter M, et al. Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nature. 13, pages1526–1533 (2010).
11. Takeuchi, T. et al. Locus coeruleus and dopaminergic consolidation of everyday memory. Nature 537, 357–362 (2016).
12. Henneberger C, Papouin T, Oliet SH, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. Nature 463:232236.
13. Adamsky A, Kol A, Kreisel T, Doron A, Ozeri-Engelhard N, Melcer T, Refaeli R, Horn H, Regev L, Groysman M, London M, Goshen I (2018) Astrocytic activation generates de novo neuronal potentiation and memory enhancement. Cell 174:59–71.e14.