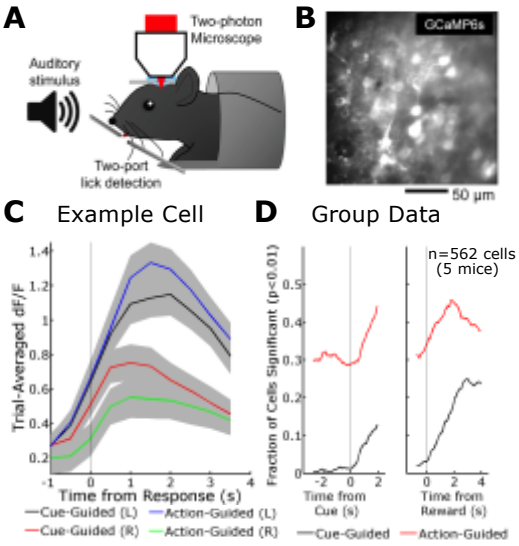
Cortical Network Mechanisms Underlying Flexible Action Selection

Background. Frontal association cortex is a brain region critical for flexible action selection in mammals. In humans and other primates, this area includes the supplementary motor complex (SMC), which has been shown necessary for suppression of inappropriate motor plans--an extreme case being 'alien hand syndrome,' in which SMC damage leads to complex and seemingly purposeful hand movements in the absence of voluntary control.[1] Premotor cortex (M2) is thought to be the rodent homolog of SMC. Lesions to M2 selectively disrupt goal-directed behavior[2], and in particular the ability to adapt choice of action to changes in reward values.[3] Despite the evidence for its causal role in behavioral flexibility, the mechanisms by which neural networks in frontal association cortex realize this vital function remain a mystery.

Preliminary Findings. To investigate the neural substrates for flexible action selection in M2, I will use a combination of rodent behavior, *in vivo* imaging of neural ensemble activity, and local silencing methods. During my first year of PhD research, I developed a two-choice decision task for mice that requires flexible switching between different action selection strategies in order to obtain optimal reward. The first phase of the task requires a cue-guided strategy in which the animal must discriminate between two distinct auditory stimuli that each indicate the availability of water reward at a corresponding lick port on either side of the animal's mouth. In the second phase, an action-guided strategy is necessary: reward is contingent upon licking a specific port regardless of the cue presented. The two phases are alternated many times within a single session without any sensory cue to indicate the phase-switch. Thus, the task requires flexible adaptation of action selection strategy to changing contingencies between cue, action, and reward.



In a first step toward understanding the neural basis of behavioral flexibility, I have begun imaging ensembles of M2 neurons at cellular resolution as mice perform this task (Fig. A&B). To measure changes in neural activity, the genetically encoded calcium indicator GCaMP6 was first transduced into layers 2/3 of M2 using an adeno-associated virus (AAV). A cranial window was implanted above M2, and fluorescence traces were recorded using 2-photon microscopy. By aligning the traces recorded from individual neurons to specific events in a trial (e.g., cue or response onset), I have correlated animal behavior with activity changes in single neurons, as well as with the aggregate activity of all neurons in the recorded ensemble. Preliminary analyses have produced two key findings that motivate detailed investigation: (1) A large proportion (>25%) of individual neurons recorded in M2 were choice-selective, i.e., these neurons showed significant differences in activity depending on which port was chosen (Fig. C). Interestingly, the fraction of choice-selective neurons increased following reward delivery, and peaked ~2-sec post-reward (Fig. D). (2) A greater proportion of neurons were choice-selective when the task required an action-guided versus a cue-guided response. Furthermore, a large fraction of neurons showed pre-response choice selectivity when an action-guided strategy was utilized.

Aim 1: Test causal role of M2 ensemble dynamics in behavioral flexibility. Our preliminary findings indicate delayed choice-selective activity in M2 that may serve as a feedback signal important for reinforcement of the current action-selection strategy. In order to test this hypothesis,

I will silence M2 in a temporally specific manner using an optogenetic approach. First, the light-sensitive neuronal silencer ArchT will be transduced bilaterally into M2 using an AAV. Light pulses will then be delivered through an optical cannula to inactivate M2 specifically during the 3-sec post reward in order to block delayed choice-selective activity. If such activity is important for reinforcement of action-selection strategy, then this manipulation should increase the number of trials taken to reach a criterion rate of correct response after phase switches, as well the number of perseverative errors. Preliminary observations also reveal early choice-selective activity during action-guided correct trials that may bias action selection toward the appropriate response. If this is the case, silencing M2 during the 3-sec prior to response should increase trials-to-criterion during the action-guided phase while having no effect on performance during the cue-driven phase of the task.

Aim 2: Investigate contribution of GABAergic inhibition to M2 ensemble dynamics and determine causal role in flexible action selection. GABAergic inhibition is known to serve essential computational roles in the neocortex. For example, fast-spiking PV^+ interneurons (PV-INs) are known to generate the gamma rhythm and sharpen feature selectivity in sensory areas.[4,5] However, the function of PV-INs in cognitive areas of cortex remains unexplored. The idea that PV-INs modulate choice selectivity within M2 ensembles is an intriguing hypothesis. To test this possibility, I will modify the *in vivo* imaging experiment described above to include PV-specific silencing, using a transgenic mouse line (PV-cre) that expresses cre-recombinase only in PV-INs. Because an optogenetic approach would preclude simultaneous imaging, a cre-dependent inhibitory receptor activated by the drug CNO (Gi-DREADD) will be transduced into M2 using an AAV, to allow PV-specific silencing as mice perform the decision task. I hypothesize that silencing PV-INs with CNO will reduce choice selectivity within the imaged ensemble by removing task-related inhibitory control of choice-selective neurons. Additionally, if PV-INs in M2 are critical for strategy reinforcement, then PV-specific silencing should disrupt adaptation of action selection strategy, and thus increase trials-to-criterion and perseverative errors after shifts in cue-action-reward contingencies.

Broader Impacts. Research on flexible decision-making will benefit a wide variety of fields that concern human and animal behavior. Economic decisions are essentially a form of goal-directed behavior in which appropriate error signals derived from expectation, reward, and punishment must play a key role. By improving understanding of how decision strategy is adapted, we might develop a more informed view of how markets operate, and possibly reduce the human toll of market dysfunction. Similarly, the justice system requires a nuanced understanding of concepts such as incentive structure, deterrence, and risk, all of which must be rooted in goal-directed behavior. As a complement to the study of normal decision-making, it will also be important to study how flexibility of the system is hampered by stress, distraction, mood, etc. A more mechanistic understanding may lay the foundation for discovery of measures we can all take to optimize our level of cognitive flexibility, in order to make better decisions as individuals and as a society. Finally, flexibility is notoriously difficult to implement in current hardware and software. Since behavioral adaptation is evidently a great talent of animals, biomimetic engineering based upon our own neural wetware may one day deliver some very smart machines.

References: 1) Nachev et al. (2008) Functional role of the supplementary and pre-supplementary motor areas. Nat Rev Neurosci, 9(11), 856-869. **2)** Gremel et al. (2013) Premotor cortex is critical for goal-directed actions. Frontiers Comp Neurosci, 7. **3)** Sul et al. (2011) Role of rodent secondary motor cortex in value-based action selection. Nat Neurosci, 14(9), 1202-1208. **4)** Cardin et al. (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature, 459(7247), 663-667. **5)** Lee et al. (2012) Activation of specific interneurons improves V1 feature selectivity and visual perception. Nature, 488(7411), 379-383.