**Katherine Furman – Graduate Research Statement**

## Nature versus nurture? Maternal responses to infant distress calls

**Introduction & Significance:** The brain has the extraordinary capability of attributing different levels of importance to the different types of input signals it receives. For example, hearing one's name, even at very low volume, elicits strong neural signals, as the brain has learned the relevance of that particular sound. The process by which this occurs is known as synaptic plasticity, which allows the brain to alter its connections based on experiences associated with particular sensory inputs. Synaptic plasticity is usually experience-dependent, and a class of molecules known as neuromodulators have the role of strengthening specific neural circuits dependent on particular situations. Here I examine the action of one important neuromodulator, oxytocin, which is involved in a multitude of social interactions, including maternal care.

Maternal behaviors are observed in all mammalian species, including mice. As infants, mouse pups are especially helpless, relying on their mother (called a 'dam') for all of their needs. Pups become scattered from the nest as the dam moves around, and must communicate with the dam that they have become isolated. To do so, they emit isolation ultrasonic vocalizations (USVs), triggering the dam to respond by retrieving the pup and returning it to the nest[1]. While dams retrieve pups with high accuracy, virgin female mice that lack prior experience with pups fail to exhibit this behavior, generally neglecting the calls of a nearby pup[2]. However, after being cohoused with a dam and pups for several days, virgin female mice can learn to retrieve pups with comparable accuracy to the dam[2]. A virgin's acquisition of this pup retrieval behavior is accelerated by administration of the neuromodulator oxytocin [2]. This behavior can be eliminated by inactivation of the left auditory cortex (A1), which contains a significantly higher amount of oxytocin receptors (OXTR) than the right A1[2].

*My proposed graduate research is concerned with the specific features of isolation USV stimuli that cause A1 to recognize the behavioral relevance of these sounds.* Specifically, I propose to examine perceptual attributes of the isolation USV encoded by the maternal A1 that enable the dam to recognize and respond to this sound, as well as the role of oxytocin-dependent plasticity in acquiring USV-induced pup retrieval behavior by inexperienced virgins. Recent work demonstrated that human A1 distinguishes screams from conversational speech by an acoustic quality known as 'roughness,' defined as the rate at which the volume of sound changes[3]. By detecting roughness, human A1 rapidly engages subcortical structures to assess danger[3]. **I hypothesize that** similar acoustic perceptual features allow the maternal A1 to distinguish, and attribute behavioral relevance to, the sound of nearby pup isolation USVs, and that learning of pup retrieval behavior through experience relies on oxytocin-dependent synaptic plasticity that strengthens the A1 response to such perceptual features.

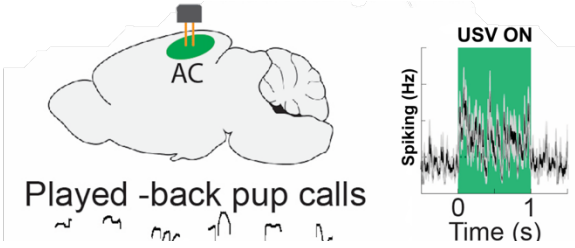


**Figure 1:** Example single-unit recordings from tetrode implants in A1. An increase in activity is observed when isolation USV stimulus begins

**Aim 1A: Which acoustic features differentiate isolation USVs from other vocalizations?** To understand how the USV response is encoded in the dam A1, I will chronically implant electrode arrays into adult female mouse A1, and obtain single-unit recordings in response to natural, pup-evoked isolation USVs from a speaker. This process (Figure 1) allows clear visualization of temporally-precise spike activity in A1 as it relates to sensory input from isolation USVs. Additionally, by observing behavior that dams exhibit when hearing the isolation USV stimulus,

I can visualize how A1 activity correlates to both sensory input and behavioral output (in the form of pup retrieval).

Using the modulation power spectrum (MPS), which can visualize sounds two-dimensionally on both spectral and temporal domains[3], I can examine the portions of acoustic space in which these naturally-produced isolation USVs reside. By comparing this to the MPS of adult USVs (which are behaviorally neutral to the dam) I will isolate which acoustic features are unique to the pup isolation USV and have behavioral relevance to A1.

**Aim 1B: Are these acoustic features relevant to A1?** If pup isolation USVs contain specific acoustic 'roughness' features distinguishable by the maternal A1, synthetically manipulated USVs which lack these features should result in a weakened response compared to natural pup-evoked isolation USVs. Using MATLAB I will synthesize audio clips which mimic pup USVs, but lack the spectral/temporal features previously identified as unique to isolation USVs. I will then play these to maternal animals, measuring behavioral and neural responses to calls with similar statistics as USVs, but varying in their frequency, temporal modulation (rhythm), and roughness.

Using single-unit recordings from electrode arrays in A1, I will observe neural activity of A1 in dams when they are exposed to synthetically manipulated USVs played from an ultrasonic speaker. If I've successfully identified the differentiating feature(s) making isolation USVs unique from other mouse vocalizations, I expect that dam A1 neurons will exhibit a stronger, more temporally-precise response to hearing pup-evoked isolation USVs (as was observed in [2]), than synthetically manipulated USVs. I also expect that pup retrieval behavior will be significantly diminished, if not eliminated, when exposed to synthetically manipulated USVs.

**Aim 2: Are these specific elements dependent on oxytocin signaling?** To test the hypothesis that oxytocin promotes maternal pup retrieval by strengthening the A1 response to unique spectral/temporal features of isolation USVs, I will observe the changes in A1 activity as a result of changes to endogenous oxytocin systems. Using transgenic *Oxy-Cre* mice will allow targeted expression of light-sensitive opsins in oxytocin-releasing neurons, the activity of which can be manipulated with light of specific frequencies. I will express channelrhodopsin-2, which is able to activate neurons in response to blue light, in pup-naïve virgin female mice. These mice will be exposed to pup isolation USVs concurrently with optogenetic stimulation of oxytocin-releasing neurons. By continuously pairing isolation USV audio with stimulation of endogenous oxytocin over a number of days, I hypothesize that the A1 activity of the naïve female will change to mimic the strong, temporally-precise response observed in dams.

**Intellectual Merit & Broader Impacts:** With the help of the NSF GRFP, I will be the first to identify the specific acoustic features, over both spectral and temporal domains, which are unique to pup isolation USVs when compared to other mouse vocalizations. By identifying the A1 single-unit activity displayed in response to isolation USVs, and by identifying the changes in activity when crucial isolation USV features are eradicated from the stimulus, I aim to observe the specific activity patterns recruited by A1 in attributing behavioral relevance to infant-related sounds. By observing the changes in neural activity induced in pup-naïve virgins after optogenetic stimulation of oxytocin, I will be able to observe the unique form of neuromodulatory plasticity evoked by oxytocin in A1 which allows experience-dependent learning of maternal pup retrieval behavior. By examining maternal auditory processing in such depth, the field can better understand the interplay between auditory input and oxytocin to yield behavioral output.

**References**: **1.** Ehret (2005) Infant rodent ultrasounds – a gate to the understanding of sound communication. *Behav Genet 35*:19. **2.** Marlin et al. (2015) Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*

*520*:499 **3.** Arnal et al. (2015) Human screams occupy a privileged niche in the communication soundscape. *Curr Biol 25*:2051.