Animals harbor a suite of innate fears, knowing them from birth in the absence of firsthand experience. These fears are rooted in evolution and are often species-specific. For example, mice respond defensively to odors related to foxes and cats, two of their most common predators. Alternatively, humans respond defensively to snakes and spiders, both of which can be highly-lethal and are endemic to East Africa, the original range of genus *Homo.* These factors strongly imply a genetic origin for these fears, though none has yet been investigated.

Aversive responses have both behavioral and hormonal components. The two brain regions necessary and sufficient for innate aversive odor responses in mice are the cortical amygdala (CoA) and the amygdalo-piriform transition area (APir). The CoA mediates innate olfactory behavior, and is organized spatially based on the emotion a given odor evokes — neurons responsive to aversive odors are located in the anterior CoA, and neurons responding to all other odors are posterior.[1] APir controls the hormonal stress response to innately aversive odors, stimulating secretion of corticotropin-releasing hormone (CRH) by the hypothalamus, raising peripheral corticosterone levels.[2] Neither region has any other known functions. A distinct, spatial organization to neuronal populations that mediate specific behavioral functions, independent of individual experience, strongly implies genetic control over the developmental programs creating these pathways. For instance, a past study examining similar populations in other regions showed each one expresses a set of marker genes specific to their own population.[3] Thus, neuronal populations in CoA and APir may similarly express their own sets of marker genes. **I propose to identify the first set of marker genes for neurons controlling innate aversive responses to a set of specific stereotyped odors.**

Aim 1: Identify neurons specifically mediating innate olfactory aversion. Hypothesis: If these regions respond to innately aversive odors, then the specific responsive neurons within these regions should be identifiable based on the population's activity in odor exposure. Method: I can mark these neurons using a transgenic mouse strain with neurons that Cre-dependently express eYFP if active within a transient, hours-long period after peripheral tamoxifen injection.[4] I will identify innately aversive odor-responsive neurons by exposing mice to either water or trimethylthiazoline (TMT), a well-validated innately aversive fox odor, shortly after peripheral tamoxifen injection. The water-responsive group represents neurons active at rest, while the TMT-responsive group represents neurons activated by innately aversive odors. Differences in response between the two conditions should reflect regional activity differences. Anticipated Results: The eYFP-expressing population should be enriched in CoA and APir in the TMT-exposed mice compared to the water-exposed mice. The regions

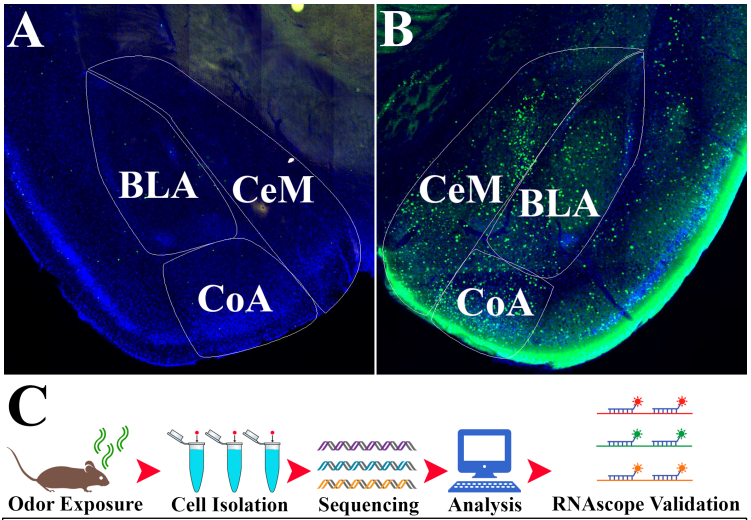

Figure 1. Preliminary Results and Research Plan. After odor exposure, cells (blue) in the amygdala express Arc (green). A. Amygdala neuron activity after water exposure. B. Activity after TMT exposure. C. Proposed research plan. BLA: basolateral amygdala nuclei, CeM: centromedial amygdala nuclei.

they innervate, the BLA, CeM, and the hypothalamus, which control general aversive responses, should be enriched as well. Preliminary data corroborates these predictions (*Figure 1A, 1B*).

Aim 2: Identify genes exclusive to innate olfactory aversion neurons. Hypothesis: Neurons active during TMT exposure should express a suite of marker genes not expressed in neurons active during water exposure throughout the brain. The eYFP-expressing activated neurons can be dissociated on ice via an optimized combination of RNA polymerase inhibitors, physiological solutions, and extracellular matrix-specific cold-active proteases to keep cells alive and eliminate gene expression artifacts.[5,6] I can combine this technique with dissection and fluorescence-activated cell sorting to isolate single live eYFP-expressing cells from CoA with high fidelity.[7]

Method: I will use an efficient, well-validated, high-resolution form of single cell RNA-sequencing to precisely assay the expression of all genes in all isolated cells (*Figure 1C*).[8] This approach closely resembles the method used in a recent series of experiments in the neuroscience literature.[9] A custom computational pipeline purpose-built for this experiment will analyze the data. Machine learning algorithms will classify cells into groups based on similarities in underlying gene expression. Differential expression analysis will identify the most highly upregulated genes in each group of cells compared to all others. Gene ontology (GO) analysis will then identify these genes' functions. RNAscope, a multiplexed single-molecule RNA fluorescent *in situ* hybridization platform, will externally validate these results.[10] I collaborate with three groups across multiple disciplines and institutions to perform these techniques: in biology at the University of Cincinnati, biochemistry at UCLA, and bioengineering at UCSD.

Anticipated Results: Using this framework, I expect to identify at least one group of neurons present in the TMT-responding population but not the water-responding population, with at least one corresponding suite of highly-expressed genes. These will both be confirmed via RNAscope. We expect these genes to display enriched neural development-related GO terms.

Intellectual Merit: This would be the first study to identify and validate the heritability of innate behaviors, the specific neurons mediating these behaviors, and their underlying genes. Finding such genes would allow the targeted stimulation and genetic access of these neurons for the first time, making modification or simulation of specific odor responses (even in the absence of prior experience) possible, a valuable future research tool. Using such tools, researchers could create far more precise experimental designs, allowing researchers to answer more specific hypotheses than ever before. The availability of such novel technologies at the intersection of psychology, molecular biology, and sensory neuroscience will have many implications for studies in all three fields and will further stimulate interdisciplinary research incorporating aspects of all three.

Broader Impacts: During this project, I will train undergraduate students from underrepresented communities at UCSD in molecular biology and behavioral techniques, as well as mentor them in research methods, both at the bench and away from it. The computational and molecular methods will be made open-source on GitHub and protocols.io. I will communicate the results in open-access peer-reviewed academic journals, and I will also write articles in popular media outlets throughout the project based on what I study and discover along the way. The marker genes identified in this project could lead to advancements in commercial research technologies and pharmacology, as each one could serve as a possible target for future drug development should any of these populations become relevant to certain research questions or diseases.

References: [1]Root et al. *Nature* 2014 515:269-273. [2]Kondoh et al. *Nature* 2016 532:103-105. [3]Kodama et al. *J Neurosci* 2012 23:7819-7831. [4]Guethner et al. *Neuron* 2013 78:773-784. [5]Wu et al. *Neuron* 2017 96:313-329. [6]Adam et al. *Development* 2017 144:3625-3632. [7]Hempel et al. *Nat Methods* 2007 2:2924-2929. [8]Torre et al. Cell Syst 2018 6:171-179. [9]Tasic et al. *Nat Neurosci* 2016 19:335-346. [10]Wang et al. *J Mol Diagn* 2012 14:22-29.