While learning a new task or skill, what changes occur in the brain that allow for progressive improvement? Since the basic sensory inputs of vision, touch etc. do not change; the neural circuits of the brain must reorganize themselves in order to better represent information relevant to the task being learned. Previous work has revealed that changes in stimulus-tuning (ref.) and population response (ref.) are associated with performance improvements in behavioral tasks, but how these changes arise from defined populations of neurons is underexplored.

Using longitudinal 2p volumetric imaging of neuronal populations in vS1 we are able to track the same set of neurons during the course of mice learning a 2-choice tactile angle discrimination over several weeks. Mice were presented with an acute (45o) or obtuse (135o) angled pole to a single whisker and trained to lick left vs. right depending on the angle. In a naïve and expert session, further pole angles were presented (45:15:135o) to determine the sensitivity of angle discrimination. Strikingly, we find that as mice improve in the task the neural response to different angles “stretches” in the high dimensional space described by the full population response, such that representations of distinct angles begin to separate from each other (fig. 1A). The magnitude of this stretching is directly related to the difference in object angle, and this relationship becomes more pronounced as the animal improves in the task (fig. 1B, C, E). Recent work from the lab has shown that the sensorimotor variables associated with this task do not change significantly during learning (Kim et al., 2020), so this neural stretching may therefore be a direct observation of a neural circuit reorganizing to better process the information required to perform a learned behavior.

Furthermore, while this process is associated with a general increase in performance over several learning sessions, the extent of the population response was predictive of performance accuracy on a trial-to-trial basis (fig. 1D, F). This was only observed in well trained expert animals, again indicating a reorganization of the cortical circuit to better represent task sensory (or choice?) information.

In what populations of cortical cells does neural stretching occur, and how do input layers of cortex drive this process? To explore this, we will utilize 2p imaging techniques to rapidly image and stimulate multiple planes of vS1 during similar tactile tasks. Piezo objective manipulation will allow for near simultaneous imaging of input (L4) and output / integration (L2/3) excitatory cells during behavior. Defined populations of cells within these networks can also be targeted based on genetics or response properties with a spatial-light modulator, allowing us to probe how circuit connectivity within and between cortical layers is shifted during task learning.

**Figure Legends**

Figure 1.

1. Neuronal population responses in neurons (n=802) recorded from one mouse matched across naïve (left) and expert (right) sessions. Population responses are plotted in a 2D PCA space and color coded according to the angle of the pole presented in each trial.
2. For the data shown in A), the Euclidean distance in population response in PCA space between each pair of trials is shown, ordered by the angle of the pole presented on each trial. White dotted lines delineate blocks of trials in which the same angle was presented.
3. Each pair distance from B) plotted against the difference in presented angle for that pair. In both naïve and expert sessions for this animal, the distance in population response between a pair of trials was correlated to the difference in angle being presented between the trials (correlation coefficient show above plot, \*\*\* p < 0.001).
4. Normalized histogram of population response distance from center point in PCA space (0 in all dimensions) for each trial, separated by correct (cyan) and incorrect (magenta) trials. In the expert session (right) population response distances on correct trials was significantly greater than distance for incorrect trials (two-sample t-test \*\*\*p<0.001).
5. Summary of analysis in C) for all mice tested (n=5) in naïve (black) and expert (red) sessions. Solid lines indicate mean pair distance, shading = SEM.
6. Summary of analysis in D) for all mice tested showing shift in mean population distance in PCA space between correct and incorrect trials. In expert sessions, mean population distance was significantly higher for correct trials (paired t-test \*p<0.05)

Figure 2

Left: Representative mean projected time series of a L2/3 imaging plane in vS1 (pAAV-CaMKIIa-GCaMP6m-p2A-ChRmine-Kv2.1-WPRE). Right: panels 1-4 show mean response (ΔF/F) in active cells to 9 targeted stimulations with different foci (cells 1-4 in left image).