Learning-induced Changes in L5 Apical Dendrites of Rbp4-cre Mice

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Background

Changes in neural circuits are a significant marker of learning. Synapses form the units of these circuits; however, the nature of synaptic changes during learning has yet to be explored. In mice, the primary sensory cortex (S1) barrel fields are somatotopically correlated to the whiskers, and it is known that S1 layer 5 (L5) neurons show enhanced activity in response to inputs from the somatosensory thalamus (POm) after Sensory Association Training (SAT) (**Figure 1**).

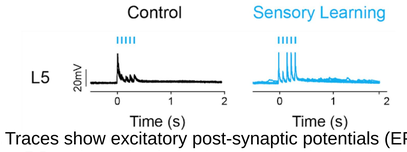

Figure 1. Traces show excitatory post-synaptic potentials (EPSPs) in response to POm photostimulation in control and trained mice.

The use of POm projections to apical dendrites of L5 neurons provides an excellent model for studying experience-dependent plasticity.

Objectives

To investigate if there is an anatomical correlate of SAT-induced changes in POm-evoked activity, we will explore two hypotheses:

1. Existing POm synapses will get stronger.
2. The number of POm synapses will increase.

Methods

Rbp4-cre mice receive three viral injections to mark pre- and post-synapses as well as L5 dendrites (**Figure 2**).

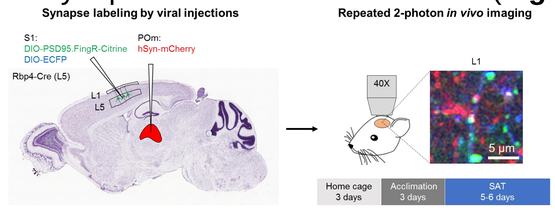

Figure 2. *Pre-synapses are marked with mCherry, post-synapses with citrine, and L5 dendrites with enhanced cyan fluorescent protein (ECFP).*

Two-photon in vivo imaging occurs over an 11-12 day period consisting of Home cage (HC), Acclimation (ACC), and SAT. During SAT, mice learn to associate a gentle air puff stimulus on the whiskers to a water reward. (**Figure 3**).

Figure 3. In 80% of trials, mice are given a 6 PSI air puff and then a water reward, while 20% of trials result in no air puff and no reward.

All image analysis was completed in ImageJ.

Results

The animals learned to lick more in stimulus trials than in blank trials, leading to improved performance (**Figure 4**).

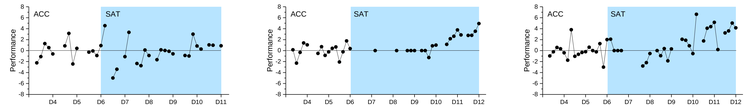

Figure 4. Behavior performance graphs for 3 animals, from left to right: GDY3, GDY8, GIB1.

There are 4 steps in the following image analysis:

First, the brightness of the images was normalized to improve the accuracy of synapse detection (**Figure 5**).

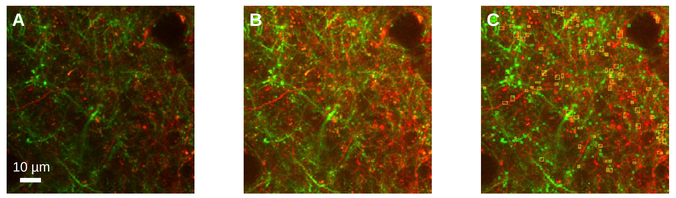

Figure 5. Raw (A), normalized (B), and counted normalized (C) in vivo images of GDY3/D6.

Second, the total number of pre- and post-synaptic spots are determined by automatic counting. Synaptic clefts are indicated by colocalized spots (**Figure 6**).

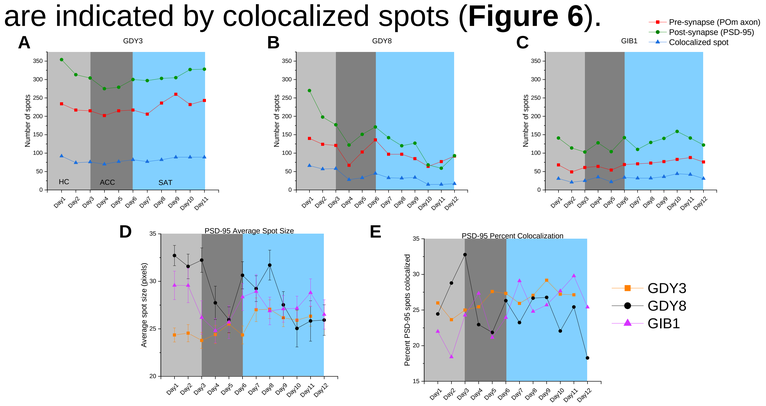

Figure 6. Total number of spots for 3 different mice (A, B, C) and analysis of average spot size and percent colocalization of these spots over time (D, E).

Third, regions of interest (ROIs) are selected as the areas with the most change in number of colocalized spots over time (**Figure 7**).

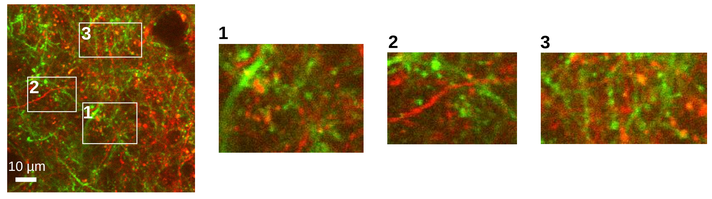

Figure 7. ROI selection on GDY3/D6 normalized image.

Results (Cont.)

Fourth, these ROIs undergo manual counting to determine the nature of the change in colocalized spots over time. Over the training period, colocalized spots will either be sustained, disappear, or appear (**Figure 8, 9**).

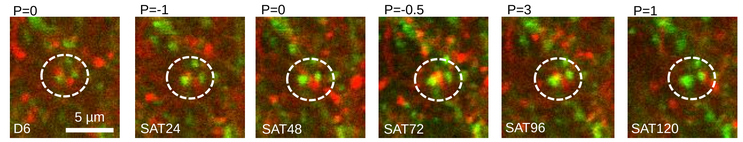

Figure 8. Tracking of a colocalized spot within an ROI over SAT; P is performance. This spot grows in intensity over time, then diminishes toward the end of the training period.

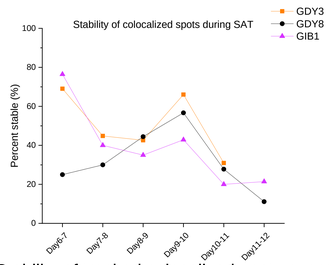

Figure 9. Stability of tracked colocalized spots over SAT.

Conclusions

In contrast to our hypotheses, there was no consistent trend across animals in either the number or area of PSD-95/POm colocalized spots induced by SAT; however, when we looked at ROIs with enriched colocalization, the stability of these colocalized spots decreased as the mice improved in performance, suggesting a substantial amount of synaptic plasticity induced by learning.

Future Directions

There are two directions we will further explore in order to explain the instability of POm synapses and the lack of trends between animals:

1. Identify POm inputs onto different types of L5 cells, which may reveal the specific synapses that are strengthened during SAT.
2. Investigate additional input types that also project to S1, such as the primary motor cortex (M1), which may discern which input synapses are increasing or decreasing in number and/or size.

Acknowledgements

Special thanks to Jiseok Lee and Alison Barth for their guidance, and to the CNBC for funding.