Sophie Sackstein

Independent Small Group Discussion Write-up

1. What was the motivation for this study? (e.g. big picture questions motivating experiments, prior work motivating experiments, etc)

This study takes a deeper look into exafferrence- touch generated sensory signal (whisker's touch) and reafference - movement generated signal (whisker movement). The target area is L4 of mouse barrel cortex - thought to be involved in somatosensation. Sensory input goes from ventral posteromedial nucleus to L4 of the barrel cortex, and each cortical barrel then processes the input from each whisker. VPM projects to both excitatory and inhibitory neurons. During tactile exploration, neural activity related to movement of digits or whiskers is suppressed to facilitate high signal-to-noise ratio encoding of touch. The authors show that in mouse this computation occurs in layer 4 of the barrel cortex and is mediated by fast-spiking interneurons. The suppression of whisker movement can allow V4 to create less "noise" making it so that the important signals are mediated.

2. List the key methodological details (species, preparation, recording/ anatomical techniques, data analysis methods)

These experiments measure spikes and membrane potential in the mouse somatosensory thalamocortical system. Mice performed an active object localization behavior (as in previous studies), they located an object through “active touch”. Importantly, whisker movements and touch forces were tracked with millisecond time-scale precision. Spikes were recorded in VPM, L4 excitatory neurons, and L4 fast-spiking, parvalbumin-expressing interneurons. Importantly, L4 recordings were made with loose-seal pipettes to avoid sampling bias. Neuron response with and without whisker movements were also analyzed. Membrane potential is in addition recorded in L4 excitatory neurons and L4 fast-spiking neurons. Various optogenetic manipulations were performed to identify neuron types and to analyze the flow of excitation in the circuit. Light gated inhibitory chloride pump eNpHR3.0 (eNpHR) in PVpositive neurons in the barrel cortex were virally expressed to suppress reafference.

3. For each figure, describe the important points including how the authors interpret the findings and what the findings may actually show.

Figure 1)

This shows activity in VPM and L4 excitatory neurons during the tasks the researcher had presented.

a) Multielectrode recording were used to measure activity in VPM and L4 circuits. The whiskers to solve the task were recorded (from the barreloids in cortex).

b) Neurons in the barreloids in cortex they were measuring produced significant responses to the task.

c) Control situation for the neurons that were recorded without this task. They increase spike rate in absence of touch.

d) Soon after the touch stimuli is presented there is a response in the spike rate. The raster plot showing 10 example trials shows less of a response. They increased spike rate with touching, not whisking movement, also shown in e.

e) Like in B, they increase spike rate in absence of touch.

f-j) loose-seal cell-attached method, corresponds to a-e. They didn't not respond to whisker movement. Also they fire still with no stimuli. Peristimulus time histograms of the same L4 E neurons corresponding to whisking also showed they didn't respond to whisking either, also seen in figure i.

Figure 2)

a)This shows an excitatory neuron (particularly a L4 spiny stellate cell), like those they measured in V4. Like in 1a, barreloid borders are shown.

b) This shows the firing for a L4 spiny stellate cell. Whisker curvature changes and movement changes the spike rate somewhat when there is no touch.

c) The whisker position didn't have much effect on the spike, not too much response without touch.

d & e) Results of neurons that didn't respond to whisking, but had relatively strong responses to touch.

f) Whisking only produces small responses in some neurons.

g) Touch shows largest suppression. Depolarization for whisker movement seems to be related to touch.

h) Figure F colors are shown for thresholds in H. Touch causes larger

Figure 3)

They added current to depolarize cells so that it seemed like an excitatory input.

a) Injected excitatory neurons show largely hyper polarized responses.

b & c) Touch responses showed inhibitory input, with current injection, without it there was only a small bump.

d) Excitation has short delay on averages for both.

e & f) Inhibitory input shown for the constant current injection neurons when mice were whisking.

g) Excitation and inhibition increase with whisking.

Figure 4)

L4 FS interneurons

a) Labeled nonspiny FS interneuron were recorded.

b & c) Membrane potential showed that there was activity without whisking or touch (without touch is shown in C).

d-h) Touch shows larger depolarizing response than whiskers and less latency.

Figure 5)

a) VPM FS (GABAergic interneurons) and E L4 neurons (Excitatory) neurons projections from VPM.

b) L4 FS shows the most response to touch and whisking, whereas L4 E flatlines.

c) Response latencies were short for VPM and L4 FS, but slightly longer for L4 E and less of a response.

d & e) VPM and L4 FS show response to touch and whisking, but L4 shows a weaker response to whisking, more to only touch.

Figure 6)

Explores if the input to the FS interneuron comes from areas other VPM.

a) Coronal section of the thalamus highlight GABAergic neurons.

b & c) TRN axons inhibit the thalamus in photo-activation.

d) In photo-inhibition there was reduced spikes.

e) Photo-inhibition reduced spike rates during touching and whisking.

Figure 7)

a) By getting rid of infraorbital nerve input they were able to look at it without mechanosensory inputs.

b) There was no response to touch in cut-off neurons and very little response to whisker movement.

c) Some response to whisker movement, depolarization were reduced.

d) Distance to threshold became nearly zero, but still small effect for whisking.

e) Coupling between whisker movement and depolarization is non-existent in the ION cut responses for mice.

f) L4 FS recording for ION cut mice diagram.

g-i) Virtually no response to touch or whisking, still some phase-locking.

Figure 8)

a) Light gated inhibitory chloride pump eNpHR3.0 (eNpHR) in PV-positive neurons in the barrel cortex were virally expressed to suppress reafference.

b & c) PV interneuron and exictatory neuron under control and then suppression conditions. During the eNpHR stimulation the L4 PV interneuron shows a lot of activity whereas the L4 excitatory neuron is inhibited.

d) Suppressing some of PV allowed L4 to excited by whisking.

e) L4 excitatory neurons show decreased or no reaction to touch with eNpHR.

f) Classifier could distinguish those that responded weakly to touch vs strongly. There was a big difference between control versus eNpHR neurons. Suppression of PV improved for classifying control and eNpHR in response to touch.

g) Touch vs eNpHR evoked responses- small AUC for those that respond to touch.

4. What is your assessment of the overall findings? Did the experimental data support the authors' interpretation? Why or why not?

In layer 4 of BC, whisker touches evoke precisely timed spikes, enforced by strong feedforward inhibition that gates thalamic input according to its degree of synchrony. Responses to slower speeds are dampened by feedforward inhibition. However they may not be considering input from other cortical layers, and input that comes directly to L4 from higher level thalamic areas. L4 and FS neurons are consistently excited by whisking and L4 FS interneurons may play an important role in suppressing movement responses in L4 excitatory neurons.

5. Describe new insights that you gained from the small group discussion of this paper.

I realized that the L4 and FS neurons are consistently excited by whisking but the excitatory neurons are selective for touch. Also, the viral method was used to express light-gated inhibitory chloride pump eNpHR3.0 in the FS cells to manipulate the cells' activity and observe the resulting effect on excitatory cell responses. One of my partners confirmed that this was to establish a causal role of FS cell-mediated inhibition of excitatory cells.

6. List all of your questions and/or points in the paper that you did not understand.

I don't understand how in 1b they measure whiskers that don't respond to touch, also why does the spike rate increase in absence of touch? I'm also confused by some of how they eNpHR stimulation and what that meant to prove. What is 2h?