THE EFFECT OF FEAR CONDITIONING ON PARVALBUMIN-EXPRESSING NEURONS IN MICE

by

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Parvalbumin-expressing (PV) interneurons are known to play key roles in the inhibiton of pyramidal neurons in the auditory cortex of the brain, but little is known about the exact circuits they function in. The auditory cortex is associated with complex temporal processing tasks and emotional learning. Dysfunctions in the auditory cortex are a major cause of age-related hearing loss. Gap detection, a task that requires the auditory cortex, involves detecting short gaps in noise. This study looks to see how PV neurons respond during gap detection before and after fear conditioning in order to determine the role of PV cells in a circuit. We identified PV neurons in optogenetically engineered mice and recorded their activity during a gap detection test. The mice were given a fear conditioning learning program, then their neural activity was recorded as they performed the gap detection test again. We found that the majority of the mice did not improve at gap detection after fear conditioning. Additionally, the PV responses did not change with fear conditioning. We did see that mice with greater PV activity (larger gap termination responses) were more likely to improve at gap detection than mice with less PV activity. We conclude that larger gap termination responses are an indicator of worse gap detection. More research on PV cells is necessary to understand the relationship between gap detection and the GTRs of

PV cells in the auditory cortex. A great understanding of these mechanisms could lead to potential treatments for those with hearing loss due to central auditory dysfunctions.

Acknowledgements

My thesis committee was very supportive in the creation of this thesis. First, I would like to thank Dr. Kip Keller for being my mentor on this project. I am grateful for his unending patience when it came to training me, answering questions, helping me prepare this thesis, and even just chatting about sailboats. Second, I would like to thank Dr. Mike Wehr for supporting this project. He always made time to answer questions and gave me many opportunities to learn and practice what I learned. Third, I would like to thank Dr. Carol Stabile for being so knowledgeable and easy to communicate with me as my CHC representative. I would also like to thank Dr. Aldis Weible for doing the surgeries on the mice, analyzing the histology results, and showing me an example of thoughtful and careful labwork.

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Introduction

Auditory Cortex

Hearing loss is estimated to affect 16.1% of people ages 20-69 in the US, and over half the population above 65 have hearing deficits associated with temporal processing of sounds. Peripheral hearing loss involves dysfunctions in the ears, and central hearing loss involves dysfunctions in how the brain processes sounds. Age-related hearing loss can be peripheral, central, or both. Many cases of age-related hearing loss are associated with complex stimuli, indicating that age-related hearing difficulties are a result of a dysfunction in the central auditory system and a generally decreased temporal acuity (Fitzgibbons & Gordon-Salant, 1996). Central hearing loss can significantly impair one's quality of life, compromising one's ability to discriminate rhyming sounds or to comprehend speech within background noise (Agrawal, Platz, & Niparko, 2008). Hearing aids and cochlear implants can help people who have peripheral hearing dysfunctions, but there is currently no treatment for hearing loss related to auditory cortex dysfunction. Research on auditory cortical processes could potentially lead to treatments for this type of hearing impairment.

Hearing begins when the peripheral auditory system, the inner and outer ear, turns sound waves into electrical signals. These signals then travel through the cochlear nuclei in the brainstem, the inferior colliculus in the midbrain, the medial geniculate body in the thalamus, and finally to the auditory cortex. The auditory cortex is located within the temporal lobes of the brain and is the site of high-level auditory processing and language comprehension processes. Temporal processing of sounds is the parsing

and representation of acoustic stimuli as they change over time. The brainstem and midbrain auditory centers can selectively respond to sound onsets or offsets, encode frequency-bands as they wax and wane, and so forth, but the most precise and selective temporal responses occur in the auditory cortex (Fitzgibbons & Gordon-Salant, 1996). Thus, for example, the complex hearing task of distinguishing sounds from background noise, which relies upon precise temporal discriminations, likely depends on intact cortical function.

Cytoarchitecture of the Auditory Cortex

Output neurons

Auditory inputs enter the auditory cortex from the thalamus. The information is then processed within the cortex and sent out to various targets. Within the cortex, there are many classes and subclasses of neurons that facilitate processing. Pyramidal neurons (PNs) are the output neurons of the auditory cortex and are excitatory onto their downstream targets.

Inhibitory interneurons

The auditory cortex of mice also contains many inhibitory interneurons which can be divided into three classes: SOM, VIP, and PV+. Somatostatin expressing (SOM) neurons comprise a very diverse range of subclasses, but they all produce an inhibitory effect on their surrounding PNs (Yavorska & Wehr, 2016). Vasointestinal peptide expressing (VIP) neurons have been found to weakly inhibit PV cells and strongly inhibit SOM cells which both inhibit PNs. This results in the ultimate disinhibition of the PNs (Karnani et al., 2016). Parvalbumin expressing interneurons (PV) belong to the

largest subclass of inhibitory neurons making up 30-50% (Tamamaki et al., 2003). PV cells are usually “fast spiking” with narrow spike waveforms. They produce a strong but short-lived inhibitory response (Yavorska & Wehr, 2016). PV cells aid in controlling the ‘gain’ of cortical responses by inhibiting their surrounding pyramidal cells (Atallah, Bruns, Carandini, & Scanziani, 2012) (Moore & Wehr, 2013). Previous research has shown that PV+ cells are also associated with enhanced plasticity and recent learning.

Gap Detection

"Gap detection" is a commonly used model for evaluating temporal acuity, which is the smallest gap that can be detected by the individual. It is used as a measure of an individual's level of auditory cortex function. In mice, detecting short gaps in noise requires the auditory cortex. Thus an analysis of the mouse's ability to detect gaps can provide a window to understand the functioning of the auditory cortex (Weible, Moore, et al., 2014). During gap detection, the mouse is presented with an ongoing white noise (a noise containing a very broad range of frequencies), with an occasional, short burst of louder noise interspersed. This 'startle burst' elicits a small jump or "startle response" from the mouse. A short gap in the ongoing noise preceding the startle burst can serve to "alert" the mouse and lessen its startle response. If the startle response is decreased, we infer that this results from the mouse having detected the gap. The experimenter can then vary the gap duration to explore the limits of gap detection. A similar paradigm has been used to test the limits of gap detection in humans, zebra finches, rats, and mice (Glasberg, Moore, & Bacon, 1987). Age related central auditory processing disorder (CAPD) involves changes in the auditory network that impair an

individual's ability to perceive sounds and use language. People who experience age-related hearing loss have a reduced ability to detect shorter gaps, suggesting a dysfunction in the cortical processing of sound. Little is known about the mechanism of CAPD, but it is linked to the auditory cortex, so learning more about gap detection could reveal more about why CAPD occurs.

Fear Conditioning

In fear conditioning, a neutral stimulus is followed by a salient event to cause learning. The most famous example of this type of learning is Pavlov's dog. In this experiment a dog was presented with a bell, and it would not salivate because the bell was a neutral stimulus. When it was presented with food, it would salivate because the food was salient to the dog. Then, the bell and the food were presented together. Later, the dog would salivate in response to the bell because it learned to associate the bell with receiving food.

In the context of this experiment, the mouse is presented with a gap which is the neutral stimulus. Following each 8 ms warning gap, a mouse is given a mild peri-orbital shock, which is the salient effect. It is expected that the gap will gain salience or predictive value for a following event. In other words, the mice are expected to pay more attention to the gap because they associate it with the shock. An earlier paper found that detection of short gaps, with durations that required an intact auditory cortex, improved after fear conditioning. Specifically, this improvement required the auditory cortex. This indicated that the temporal association of salient 'warning' sounds and emotionally significant events (shocks, or startle bursts) may occur within the auditory cortex (Weible, Liu, Niell, & Wehr, 2014). Fear conditioning has been shown to target

cell differentiation to increase PV+ expression in adult mice while decreasing expression of inhibitory basket cells (Donato, Rompani, & Caroni, 2013). There is no current research on the effect of fear conditioning on PV+ cells in the auditory cortex specifically, or how PV+ expression varies independent of basket cell expression.

Gap Termination Response (GTR)

In mice, neurons in the auditory cortex respond with a burst of activity when the gap in noise ends, as seen in figure 1. This is known as the gap termination response (GTR). We use the amplitude of small 'startle' jumps by the mice as well as the size of the GTR as measures of temporal acuity (Weible, Moore, et al., 2014).

The role of PV cells in auditory cortical function has been a focus of the Wehr lab. One study, published in 2018, found that PV cell responses to gaps reflected the responses of pyramidal neurons in the auditory cortex during gap detection (Keller, Kaylegian, & Wehr, 2018). It is still unknown how the GTRs of PV cells during gap detection might change before and after fear conditioning.

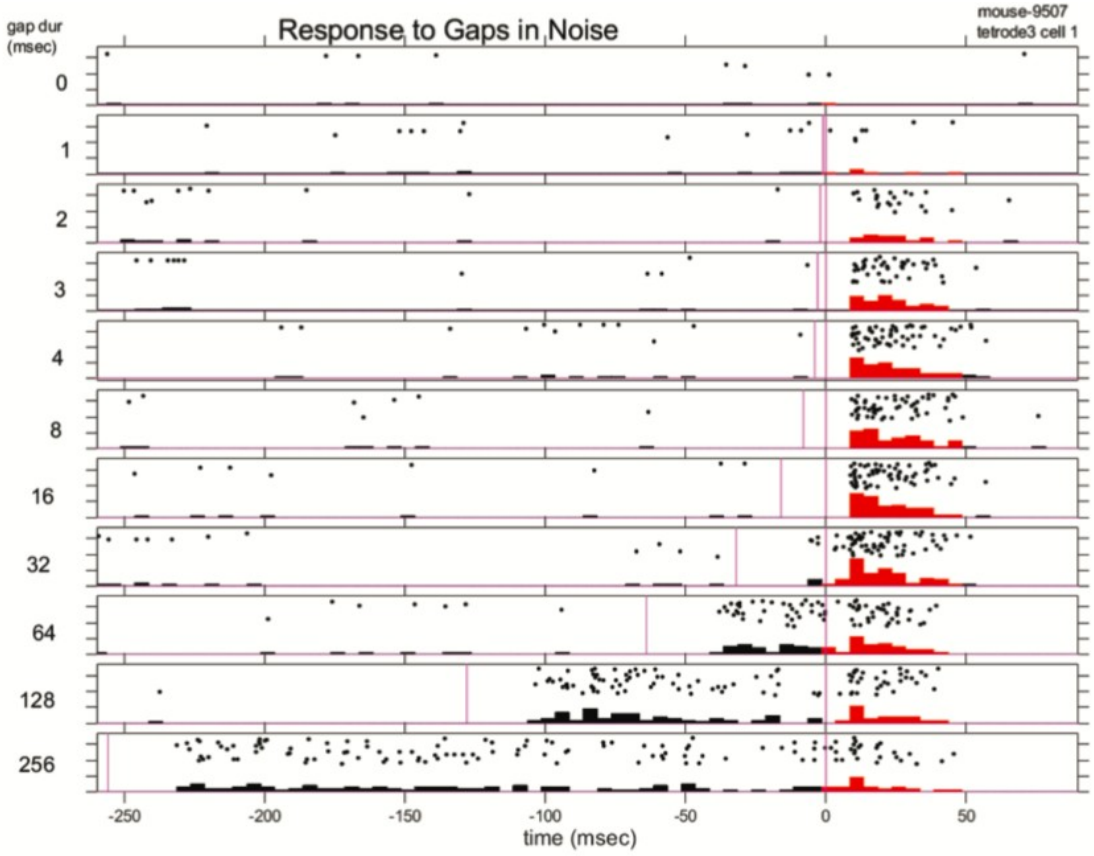

Figure 1. Gap termination response

The activity of a cell in the auditory cortex is plotted over time during gaps of varying lengths. When the gap ends, indicated by the right-hand vertical pink line, there is an increase in the cell's firing. These spikes are quantified with a histogram, where the GTR is in red.

Research Question

Repeating previous experiments, gap detection was measured before and after fear conditioning. However, adding to the procedures of previous experiments, the specific responses of PV cells were also recorded and characterized. PV+ cells were identified by adding channelrhodopsin-2 as an optogenetic tag. This caused all of the PV+ cells to fire in response to light, which was used as a tool to identify them. The

purpose of this experiment is to determine how the responses of PV+ cells to gap detection in the auditory cortex might change after fear conditioning.

Hypothesis

We hypothesize that the mice will improve at gap detection after fear conditioning. We expect that the shock will cholinergically activate SOM cells (via acetylcholine) which, in turn, will inhibit the PV cells in layers 2 and 3. If this occurs, we will see that the PV cells fire less (have smaller GTRs) after fear conditioning.

Methods

Mice used

We used genetically modified mice made by crossing homozygous Pvalb-IRES-Cre mice ("PV"; stock no. 008069; The Jackson Laboratory) and homozygous CAGChR2-eYFP mice ("ChR2"; stock no. 012569, line Ai32; The Jackson Laboratory). The resulting offspring expressed the protein ChR2 in PV interneurons in their brains, with 97% specificity (Moore and Wehr 2013).

Surgery

The mice were surgically implanted with an array of eight tetrodes (32 electrodes), a pair of shock electrodes, and an optic fiber for the laser by my colleague Aldis Weible. Before surgery, to reduce inflammation and respiratory stress, the mice were treated with dexamethasone (0.1 mg/kg) and atropine (0.03 mg/kg). They were then anesthetized with isoflurane (1.25–2.0%). A small opening was cut into the skull (2 mm - 1 mm) dorsal to the left auditory cortex. The array of eight tetrodes was inserted into the opening and glued into place with Grip Cement (Dentsply, Milford, DE). The optic fiber (200-m) was placed above the auditory cortex, covered with antibacterial ointment, and secured into place with more Grip Cement. Teflon-coated stainless-steel shock electrodes were placed just behind the left eye in muscle. After the surgery, the mouse was given Ketoprofen (4.0 mg/kg) to alleviate discomfort. Mice were housed individually after surgery.

Tetrodes inserted into the brain were made of 18-micron (25 micron coated) tungsten wire (California Fine Wire). The tetrodes were passed in pairs through

hypodermic tubing (28-gauge) creating 4 arrays. The 4 arrays were mounted together on a custom-built miniature microdrive. Continuous and peri-spike data from the tetrodes were collected with 32-channel RHD2000 hardware (Intan Technologies) and Open Ephys software (https://www.open-ephys.org).

Screening for cells

Cell screening took place in a sound-attenuating chamber where the mouse could roam around in a plastic tub (diameter 15cm) with litter covering the bottom and a free-field speaker placed directly overhead. The speaker was calibrated to 70 dB +/- 1 dB using a Brüel and Kjær type 4939 1/4-in. microphone. Throughout the arena, sound intensity varied up to 8.5 dB. Every 500 ms, the mouse was alternately presented with either a 50 ms white noise burst from the speaker or a blue light pulse through the optic fiber (laser duration 100ms, 445 nm wavelength, 5-30 mW power). Spikes were recorded if they exceeded a minimum threshold of 50 microV. Offline, the spikes of individual neurons were isolated using Simpleclust and MClust. We used firing rate vs time histograms with and without laser illumination to determine if the cell was a PV cell. Cells that responded both to laser and the sound were classified as PV+ cells located in the auditory cortex, while cells that did not meet both of these criteria were classified as PV- cells or as PV+ cells outside of the auditory cortex. Figure 2 shows an example of a PV+ cell responding to the laser pulse.

Once a PV+ cell was isolated, the mouse was ready for testing and conditioning. Cell responses to various gap durations ('Gap duration tuning') was tested before and approximately 6 and 24 hours after conditioning. Behavioral gap detection was also tested immediately before and six hours after conditioning.

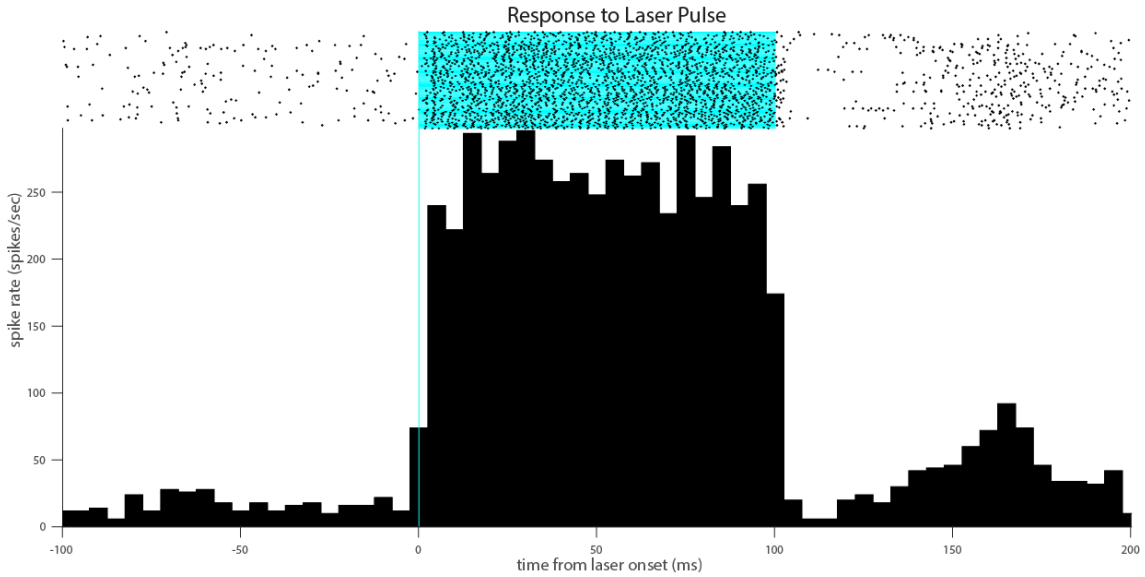

Figure 2. A firing rate vs. time histogram of a cell that clearly responds to the laser with a short (non-synaptic) latency.

Gap duration tuning

An ongoing white noise was played and briefly interrupted every 1000 ms by a gap with duration of 0, 1, 2, 3, 4, 8, 16, 32, 64, 128, or 256 ms, chosen pseudo-randomly (to test each duration the same number of times). There was no startle pulse or laser.

Behavioral testing and training

The mouse was moved from the recording tub to a small tube to restrict its motion. The tube was mounted on a small hinged platform that allowed recording of the mouse's movements (figure 3). We measured the mouse's startle response when presented with brief (50 ms), loud (100 dB) startle sound pulses in an ongoing white noise background (70 dB). Startle pulses were presented both with (test stimuli) and without (control stimuli) a preceding 8 or 16 ms gap in the noise. The mouse's startle amplitude was quantified as the area of the rectified platform movement within a 100

ms window following the startle pulse. Responses to test pulses (preceded by a gap) were expressed as z-scores relative to the responses to control (no gap) pulses.

After initial testing, the mouse was presented with a 'learning paradigm' where 8 millisecond gaps within ongoing white noise were paired with mild shocks. No startle pulses were presented. Thereafter, the mouse was placed in a dark, sound-attenuating box to limit external stimuli, for 6 hours between tests. This has been shown sufficient time to allow consolidation of 'learning' to occur with this paradigm (Weible, Liu, et al., 2014).

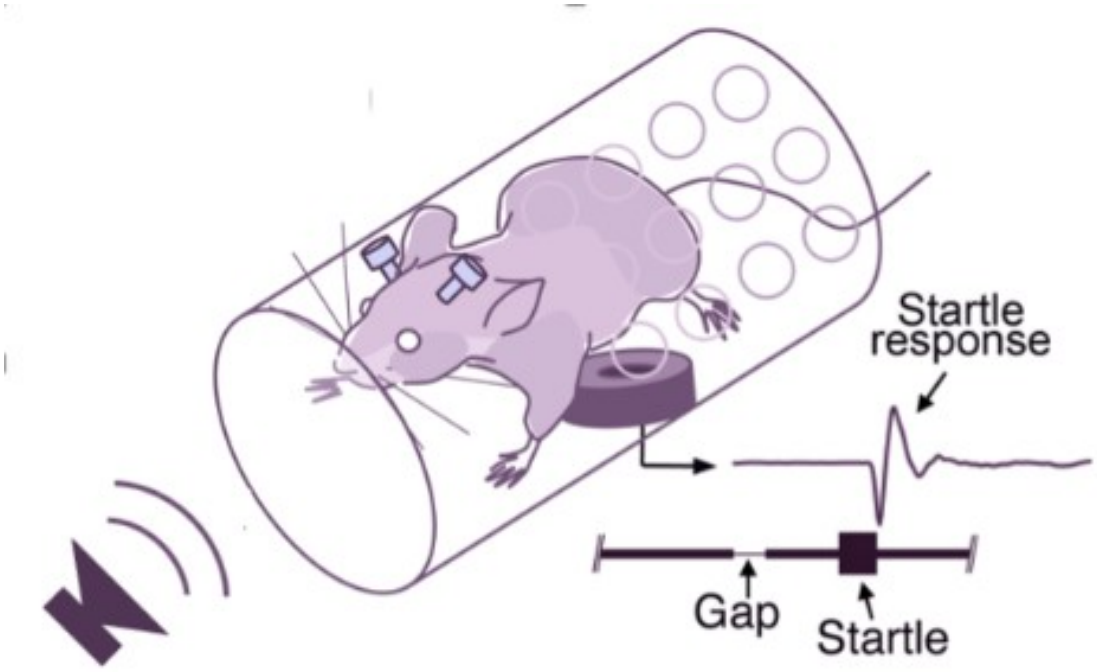

Figure 3. The mouse is put in a tube mounted on a pressure sensor during the behavior and fear conditioning portions of the experiment.

Histology

For confirmation of electrode placement, the mice were euthanized and perfused, and their brains were removed and cut into coronal cross sections with a

vibratome. The sections were placed onto slides, cover slipped, and photographed. The photographs were analyzed for electrode placement.

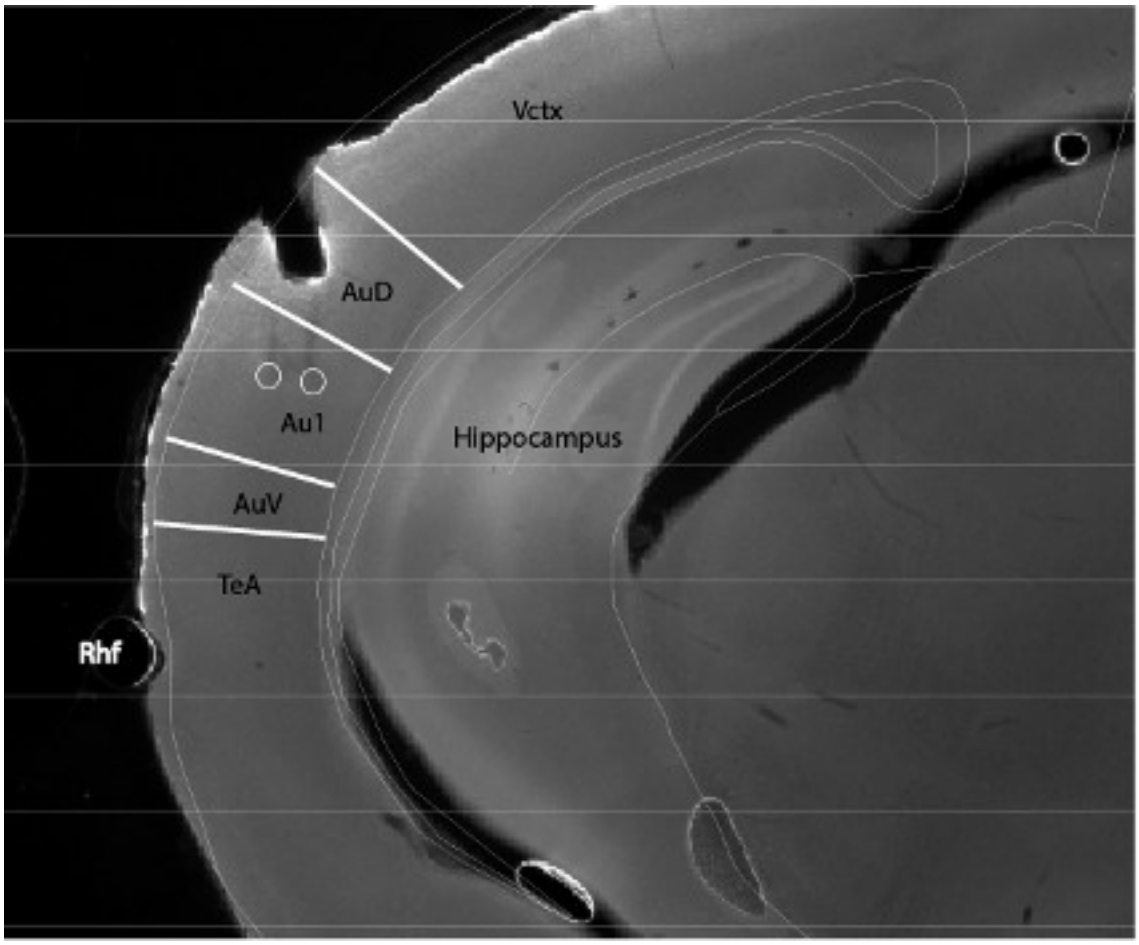

Figure 4. An example of a photograph of a coronal section, highlighting the auditory cortex.

The two circles show the end of the tetrode tracks within the cortex. Abbreviations: **Au1** - primary auditory cortex, **AuD** - dorsal auditory cortex, **AuV** - ventral auditory cortex, **Rhf** - rhinal fissure, **TeA** - temporal association cortex, **Vctx** - visual cortices.

Behavioral analysis

Behavioral tests comprised 40 startle pulses, half preceded by gaps ('test'), and half without gaps ('controls'). Within a session, the control responses were averaged ('averaged control'), and then, each test startle response was subtracted from the

average control and then divided by the averaged control. These ‘normalized’ responses for the pre-shock morning session and the post-shock afternoon session were compared by t-test. This separated the mice into three groups: ‘learners’: significantly smaller startles in the post-shock session ($p < 0.05$), ‘anti-learners’: significantly larger startles in the post-shock session ($p<0.05$) and ‘no change’ ($p>0.05$).

Results

We tested behavioral and cellular responses during gap detection before and after fear conditioning in 31 mice. Eight of these mice were tested and conditioned with 16 ms gaps, the remaining 23 mice employed 8 ms gaps. We found no systematic differences in either behavioral or cellular responses between the two groups and therefore we pooled all these mice together in the following analyses.

Based on previous research by Weible, Liu, Niell, & Wehr, 2014, we expected to see the majority of our mice improve at gap detection after fear conditioning. However, nineteen of the 31 mice tested were classified as showing 'no change' ($p > 0.05$, paired t-test) in their ability to detect gaps before and following fear conditioning. This was primarily due to the large within-mouse variance in startle responses across both the 20 no-gap and 20 with-gap trials. In the remaining 12 mice, gap detection improved after fear conditioning (startles with gaps were more attenuated) in 6 mice ('learners') and worsened (startles with gaps were less attenuated) in 6 mice ('anti-learners', figure 5, $p < 0.05$). 'Learners' had generally worse gap detection before conditioning, and therefore more 'room for improvement', than 'anti-learners.' Three of the six 'anti-learners' had quite strong gap detection before conditioning and thus, perhaps, almost no 'room for improvement.' Although there was a higher percentage of females amongst the 'no-change' mice than the other groups, we found no significant differences in learning related to the mouse's age or gender (figure 6).

We recorded from 40 PV+ cells and 96 PV- cells as identified by their responses to laser stimulation. We further categorized the PV- cells as having 'broad' (n = 79) or 'narrow' (n = 17) spike shapes (Figure 7). Broad-spiking cells are assumed to be

pyramidal (excitatory) neurons and narrow-spiking cells are presumably inhibitory interneurons (Moore & Wehr, 2013). Five of the narrow spiking PV- cells responded to laser stimulation with long latency (> 4 ms), suggesting that they were probably synaptically driven by PV+ cells and not directly driven by the laser.

We defined the Gap Termination Response (GTR) as the summed spiking response between 0 and 50 ms following the offset of the gap (figure 1). We compared GTRs for each cell-type and learning category and found that no group showed a significant change in size or timing of their GTR before versus after conditioning (Figure 8).

The averaged peri-stimulus time histograms (PSTHs), shown in Figure 9, demonstrate a major difference between the responses of PV+ cells from ‘learner’ and ‘anti-learner’ mice. On average, the GTRs of ‘learners’ were more than 5-fold greater than those from ‘anti-learners’ ($p = 0.0009$, Wilcoxon ranksum). This was true even before conditioning, as there was no significant change in the GTR in either group due to conditioning (Figure 8). Learner GTRs also had greater peak amplitudes ($p = 0.003$) and were longer lasting ($p = 0.0024$) than those of anti-learners. The GTRs of 'no-change' mice were more variable between mice and their average fell intermediate between the average GTRs of ‘learners’ and ‘anti-learners’. Despite this inter-cell variability, ‘no-change’ GTRs were broader ($p = 0.039$) and higher-peaked ($p = 0.013$) than those of anti-learners and narrower ($p = 0.032$) but not significantly different ($p = 0.351$) in peak height than the GTRs of ‘learners.’

The GTRs of PV- cells of all behavioral categories were much smaller than those of PV+ cells from ‘learner’ and ‘no-change’ mice ($p < 10^{-13}$, $p < 10^{-6}$) and similar to

those of PV+ ‘anti-learner’ mice ($p>0.46$, Figure 10). While there were no significant differences between PV- narrow and PV- broad cell GTRs, the number of PV- narrow cells in each behavioral category is quite small and these results are inconclusive.

We confirmed the locations of all tetrodes histologically, and all cells included in these results were located within the auditory cortex. We did not find any pattern relating gap detection ability or GTR to cortex layer or region of the auditory cortex.

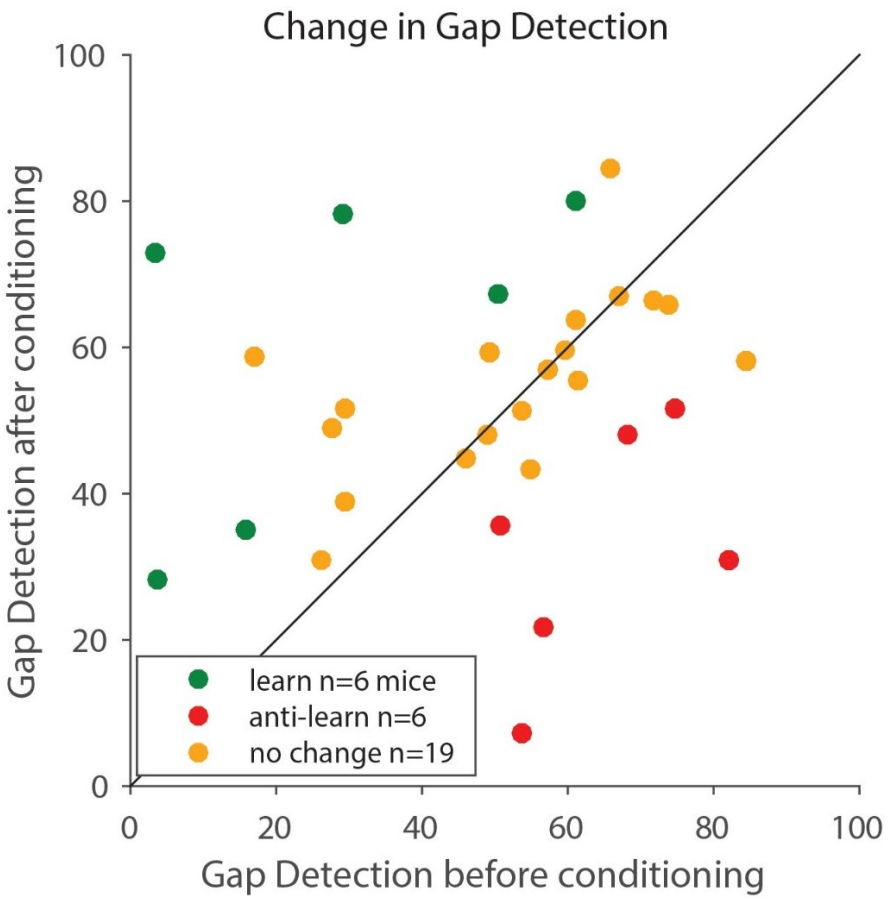

Figure 5. Effect of fear detection on gap detection performance.

Gap detection recorded before and after fear conditioning. Mice whose gap detection improved with conditioning (green) had a generally lower decline in startle response when a gap was present (compared to no gap) before conditioning (max = 61%) than mice whose gap detection diminished with conditioning (red, min = 51%).

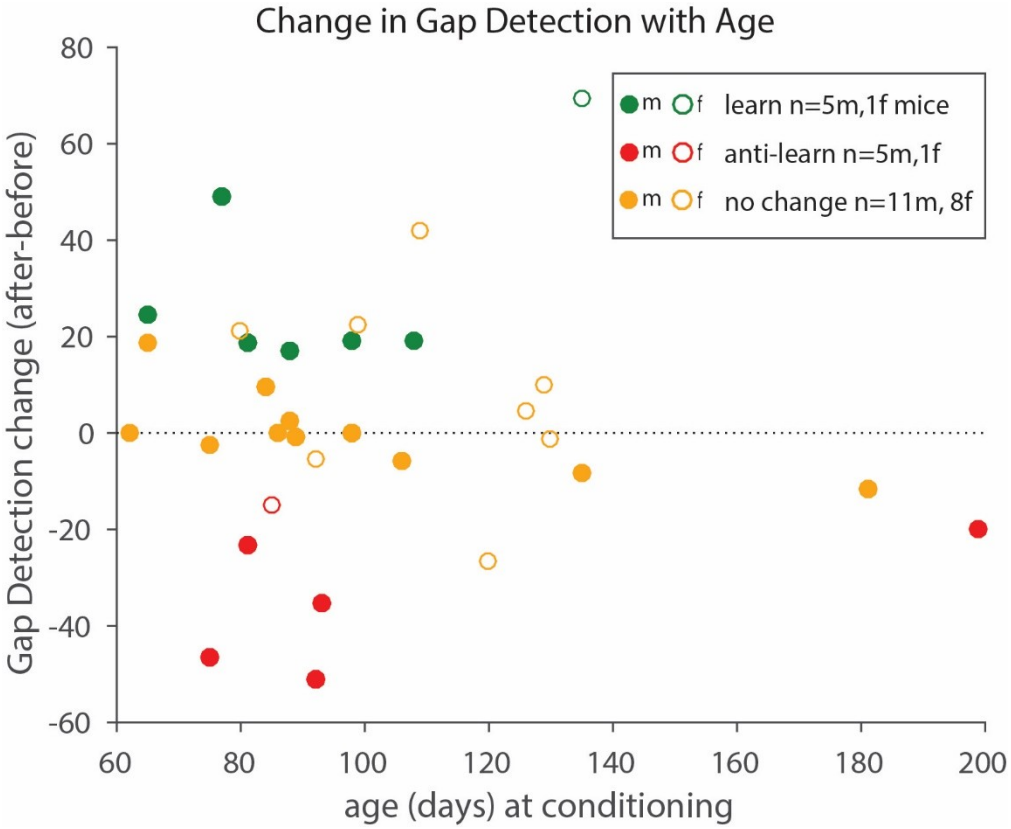

Figure 6. The effect of age and gender on gap detection.

Change in gap detection with age at the time of conditioning for males (filled circles) and females (open). There is no clear relationship between age or gender and changes in gap detection.

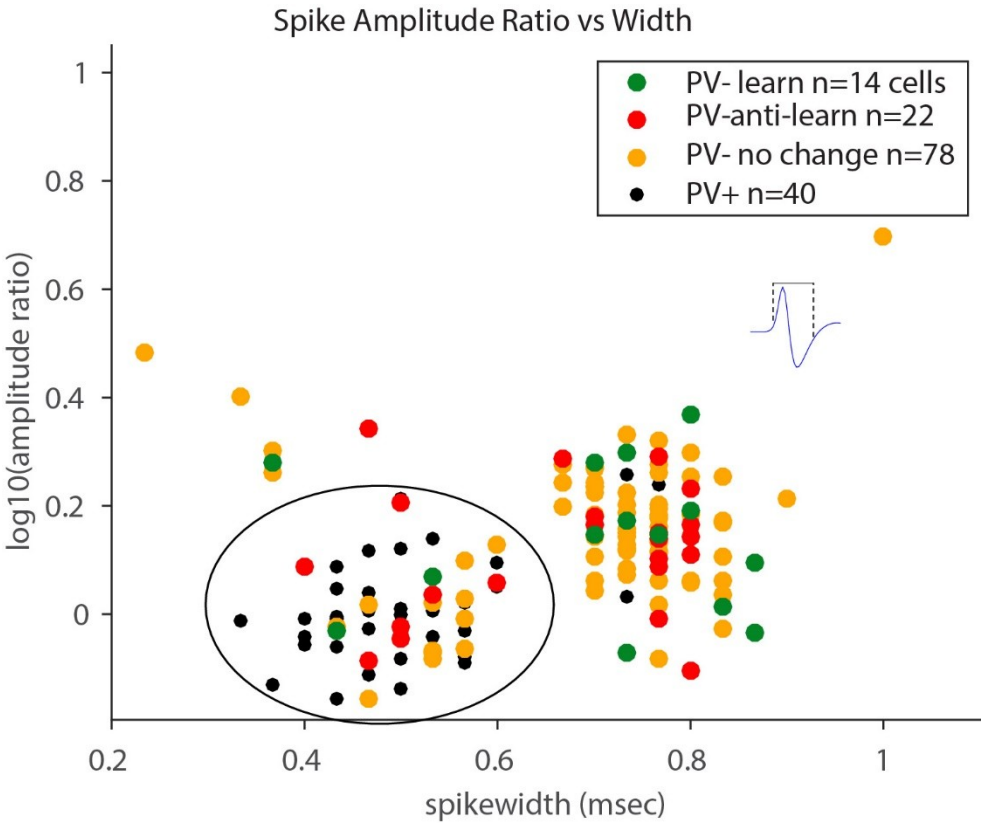

Figure 7. Spike amplitude ratio versus spike width.

Average spike shape for each cell measured as the log (ratio of positive amplitude / negative amplitude) plotted against the spike width measured as shown in the inset. The ellipse encloses cells defined as 'narrow' (after Keller et al. 2018).

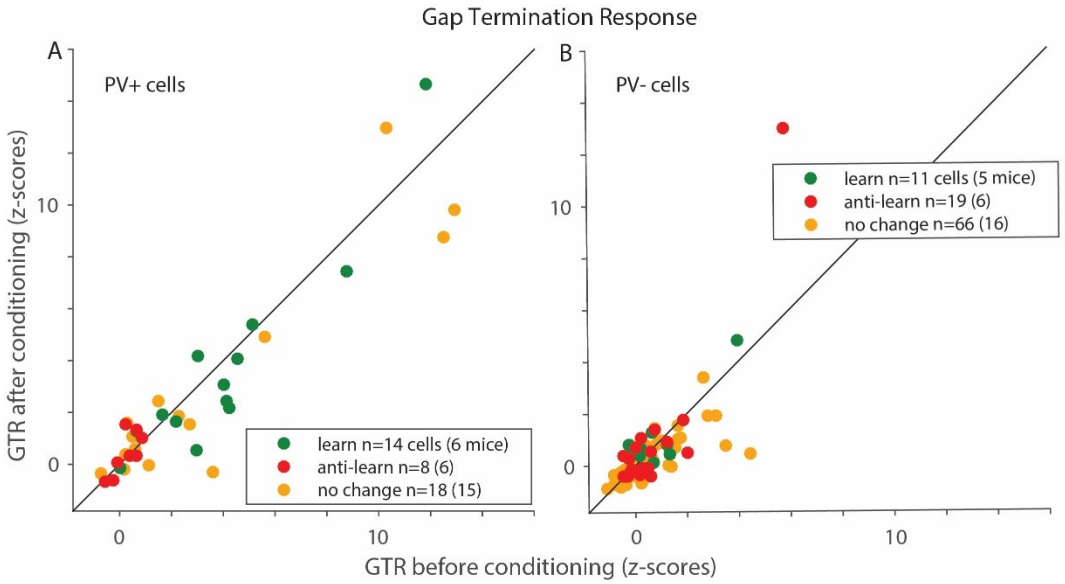

Figure 8. Gap Termination Responses (GTRs) before and after fear conditioning.

Average GTRs measured before and after fear conditioning. There were no significant changes in GTR in any of the three behavioral groupings for either PV+ or PV- cells. A cell's GTR is its summed activity between 0 and 50 ms after the gap offset, expressed as a z-score relative to the cell's responses to 0-ms duration gaps. **A)** PV+ cells **B)** PV- cells

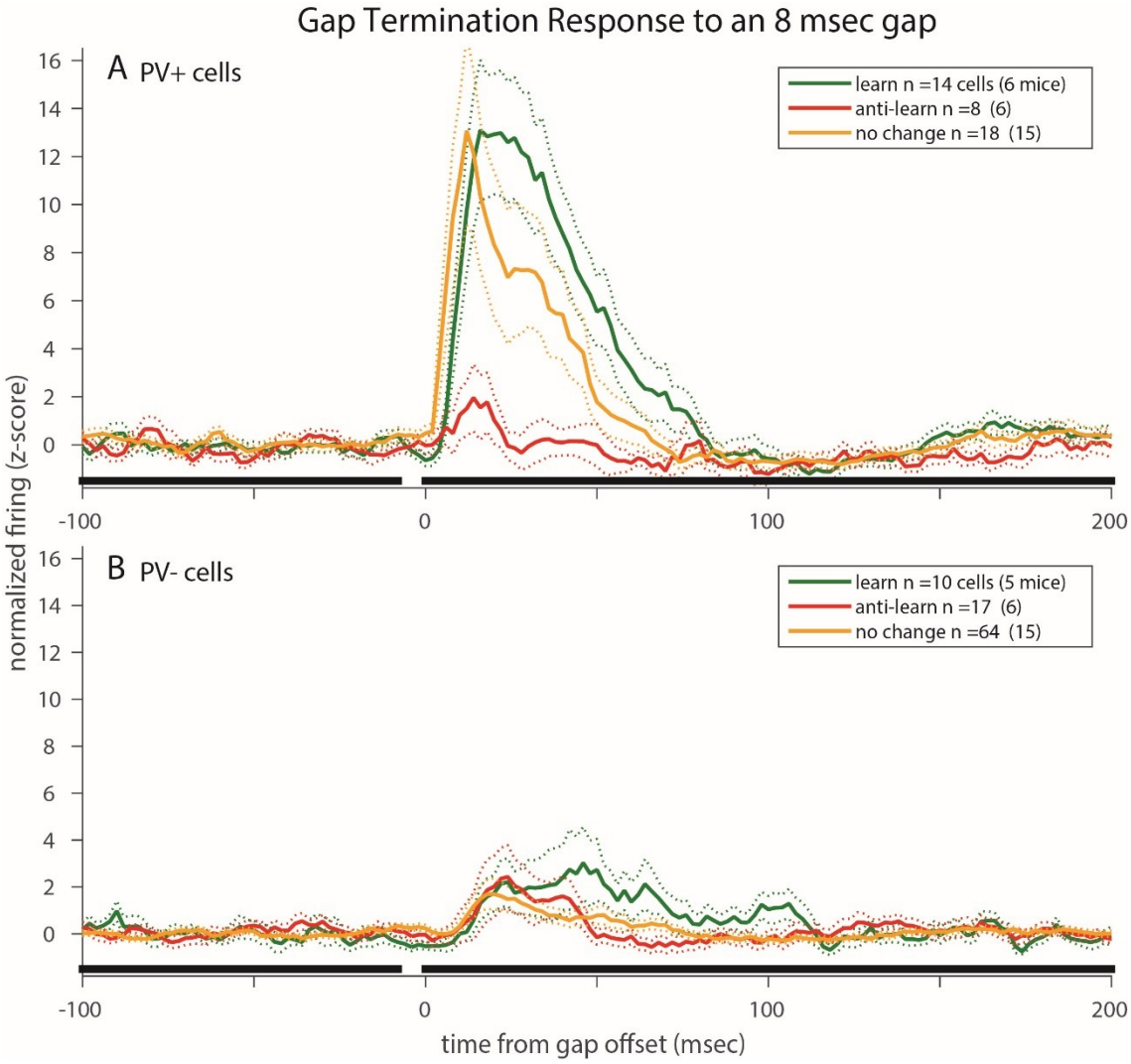

Figure 9. Averaged Peri-Stimulus Time Histograms (PSTHs) to an 8 ms gap before fear conditioning.

Each cell's PSTH was converted into running z-scores relative to the no-gap response and then averaged (thick lines, +/- s.e.m. dotted lines) within each cell type and learning category. Thick black line below the traces indicates time course of noise stimulus and the 8 ms gap ending at time 0. **A)** PV+ cells **B)** PV- cells

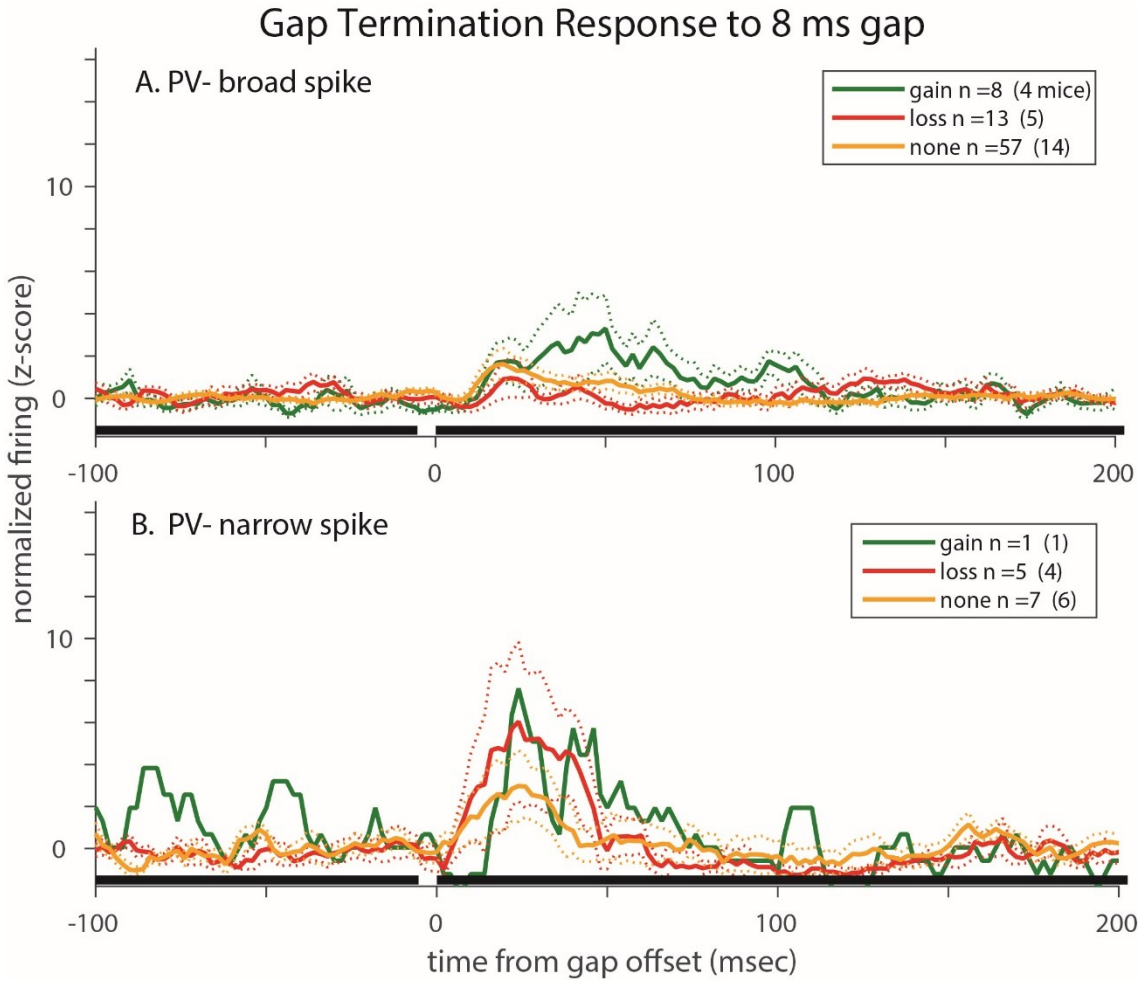

Figure 10. Averaged PSTHs for PV- cells to an 8 ms gap before fear conditioning.

Presentation as in Figure 6. A) PV- broad spiking cells, B) PV- narrow spiking cells.

Discussion

In this study we recorded from PV+ cells during gap detection before and after fear conditioning. Only 6 of 31 mice learned due to fear conditioning, while 6 mice were anti-learners, and 19 did not change. Mice that learned had GTRs both before and after conditioning that were about 5 times larger than those of anti-learner mice. There was no significant change in GTRs following conditioning.

Our hypothesis was that during fear conditioning, shocks would inhibit PV+ cells via a cholinergic pathway onto the superficial layers of auditory cortex. Based on this hypothesis, we expected to see smaller GTRs from the PV+ cells after fear conditioning. Instead we saw that GTRs did not change significantly after fear conditioning and we found no evidence that the shocks modified PV+ cell responses. These results cast doubt on our hypothesis.

An earlier study from our lab using very similar methods found that 13 of 14 mice learned to associate a peri-orbital shock with a brief 10 ms gap in noise, which significantly improved gap detection (Weible, Liu, et al., 2014). This study found that most mice learned, while our results found that most mice did not, and showed no change in gap detection (figure 11). Thus, the data reported here are inconsistent with these earlier findings. Why might these two studies give such different results? Both studies used PV-ChR2 mice (although the earlier study included some from another strain as well). Housing of mice, measurement of startle, and our method for shock delivery were similar (but not identical) between the studies. A subset of mice in each study showed no significant changes in gap detection (n=19/31 present data). Startle response reduction as a measure of gap detection is highly variable both within and

between mice. The current study used a perhaps more stringent criterion to determine significance of change in gap detection, but re-analysis of the current data with the earlier criteria did not change our conclusions. The result that mice with higher GTRs were worse at gap detection was consistent with both analyses. This left relatively small numbers of mice in each study that showed significant changes in gap detection (n = 14, Weible et al., n = 12 current data). In both studies, mice with relatively weak gap detection (higher initial startle amplitudes, figure 11) before conditioning improved and those with relatively strong gap detection (lower initial startle amplitudes, figure 11) before conditioning worsened. In other words, the learners had more room to improve. The differences between studies may simply reflect differences in these starting strengths of gap detection. It is possible that the learner mice that came into each study were already likely to improve, despite the fear conditioning.

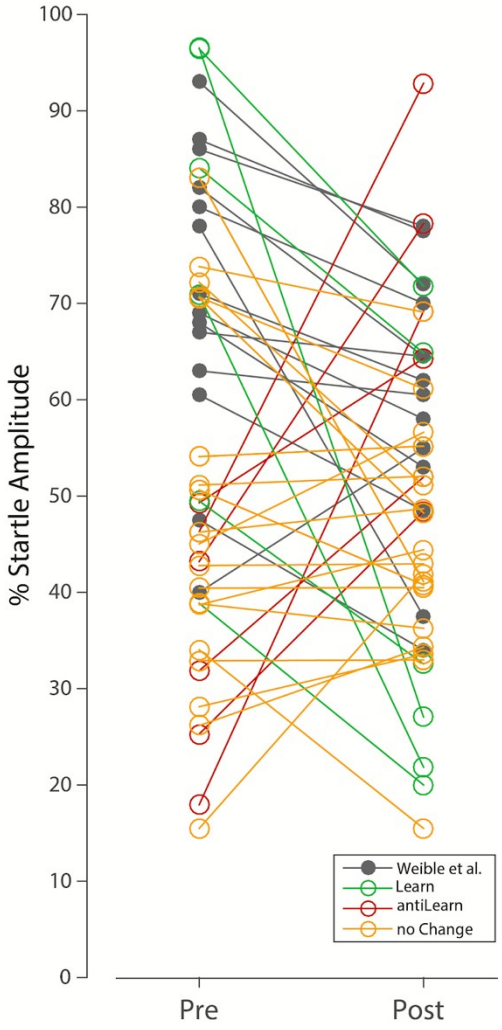

Figure 11. Comparison of startle amplitude before and after fear conditioning from this study and previous study (Weible, Liu, et al., 2014).

The gray points represent data from the past study, and the colored points represent data from this study.

Fear conditioning by foot-shock in mice is associated with increased cholinergic input to auditory cortical layer 1 cells, which suppresses spontaneous activity in PV+ cells in layers 2/3 (Letzkus et al., 2011). PV+ cells are known to inhibit principal neurons (PNs), so the end result of conditioning is thought to be a disinhibition of PNs (i.e. a net activation that results from the inhibition of inhibitory cells). The broad-

spiking PV- cells in the current study are probably mostly PNs. Thus, based on previous work (Letzkus et al., 2011), we expected to see a suppression of PV+ cell gap responses, and an increase in PN cell gap responses in mice that learned, but we saw neither. Similarly, we might have expected the gap termination responses (GTRs) of 'anti-learners' to change in the opposite way, but we saw no changes in the GTRs of PV+ or PV- cells in these mice either.

Our most striking finding was that the GTRs of the 'learner' and 'no change' mice were much stronger than the GTRs of 'anti-learners' (Figure 9A). The GTRs before and after fear conditioning did not change, so PV+ GTR strength appeared to be a predictor of whether a mouse was a 'learner' or 'anti-learner.' Since 'learner' mice generally had weaker initial gap detection, a strong GTR in PV+ cells might correlate with weaker gap detection before conditioning. GTR strength in any given mouse varied widely, and thus across cells the correlation between GTR strength and gap detection was weak ($r^2 = 0.17$ when including only learners and anti-learners). We therefore wondered if we could identify a trial-by-trial relationship between GTR strength and initial gap detection. Figure 12 shows this trial-by-trial relationship in PV+ 'learner', 'anti-learner', and 'no change' mice. Each line represents the best linear regression between gap detection and GTR for all trials for a given cell. A relationship between gap detection and GTR on individual trials could be inferred if each line within a behavioral category showed a similar slope. This, however, is not the case. Thus, GTR strength in PV+ cells is not predictive of startle size on a single trial, but instead is predictive of the mouse's learning capacity. Moreover, the trial-by-trial relationship

between GTRs and gap detection was similar for PV- broad spiking cells. Thus, PN GTR strength was also not predictive of gap detection on a trial-by-trial basis.

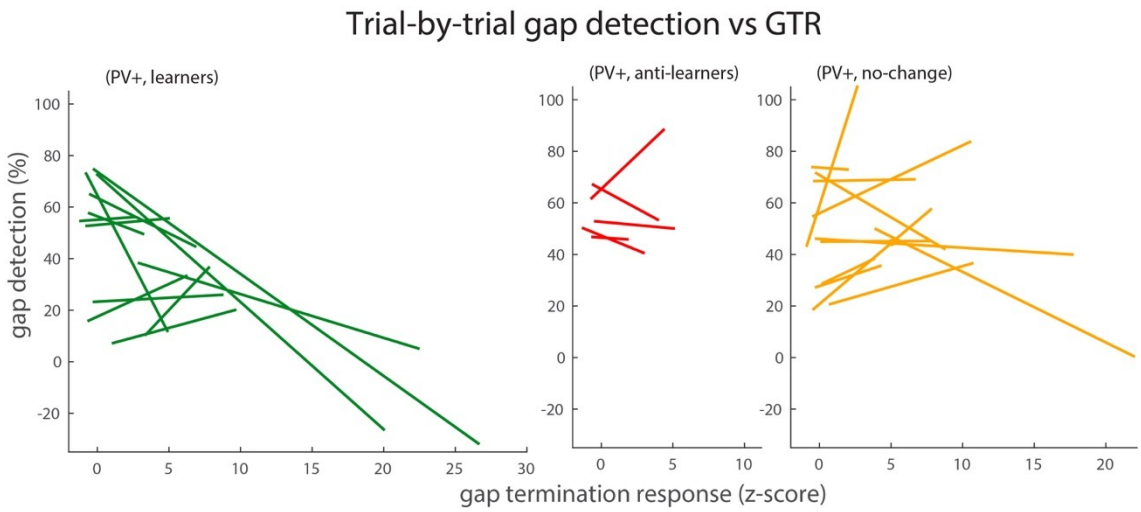

Figure 12. Trial-by-trial comparison of PV+ cell GTRs and gap detection before fear conditioning.

Each line is a regression for a cell across trials. There were usually very few spikes and most trials had no spikes. Most, or all, regressions are not significant. No clear pattern is observed between GTR size and gap detection ability before fear conditioning.

In preliminary, unpublished data from our lab (Weible and Wehr) a subset of PNs (6 of 17 cells in 2 mice) showed an increased GTR after fear conditioning. In the present data we found that 2 of the 8 PV- broad cells from 'learner' mice also showed strong increases in GTR after fear conditioning. Overall, this class of cells showed a small, but non-significant, strengthening of the GTR (figure 13).

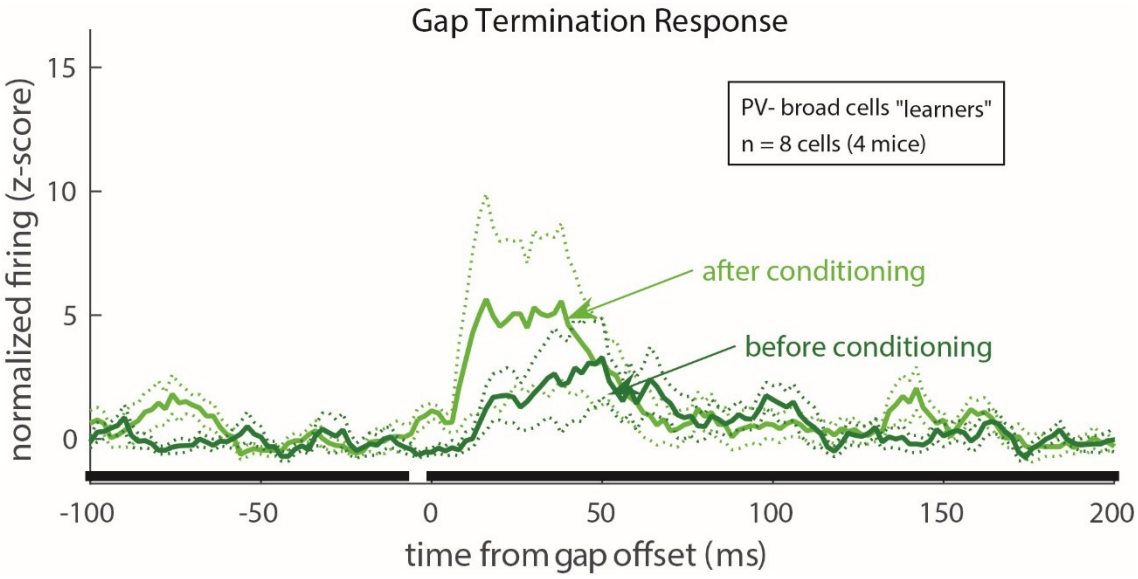

Figure 13. Average GTR before and after conditioning for broad-spiking PV- cells from 'learner' mice.

The averages across cells (bold, +/- s.e.m. dotted) of the PSTH are plotted for before and after conditioning. For each cell, the PSTH is computed as a running z-score of the spike-rate relative to the pre-gap spiking activity.

In the future, increasing the number of subjects would be valuable to increase our confidence in these surprising results. Also, more trials for each subject during the gap detection tests would make the statistics stronger. This might cause some of the no

change mice that got slightly better at gap detection to be statistically classified as learners. Additionally, this experiment requires a more thorough investigation of the relationship between the size of the GTR and gap detection. It appears that fear conditioning does not influence this relationship, so I would like to explore whether the connection between a high GTR and learning gap detection is innate. Finally, because we were not able to closely replicate the results of the experiment that looked at how fear conditioning affects gap detection and PNs, this result should be looked at more thoroughly.

This study tested a proposed circuit for how PV cells function in the auditory cortex for gain control. Although our data did not match the predictions based on that circuit hypothesis, we now have a better idea of the relationship between learning and GTRs in auditory cortex. This serves as a foundation for further research, before any broader conclusion about the circuit can be made. Understanding gap detection and dysfunctions within these processes directly connects to understanding more about speech perception and why elderly listeners might have speech deficits. Further knowledge on how learning affects the auditory cortex and gap detection ability is important in the process of figuring out therapies for centrally related hearing loss.

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