# AN EVALUATON OF THE GAP DETECTION ABILITY OF MICE USING TWO-ALTERNATIVE FORCED CHOICE TASKS

by

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A THESIS

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The ability to understand speech becomes more difficult with normal aging and with diseases such as Alzheimer's. This difficulty is caused in part by speech processing deficits. Speech processing is an aspect of hearing that converts the speech you hear into electrical signals your brain can understand. These speech processing deficits are thought to reflect problems with the neuronal connections in auditory cortex. To remedy speech processing deficits and improve the quality of life of people living with speech processing deficits, we first need to understand the complex speech processing pathway.

One aspect contributing to speech processing deficits is a specific deficit in detecting the gaps in between words, syllables, and phonemes. Gaps are important cues used to identify the boundaries between words in fluent speech. Moreover, many phonemes (such as /b/ and /p/) are distinguishable based on the timing of gaps within these speech sounds. The ability to detect brief gaps is known as gap detection. Since gaps play an important role in speech processing, the neuronal circuits used in gap detection provide a simplified model of the neuronal circuits used in speech processing.

Thus, we focus on gap detection to learn about one part of the speech processing pathway in auditory cortex.

To study the pathways used for gap detection, we are assessing the gap detection ability of mice. Once we establish the gap detection ability of mice, successfully manipulating their gap detection ability with optogenetics will imply that gap termination responses do mediate gap detection.

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# INTRODUCTION

## Hearing loss

The World Health Organization reports that 430 million people in the world have disabling hearing loss. There are a few different types of hearing loss. It can be caused by problems with the peripheral auditory system, the central auditory system, or a combination of both systems. The peripheral auditory system consists of the outer, middle, and inner ear. Hearing begins when sound enters the ear canal. The sound travels as a wave through the peripheral auditory system to the cochlea of the inner ear. The cochlea transforms the sound wave into electrical signals which travel towards auditory cortex, which is in the outermost layers of the temporal lobe. The auditory cortex, along with a variety of structures within the brain, comprises the central auditory system. This is where sound is processed by the brain, allowing you to understand and assign meaning to the sounds you hear. Devices such as hearing aids and cochlear implants can help restore hearing capability to people with dysfunction of the peripheral auditory system. However, there is no treatment for hearing deficits due to dysfunction of the central auditory system. Research into the complex processes of auditory cortex is a step towards finding a treatment for those who suffer from central auditory processing deficits.

Deficits with auditory processing occur with natural aging, but they also occur with diseases such as Alzheimer's. 50% of the population over 65 have hearing deficits associated with processing sounds (Kim 2013). These processing deficits cause people to have trouble identifying and understanding speech sounds amongst background noise, difficulty following rapid speech, and difficulty localizing sound (Humes 1991).

These symptoms represent problems with auditory discrimination, temporal processing, and binaural processing. For this thesis, we focused on temporal processing deficits, specifically temporal gap detection.

## Gap detection

Gap detection is the ability to quickly detect brief gaps in sound. Gap detection is a simplified model for the various complex pathways used in speech processing. To perform gap detection, you must distinguish between the presence of a gap in background sound vs the absence of a gap in background sound. Deficits in gap detection are important to study because deficits in the gap detection pathway contribute to the deficits in speech perception. Detecting gaps in sound allows you to distinguish between different phonemes and words. Gap detection is crucial for being able to pick out speech in conversation from background sound.

### *Minimum gap threshold*

The minimum gap threshold (MGT) is the briefest gap the mice can detect. This means that it is difficult to perceive gap durations shorter than one's detection threshold. The threshold will vary from subject to subject due to natural variance. A 2020 study showed that the threshold of the duration of gap which mice can process and perceive was 2ms. This MGT was determined by using the gap duration at the midpoint of the logistic fit (Weible 2020). This literature focused primarily on testing the mice's ability to detect brief gaps in background sound. To this end, they assessed the mice's gap detection ability for gap durations of 0ms to 256ms. For this thesis, we will repeat the

steps to find the MGT by again testing the mice's ability to detect gap durations ranging from 0ms to 256ms.

*Voice-onset time*

As mentioned above, gaps of different durations are used to distinguish different phonemes and words in speech. This becomes especially applicable when thinking about voice-onset time (VOT), which is the gap between the movement of the speech organs and the vibration of the vocal cord. The characteristics of this gap distinguish different speech sounds, such as different phonemes like /b/ and /p/. Learning more about the gap detection pathway will lead to more questions about the VOT pathway.

**Mice as a model**

Mice are an effective model to use for studying the speech processing deficits in humans because mice have similar anatomy to humans in the central nervous system. They also age to adults quickly, allowing for experiments to be carried out rapidly. Additionally, mice can learn tasks efficiently and execute them well. Thus, we can train mice in behavior tasks that evaluate their perception of sound and learn about the neuronal pathways involved with that perception. Another key advantage to using mice over humans is that there is a wide array of powerful genetic and neurophysiological tools that we can use to study brain function in mice that are not available in humans.

Studies in mice have already been used to study gap detection and hearing loss. One study showed that hearing loss in mice is a sign of Alzheimer's (Liu 2020). Another study in 2019 examined gap detection ability in 5XFAD mice, mice bred with human Alzheimer's genes, and it was found that their gap detection ability deteriorated

as they got older and the effects of Alzheimer's set in (Kaylegian 2019). The connection between hearing loss due to Alzheimer's and gap detection deficits suggests that in humans, gap detection deficits might be an early sign of Alzheimer's. This is significant because it shows how the mouse model leads to further human research that could impact the quality of life of people.

**Role of auditory cortex**

The auditory cortex is located in the temporal lobe and is an integral piece of the central auditory system. In both mice and humans, the neurons in primary auditory cortex are organized as a tonotopic map of the cochlea (Bandyopadhyay 2010). As seen in Figure 1, each section of the primary auditory cortex corresponds to afferent stimuli from a specific part of the cochlea, and thus a specific frequency of sound.

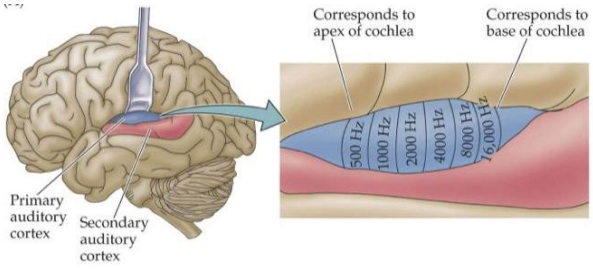


Figure 1. Tonotopic map of auditory cortex (Bandyopadhyay 2010)

It is important to note that auditory cortex is only one of the brain structures that contributes to the pathway of speech processing and gap detection. Other structures, such as the inferior colliculus and superior colliculus, are also heavily involved downstream of the processing that occurs in auditory cortex (Tokuoka 2020).

*Gap termination response*

When a gap in background sound ends, the neurons in auditory cortex respond with a spike of activity. This is known as the gap termination response (GTR). The GTR occurs in the 50ms immediately following the end of the gap. Previous studies have shown that GTR is crucial for the detection of brief gaps (Weible 2014). However, more research needs to be down to show that GTR mediates gap detection.

For this thesis, we focused on the processing in auditory cortex because previous studies have made it evident that mice use auditory cortex to process gaps in background sound (Weible 2020). This study suggests that auditory cortex aids in the detection of gaps during both the on-responses, when the neurons are firing, and during the off-responses, when the neurons are silent. The off-responses contribute to gap detection because the contrast between the off-responses and the on-responses amplifies the on-responses.

*Auditory cortex and behavioral learning*

Other previous findings about auditory cortex in mice highlight the role that auditory cortex plays in behavioral learning. The literature shows that auditory cortex encodes sounds in a way that is directly relevant to behavior (King 2018). This means that the way auditory cortex processes sound depends on the behavioral context. This enables a subject to learn and remember behavior associated with complex acoustic stimuli. This is crucial to know for this thesis as it indicates that the auditory cortex influences not only the processing of gaps, but also the behavior the mouse performs in association with the gap. Another study showed that auditory cortex is used specifically for learning gap detection tasks involved with fear conditioning (Weible 2014). While

this thesis does not include fear conditioning in its methodology, this 2014 study is relevant because the ability to learn fear conditioning with auditory cortex suggests the mice will be able to learn a two-alternative forced choice task with auditory cortex.

**Two-Alternative Forced Choice tasks**

The primary methodology that I'm using for my thesis is 2-alternative forced choice (2-AFC) tasks. This type of behavioral task requires the subject to make a choice based on the stimulus they're presented with. In this case, they make their choice by poking their noise in one of two response ports. If they hear a gap they go to the left port, and if they don't hear a gap, they go to the right port. From their behavioral response, we can determine whether they perceive the gap or not.

2-AFC tasks are a well-established method for analyzing the perception of mice (Ashwood, 2020). 2-AFC tasks are often referred to as a "go/go" task because the subject is forced to make a choice regardless of the stimuli. This contrasts with "go/no go" tasks where the subject would only perform a behavioral response for one stimulus. "Go/no go" tasks often lead to a bias towards the "go" response (Shenoy 2012), which is not ideal for our aims in this thesis. In the 2-AFC task, by analyzing which stimuli lead the mouse to make the correct choice and the percentage of trials in which they make the correct choice, we can figure out the MGT.

Results from 2-AFC tasks are displayed as psychometric curves. Psychometric curves show the subject's response as a function of the stimulus intensity. For this thesis, our psychometric curves show the percentage of times the mice went to the left or right port as a function of gap duration.

*Psychometric curves do not only represent perception*

One issue with analyzing 2-AFC results is that the mice's performance does not only depend on perception, but it is also affected by the probability of reward (Stuttgen 2011). For example, for the stimuli near the gap duration threshold (the gaps that are just too short for the mice to hear), the mice will have to guess whether they heard a gap. In Figure 2, these borderline stimuli near the threshold are represented by the striped area under the curves. The mice do not perceive a gap both when there is no gap and when there is a gap duration that is shorter than their MGT. When the stimulus is no gap, they get a reward for going to the right. However, when the stimulus is a very short gap duration, they get a reward for going to the left. Thus, when the mouse doesn't perceive a gap, they make their choice based on the probability that they will receive a reward, not on the gap duration. This skews the psychometric curve plotting their performance.

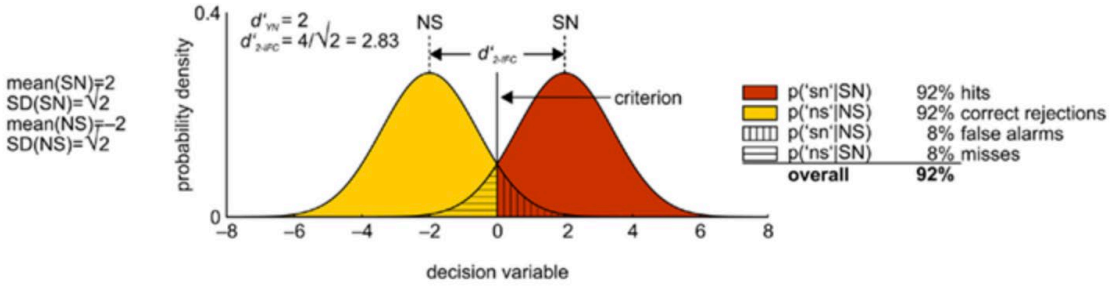


Figure 2. Critique of Psychometric Curves (Stuttgen 2011)

This chart shows that in a psychometric curve, not all the subject's choices are accurate representations of their perception. Statistically, 8% of the hits are false alarms and 8% of the rejections are misses. These percentages can increase with other factors, such as distraction, that interfere with the mice's perception of the stimulus.

The mice's performance is also impacted by factors like the amount of time the mice can focus on the task and how frequently they get distracted. When the mice get

distracted, they may not pay as close attention to the stimulus and guess, which also skews the psychometric curve. Thus, when evaluating a psychometric curve, one must remember that is does not only represent perception. Even still, psychometric curves are the most common way to represent data from choice task behavioral trials.

*Water as a reward*

Another critique of 2-AFC tasks is that water is the reward for correct trials. To motivate the mice to do trials, they only receive water when they are doing trials. On non-training days, mice receive supplemental water. Some literature suggests that instead of putting mice on water restriction, mice can have free access to citric acid (CA) water (either only on non-training days or continuously) without significantly reducing their thirst or decreasing the number of trials they perform (Urai 2021). Using CA keeps the mice at a healthy weight and allows them to self-regulate their drinking patterns. It also keeps them thirsty enough to be motivated to perform the task. Additionally, providing free access to CA would eliminate the responsibility of giving mice supplemental water on their non-training days, thus CA is a more non-labor-intensive option. While this methodology would work in our lab, there is not a worthwhile cost/benefit ratio to switching from our water restriction protocol to free access CA. In our water restriction protocol, the number of trials mice perform does tend to decrease in the days following non-training days, since the mice are less thirsty after receiving supplemental water. However, this was not a problem for this thesis because the mice performed behavioral trials over a span of multiple months, so there was no shortage of trials. Also, the researcher responsibility of ensuring the mice receive supplemental water on non-training days was also not a problem for our lab.

Lastly, the mice's health is closely monitored on our water restriction protocol to ensure the mice are hydrated enough, so the CA access would not significantly increase their health. Thus, the effort to switch to CA access was not worth it for this thesis.

**Optogenetics**

The other main methodology we used is manipulation of the brain through optogenetics. Optogenetics is a genetic technology that lets us control the activity of specific neurons and circuits with light, using genetically engineered light-sensitive genes. This means the presence of light will alter the mice's neural activity during a 2-AFC task. We are trying to see if the manipulation of neural activity impacts behavior. In one experiment, we're attempting to activate neurons to fool the mice into perceiving a gap that isn't there, and in another experiment, we're attempting to silence neurons to fool the mice into missing a gap that really was there (Keller 2018). Using mice with optogenetic genes is a powerful method for manipulating behavior.

It has been shown that optogenetic suppression of neurons during the GTR will inhibit gap detection ability (Weible 2014). Manipulating the gap detection ability by manipulating neuronal activity during the GTR with optogenetics, will suggest that GTR does mediate gap detection. One limitation to consider is that cortical manipulation during the GTR following gap durations close to MGT may have little effect because mice perform poorly close to their detection threshold (Weible 2020). Even still, the effect of the presence of light should be evident in the behavior response.

## Research questions

The primary question this thesis addresses is, “Does the gap termination response (GTR) mediate gap detection?” To test for this, we must first ask ourselves “What is the gap detection ability of mice?” To find this, we are specifically asking, “What is the shortest gap duration that the mice can detect?” because this will tell us the MGT of the mice’s gap detection ability. Another way to test if GTRs mediate gap detection is by asking, “How can we use optogenetics to manipulate gap detection?” To answer this, we are specifically asking, “In mice with inhibitory optogenetic genes, will gap detection ability be impaired when light is present in auditory cortex during the GTR?” and, “In mice with excitatory optogenetic genes, will gap detection ability be enhanced when light is present in auditory cortex during the GTR?” Once we establish the gap detection ability of mice, successfully manipulating their gap detection ability with optogenetics will imply that gap termination responses do mediate gap detection.

### *Hypothesis*

Gap detection is mediated by gap termination responses, and this is evident through the optogenetic manipulation of neuronal activity in the auditory cortex of mice during gap termination responses.

# METHODS

## Mice used

There were 28 genetically bred mice involved with these trials, 10 bred with excitatory optogenetic genes, and 18 bred with inhibitory optogenetic genes. The mice with excitatory optogenetics were bred by crossing ChR2 x Gpr26-cre lines. The mice with inhibitory optogenetics were bred by crossing ChR2 x PV-cre lines. These genotypes were confirmed with testing.

ChR2 stands for Channelrhodopsin-2 which is a gene that encodes for the expression of a blue light activated cation channel. As seen in Figure 3, the presence of blue light causes a conformational change in the protein channel, allowing cations to passively diffuse down their concentration gradients (Tan 2015).

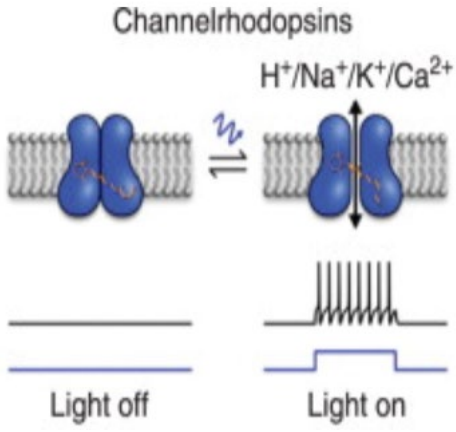


Figure 3. Effect of the presence of light on channelrhodopsin proteins (Tan 2015)

Gpr26 stands for G-Protein Coupled Receptor 26 which is a gene that encodes for a membrane protein that is involved with cellular responses to environmental stimuli, neurotransmitters, and hormones (Watkins 2020). In this case, crossing ChR2 and Gpr26 should cause the presence of light to increase the expression of this protein

which will excite the neuronal transmission of signals responding to environmental stimuli.

PV stands for parvalbumin which is a gene present in some fast-spiking interneurons. Interneurons are cells that transmit signals between neurons. The parvalbumin expressing fast-spiking interneurons are GABAergic, meaning they are influenced by the neurotransmitter GABA, which inhibits neuronal transmission of signals (Nahar 2021). In this case, crossing ChR2 and PV should cause the presence of light to increase the activity of these GABAergic interneurons, which will inhibit the neuronal transmission of signals responding to environmental stimuli.

**Surgery**

Figure 4. Depiction of mouse implanted with optic fibers

The 28 mice were surgically implanted bilaterally with optic fibers by Dr. Aldis Weible, a postdoctoral Senior Research Associate in the Wehr Lab. The fibers protrude from the skull, as seen in Figure 4, so that tethers can be attached to them. During the 2-AFC task, laser pulses travel through the tethers, to the optic fibers and then into the brain via the optic fibers. These optic fibers were positioned by Dr. Weible to rest just above auditory cortex, as seen in Figure 5. This means the laser shines specifically on

neurons in auditory cortex, so that the presence of light impacts neural activity in auditory cortex.

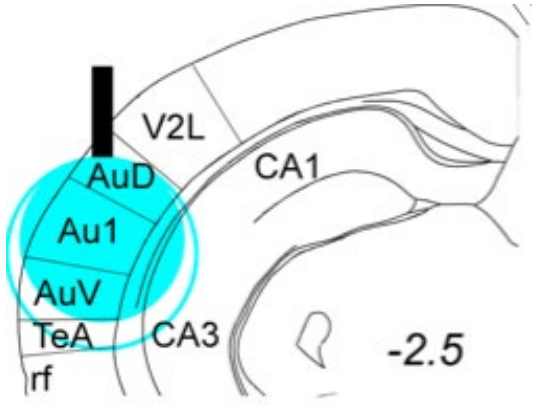


Figure 5. Diagram of optic fiber resting over auditory cortex

The optic fiber is represented by the thick black line and the area of cortex the laser will shine on is represented by the blue circle.

**Hardware and software**

The mice perform the 2-AFC behavioral tasks within a sound attenuating chamber. These chambers are comprised of an outer box, which is lined with soundproofing material, and an inner box, where the mouse is set up. There are 10 of these chambers, as seen in Figure 6. In the outer box, there is a speaker that delivers the background white noise and the gaps. As seen in Figure 7, the walls of the inner box have many holes in them to allow this sound to reach the mouse with limited interference. Within the inner box, there are three ports which deliver water as a reward for correct trials, which you can see in Figure 8. The water delivery through each port is controlled by solenoids, which can be seen in Figure 9.

Figure 6. Ten sound attenuating chambers, optic

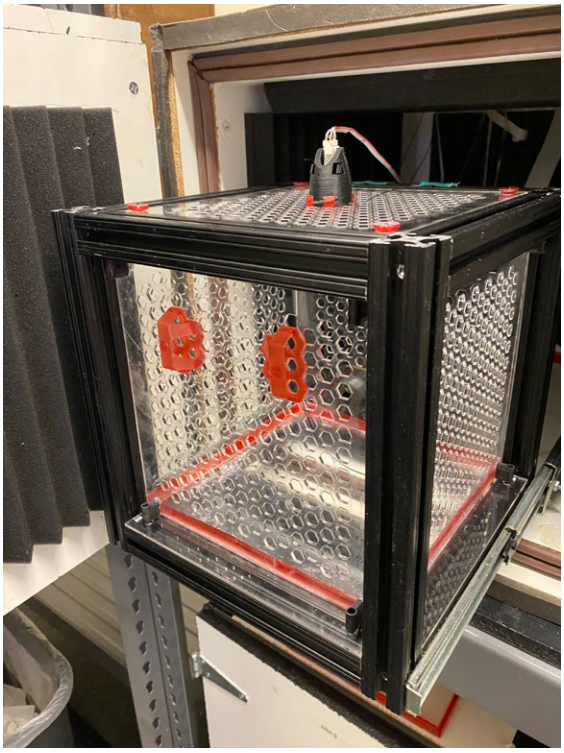

Figure 7. The inner box within the outer box

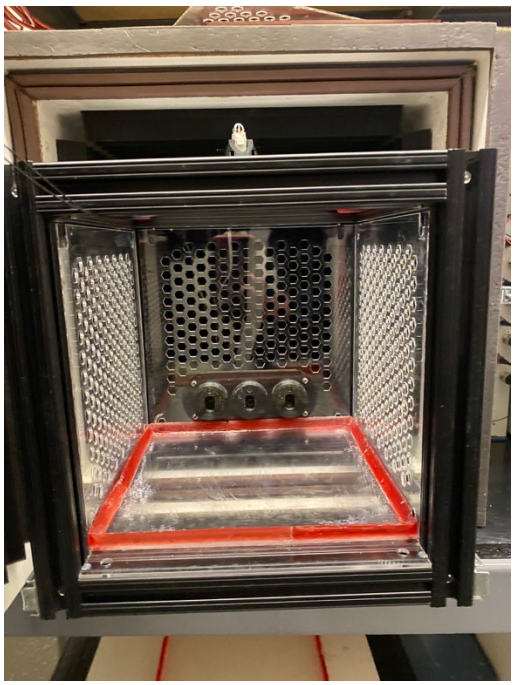

Figure 8. Image of the inside of the inner box and the three water ports

The light from the lasers has a wavelength of 450nm and it travels to the implanted optic fibers through a tether at maximum power output of 6.3mW. There is an LED light set up in the box that flashes during trials to mask the laser delivery to prevent the mouse from using the laser as a cue that could influence their behavioral response.

Each chamber has a Raspberry Pi, a small single board computer as seen in Figure 9. The ten Raspberry Pi's connect to the software that runs and stores the behavioral task, Autopilot. Autopilot is a program that was created by Jonny Saunders, a PhD candidate in the Wehr Lab, to run behavioral tasks, like the 2-AFC task used in this experiment (Saunders and Wehr, 2019).

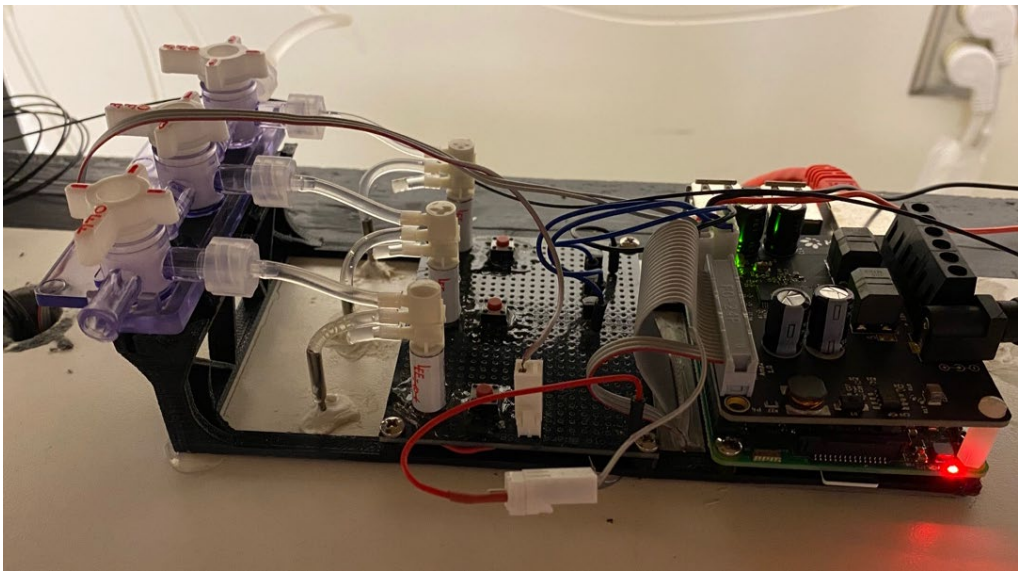

Figure 9. Arrangement of hardware and software connections for each chamber

Left to right: the water line connections for the three ports, the solenoids for each water line/port, and the Raspberry Pi

## Behavioral testing and training

### *Water restriction*

During the behavior trials, the mice are put on a water restriction protocol. This means that they do not have access to free water in their cage, instead they only have access to water while they are doing the 2-AFC behavior task. To ensure the mice maintain a healthy level of hydration, we closely monitor their weight, ensuring they stay above 80% of their baseline weight. On any non-training days, the mice received access to supplemental water in their cage for at least 30 minutes.

### *2-Alternative Forced-Choice tasks*

As previously discussed, 2-Alternative Forced Choice tasks (2-AFC tasks) are a type of behavioral task that requires the subject to make a choice based on the stimulus

they're presented with. In this case, the mouse is the subject, and the stimulus is the presence or absence of a gap in background sound. The choice the mouse must make is whether they perceived a gap or perceived no gap.

We set up each mouse in the inner box of a sound attenuating chamber and white noise plays constantly in the background. The mouse initiates the trial by poking their nose in the center port. Once the stimulus (a gap or no gap) is delivered, the mouse chooses what they perceived by poking their noise in one of two response ports. The set-up of these ports can be seen in Figure 10. If they perceived a gap of any duration, they go to the left port for a water reward, and if they did not perceive a gap, they go to the right port for a water reward. The function of each port is outlined in Table 1. From their behavioral response, we can determine which gap durations they can perceive and find the MGT.

Figure 10. Diagram of water ports

This shows that the mouse initiates a trial first by poking the middle port and then chooses the left or right port depending on the stimulus they perceive.

| Water Port | Reason port delivers reward |
|---|---|
| Middle Port | Water reward for initiating a trial |
| Left Port | Water reward when stimulus is a gap of any duration |
| Right Port | Water reward when stimulus is NO gap |

Table 1. The function of the three ports during the 2-AFC task

*Learning how to do the 2-AFC task*

Before the mice can begin the 2-AFC task, they must learn how to use the sound attenuating chamber. The steps are outlined in Table 2. The first step they must pass is "Free Water" when they learn that they can receive water from poking their noses into the ports. There is no gap stimulus and no background sound playing during this step. After they complete 200 trials on this step, they graduate to a step called "Request Reward." In this step, there is constant white noise playing in the background, but no stimulus. The mice get rewarded from the center port for going there first, and then they get rewarded for going to either the left or right. This teaches the mice that they must go to the middle port first to receive a reward from the left or right port. This is important because in the 2-AFC task, the mice must initiate a trial by going to the middle port, and then make their choice by going to the left or right port.

Once they pass "Request Reward," they begin the 2-AFC task, with no laser trials. They begin with an easy gap discrimination, the "Long Gap" task. For these trials, the mouse will have to decide if they heard a gap of 256ms or no gap. Once the mice get 80% of 1000 of these trials correct, the mouse will graduate to the next step. This means that a shorter gap will be added, so now the mice will have to distinguish between a gap (which could be 256ms or 128ms) and no gap. After graduating from that

step, another shorter gap duration is added so that now the mice must distinguish between a gap (which could be 256ms or 128ms or 64ms) and no gap. This process of slowly adding the shorter gap durations allows the mouse to get increasingly good at the task and slowly learn that shorter gaps still count as gaps. After this step, the mouse graduates to the "Gap All" task, in which gaps of all durations are available to be played at equal probabilities. The following step, "Laser 2-AFC," is when the laser trials begin. Similar to the "Gap All" task, gaps of all durations are played at equal probabilities, but now the laser shines on 10% of the trials. The details of this step will be discussed in the *Laser delivery* section.

Additionally, there are also correction criteria set in place to aid the mice in learning how to do the task. When a mouse gets a trial wrong, a light flashes for a few seconds to alert the mouse that its choice was wrong. Then, the mouse repeats that trial until it gets it correct. This forces the mouse to listen to the stimulus and prevents them from randomly guessing.

| TRAINING STEP | DESCRIPTION | ADVANCEMENT CRITERIA |
|---|---|---|
| 1. Breeding | For inhibitory optogenetics - cross ChR2xPV<br>For excitatory optogenetics- cross ChR2xGpr26 | Mice age to adulthood<br>Genetic testing confirms their genotype |
| 2. Surgery | Optic fiber implanted bilaterally so they rest above auditory cortex | 5-day recovery<br>Begin water restriction 1 day before beginning behavior trials |
| 3. "Free water" step | No stimulus, mice learn they can receive water from the ports | 200 trials |
| 4. "Request reward" step | Constant background white noise plays, no stimulus<br>Mice learn to go to middle port first to initiate trial | 80% correct for 1000 trials |
| 5. "Long gap" step | 2-AFC task begins<br>Mice chose between no gap and gap (gap durations: only 256ms) | 80% correct for 1000 trials |
| 6. "128ms gap" step | 2-AFC task<br>Mice choose between no gap and gap (gap durations: 256ms and 128ms) | 80% correct for 1000 trials |
| 7. "64ms gap" step | 2-AFC task<br>Mice choose between no gap and gap (gap durations: 256ms, 128ms, 64ms) | 80% correct for 1000 trials |
| 8. "Gap all" step | 2-AFC task<br>Mice choose between no gap and gap (gap durations: 256ms, 128ms, 64ms, 32ms, 24ms, 16ms, 8ms, 6ms, 4ms, 2ms, 1ms) | 80% correct for 1000 trials |
| 9. "Laser 2-AFC" step | 2-AFC task with lasers on for 10% of trials<br>Mice choose between no gap and gap (gap durations: 256ms, 128ms, 64ms, 32ms, 24ms, 16ms, 8ms, 4ms, 2ms, 1ms) | Continue running mice through behavior tasks 7days/week until results stabilize |

Table 2. Procedure for learning how to do 2-AFC trials

*Optogenetic manipulation*

To test our hypothesis that GTR's mediate gap detection, we attempt to manipulate the gap detection ability of mice with optogenetics. As discussed above, optogenetics is a genetic technology that lets us manipulate the activity of specific neurons with the presence of light by using genetically engineered light-sensitive genes. We predict that shining light on neurons in auditory cortex during the GTR will alter the mice's gap detection ability.

The optogenetic manipulation occurs on the final step in Table 2, the "Laser 2-AFC" step. The 28 mice (10 mice with excitatory optogenetic genes, and 18 mice with inhibitory optogenetic genes) did trials on the "Laser 2-AFC" step for 7 days a week for over two months.

*Laser delivery*

During the "Laser 2-AFC" step, the laser shines on 10% of the trials at varying intensities. The laser works differently to manipulate neuronal activity in the two cohorts of mice. In both cohorts, the laser shines during the GTR which occurs in the 50ms immediately following the end of a gap, as seen in Figure 11.

For the Gpr26 cohort, the mice with excitatory optogenetic genes, the laser turns on for 25ms after a gap finishes, as seen in Figure 11. The laser turns on during trials with gaps of any duration and during trials with no gaps. The intention here that the light from the laser will excite the neurons during the GTR. During the GTR when the laser is off, the Gpr26 mice do not process gap durations shorter than their MGT. When the laser is turned on, we expect the excitatory optogenetic genes to allow the mice to

perceive gaps shorter than their MGT and to perceive gaps that are absent (a gap of 0ms).

For the PV cohort, the mice with inhibitory optogenetic genes, the laser shines for 50ms after a gap finishes, as seen in Figure 11. The laser only turns on during trials with a gap. The intention here is that the laser shining with suppress the neurons during the GTR. During the GTR when the laser is off, the PV mice are able to perceive gap durations longer than their MGT. When the laser is on, we expect the inhibitory optogenetic genes to prevent the mice from perceiving gaps they normally can perceive (gaps longer than their MGT).

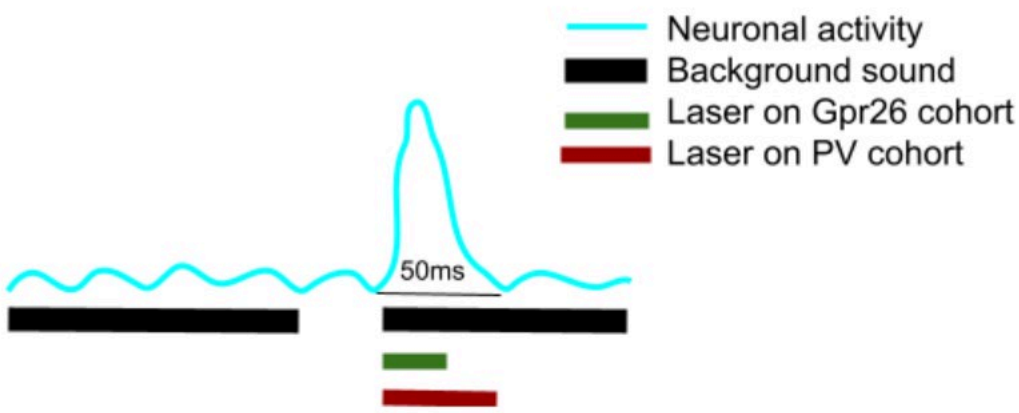


Figure 11. Diagram of laser delivery timing relative to GTR

This GTR is represented by the spike in neuronal activity following the gap in background sound. This shows the lasers for both cohorts are turned on during the 50ms when the GTR occurs. For the Gpr26 cohort, the laser is turned on for 25ms, shown in green, and for the PV cohort, the laser is turned on for 50ms, shown in red.

**Behavioral data analysis**

To analyze the data from the 2-AFC tasks, we used psychometric curves to plot the data from each mouse individually. Psychometric curves plot the data as the percentage of times the mouse went to the left or right port as a function of gap

duration. We looked at data from the most recent 3000 trials the mice did on the "Laser 2-AFC" step.

*Determining gap detection ability*

Based on the shape of the psychometric curve, we can determine whether the mice performed gap detection. Gap detection will be evident if the mouse went to the left port approximately 100% of the time when the gap is 256ms and went to the left port approximately 0% of the time when the gap is 0ms (no gap). Additionally, their performance should be the least accurate (lowest % correct) for gap durations shorter than their MGT and most accurate (highest % correct) for the most obvious gap durations, 0ms and 256ms.

*Determining minimum gap threshold*

To find the MGT, the shortest gap duration that the mouse can perceive, we looked at the midpoint on the logistic fit of the psychometric curves. This could only be done on the psychometric curves for the mice who performed gap detection. Once we found the MGT of each mouse, we calculated the average and standard deviation of the MGT.

*Determining laser effect*

A clear laser effect would have to show significant difference between the behavior with the laser turned on and the laser turned off, as seen in Figure 12. The Gpr26 cohort and PV cohort should see opposite shifts in the psychometric curve due to the laser. Since the excitatory optogenetic genes should lower the MGT, the Gpr26 psychometric curve should shift to the left when the laser is on. On the other hand, the

inhibitory optogenetic genes should raise the MGT, so the PV psychometric curve should shift to the right when the laser is on. If these shifts are clear, it will show that the laser influenced the neuronal activity and the behavior.

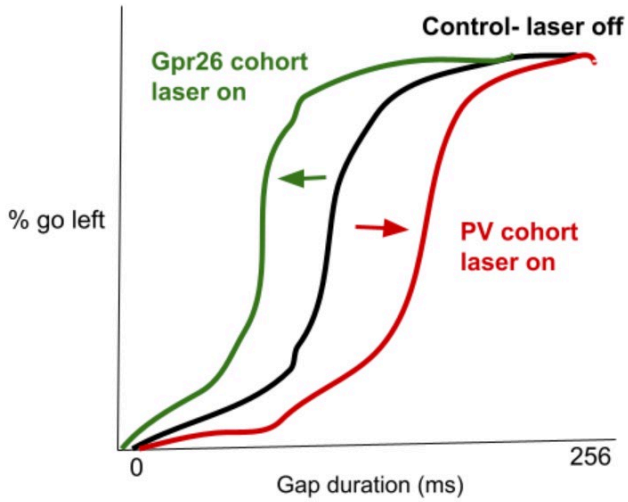


Figure 12. Expected results of laser data

This graph shows how the presence of light from the laser is expected to shift behavior response. This psychometric curve represents the % of trials the mouse went to the left for each gap duration. The black line represents the mouse's behavior when the laser is off, the green line represents the behavior of the Gpr26 cohort when the laser is on, and the red line represents the behavior of the PV cohort when the laser is on.

# FINDINGS

## Results of Gpr26 cohort

*Behavior*

| Gpr26 Mouse Behavior Performance | Number of Mice (total=10) |
|---|---|
| Performed gap detection | 8 |
| Right port bias<br>(Went to right port >50% trials regardless of stimulus) | 1 |
| Left port bias,<br>(Went to left port >50% trials regardless of stimulus) | 1 |

Table 3. Summary of Gpr26 Mouse Behavior Performance

As seen in Table 3, out of the ten Gpr26 mice, eight of them performed gap detection. The mice who performed gap detection were determined using the criteria outlined in the methods section: the percent they go to the left changes as expected with the gap duration, as seen in Figures 13 and 14, and the percent of trials they get correct is low for the gap durations beneath their MGT and highest for the most obvious gap durations (0ms and 256ms), as seen in Figure 15. Figures 13-15 only represent two mice, but the data for each of the eight mice that performed gap detection followed this trend.

The other two mice showed an extreme bias towards one port regardless of the stimulus, as seen in Figure 16. This means that the mouse went to the same port the same percent of trials even as the stimulus changed, suggesting that the stimulus was not a factor in the mouse's decision.

The MGT's for these eight mice were inconsistent. The average minimum gap threshold was 34ms +/- 42ms. While most of the mice had MGT's near 16ms, there

were some outlier mice who had much higher gap detection thresholds, such as the 64ms MGT seen in Figure 13.

This variance shows how behavior can be variable between mice. Each mouse has a different threshold for gap detection because each mouse is different. Even though there is natural variance in behavior, the large standard deviation calculated and the significant difference from the MGT of 2ms found in previous studies (Weible 2020), suggests this experiment must be repeated with a larger sample size to attempt to find a value for MGT that has more precision and accuracy.

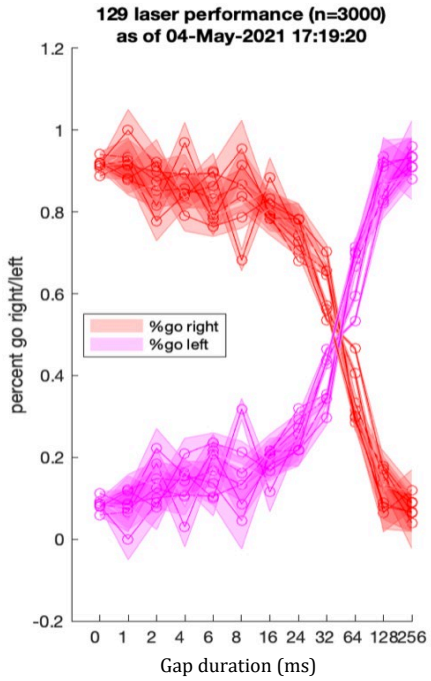


Figure 13. Gap detection performance of mouse 129 (Genotype: Gpr26)

This psychometric curve depicts the percentage of times the mouse went to the left or right port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The graph plotting the data of the % of times the mouse went right is the mirror of the graph showing the % of times the mouse went left. This shows a MGT of a 64ms gap, meaning the shortest gap that the mouse can perceive is 64ms. This is one of the outlier mice.

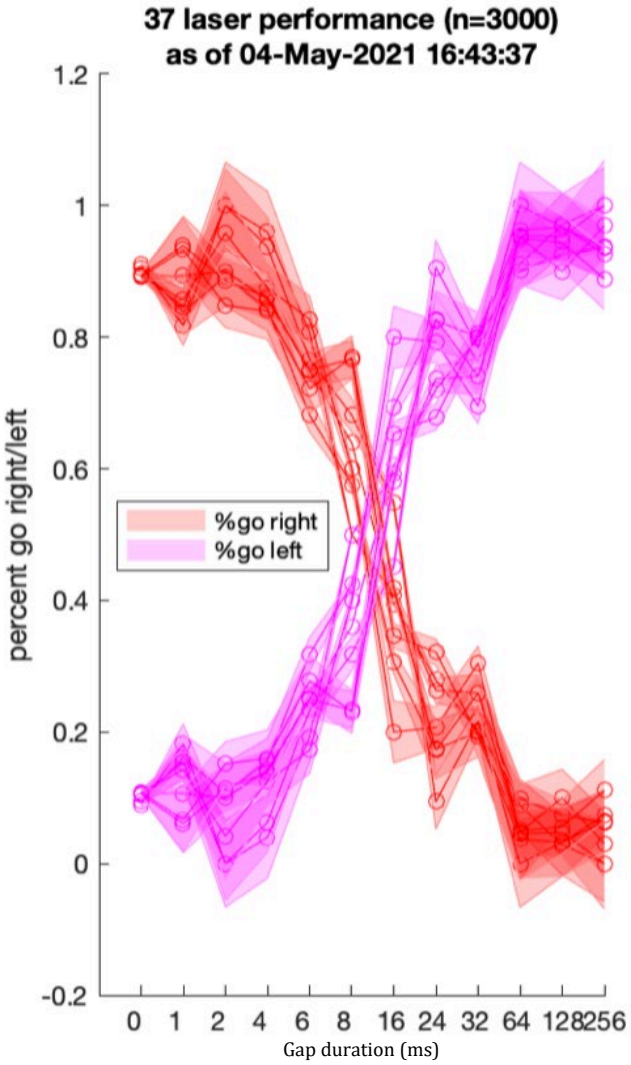


Figure 14. Gap detection performance for mouse 37 (Genotype: Gpr26)

This psychometric curve depicts the percentage of times the mouse went to the left or right port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The graph plotting the data of the % of times the mouse went right is the mirror of the graph showing the % of times the mouse went left. This shows a MGT of 16ms, meaning the shortest gap that the mouse can perceive is 16ms.

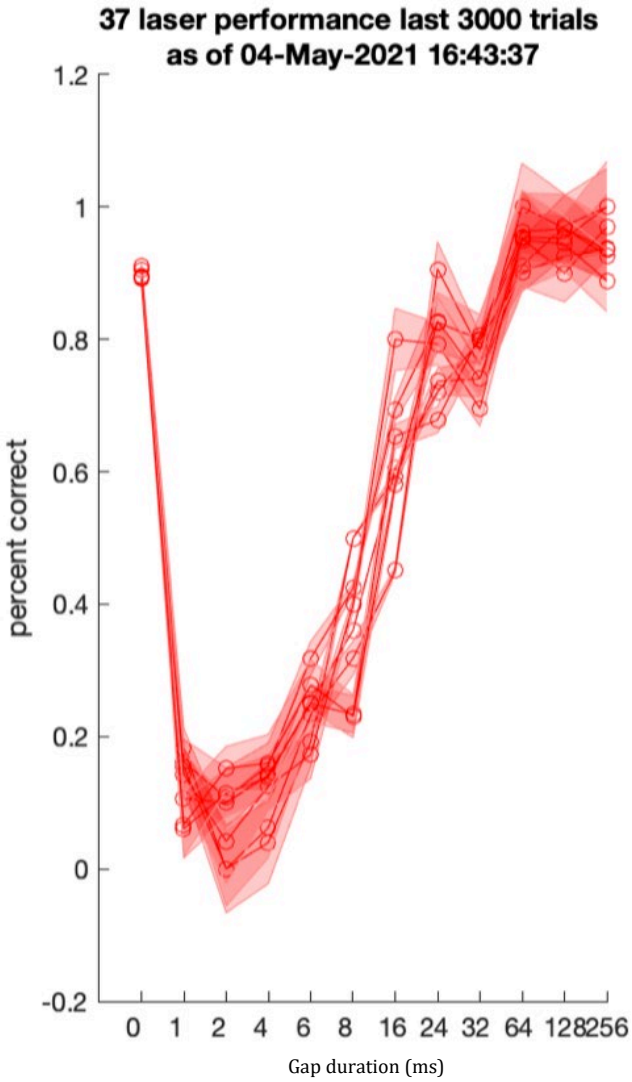


Figure 15. Percent of trials correct for mouse 37 (Genotype: Gpr26)

This psychometric curve depicts the percent of trials the mouse got correct for each gap duration (ms). This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. This shows that for a 256ms gap, the mouse chooses "gap" almost 100% of the time and for a 0ms gap, the mouse chooses "no gap" almost 100% of the time. The data shows that the mice rarely get the trials where the stimulus is a shorter than their MGT (mouse 37's MGT is 16ms). This data supports the fact that this mouse performed gap detection.

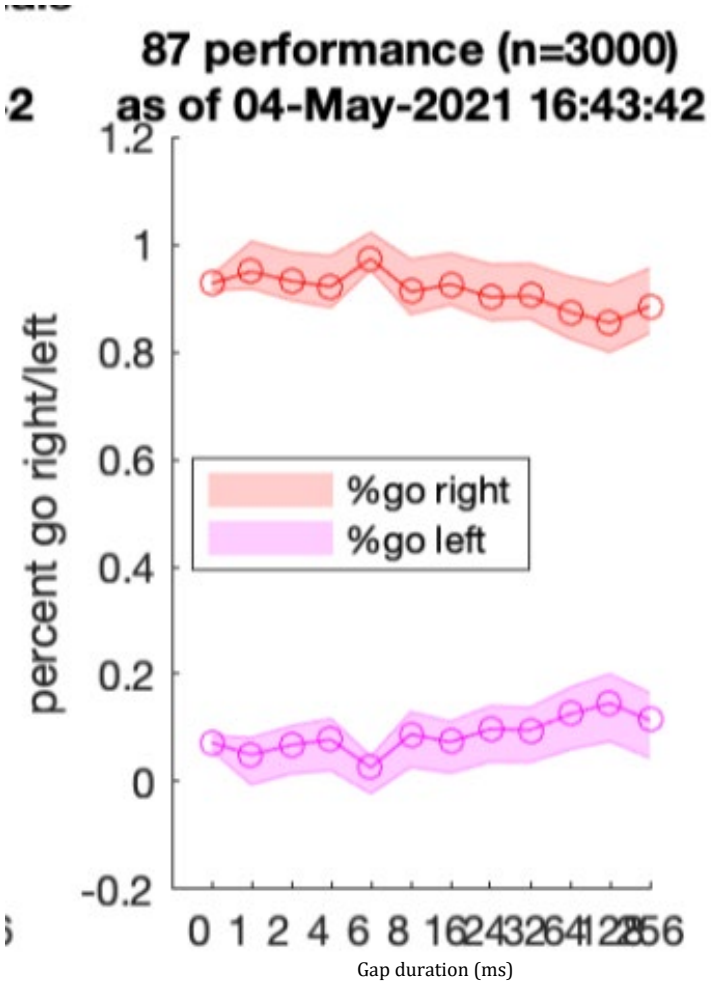


Figure 16. Gap detection performance of mouse 87 (Genotype: Gpr26)

This psychometric curve depicts the percentage of times the mouse went to the left or right port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The graph plotting the data of the % of times the mouse went right is the mirror of the graph showing the % of times the mouse went left. This mouse went to the right almost 100% of the time, regardless of the gap duration, indicating that the mouse was not paying attention to the stimulus. This shows a lack of gap detection ability.

*Effect of laser*

None of the Gpr26 mice had a clear laser effect. This is evident because the laser did not shift the psychometric curves as expected in Figure 12. Instead, the curve for the gap detection ability for when the laser was off and the curves for the various laser intensities were all overlapping, as seen in Figures 17 and 18. These two graphs only represent two mice, but the data for each mouse followed this trend and did not show a clear laser effect.

This is further evident because we also expected to see that a mouse would get a higher percentage of trials correct for the shorter gap durations, because the laser would cause it to perceive those short gaps as a gap, when it could not perceive them without the laser. However, as seen in Figure 19, there was no impact of the laser on the percent of trials the mice got correct. Figure 19 only represents one mouse, but the data for each mouse followed this same trend and did not show a clear laser effect.

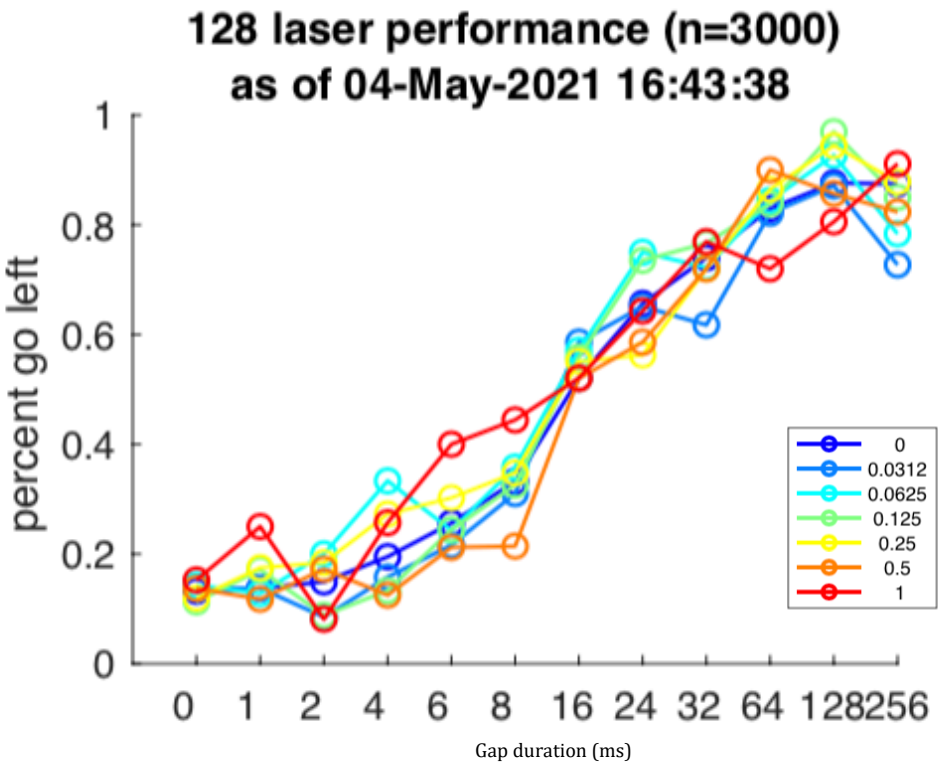


Figure 17. Gap detection for each laser strength in mouse 128 (Genotype: Gpr26).

This psychometric curve depicts the percentage of times the mouse went to the left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The dark blue line shows the trials when the laser was off, and the red line shows when the laser was at full strength. This shows the laser did not influence gap detection ability.

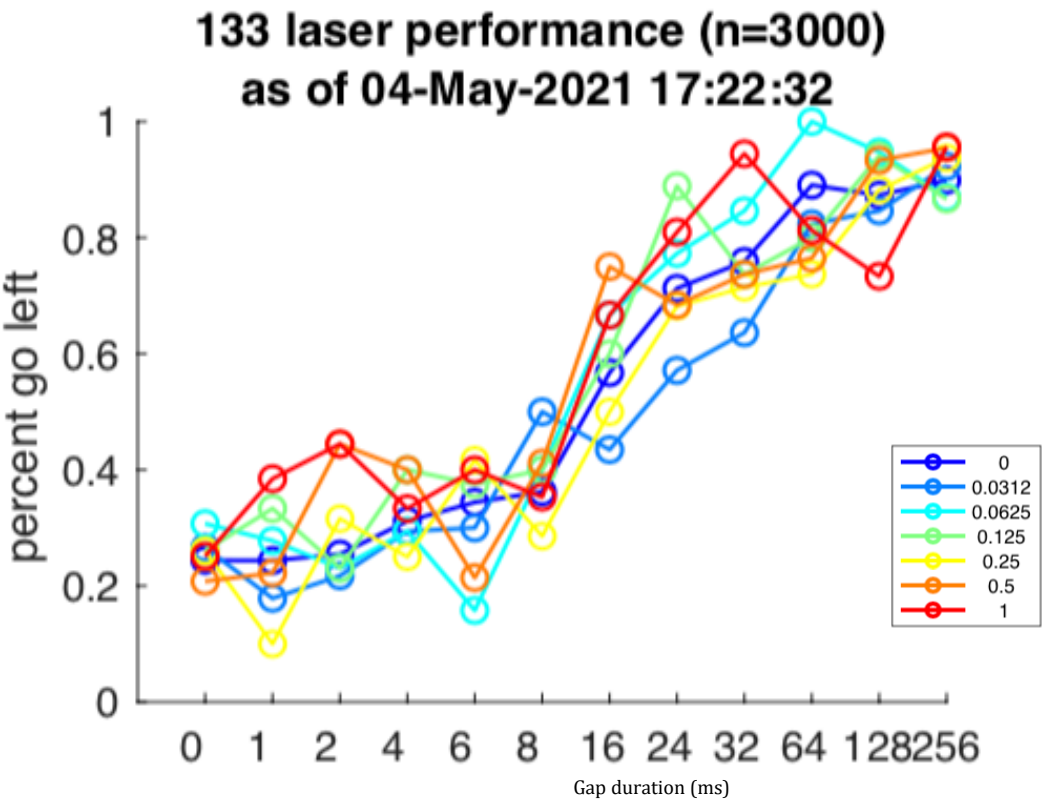


Figure 18. Gap detection for each laser strength in mouse 133 (Genotype: Gpr26)

This psychometric curve depicts the percentage of times the mouse went to the left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The dark blue line shows the trials when the laser was off, and the red line shows when the laser was at full strength. This shows the laser did not influence gap detection ability.

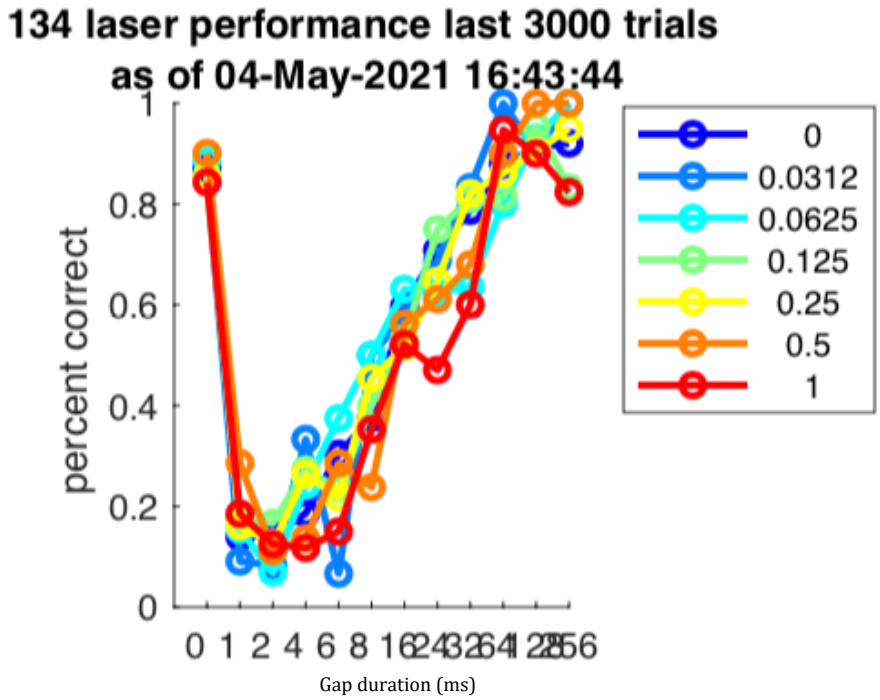


Figure 19. Percent of trials correct for each laser strength in mouse 134 (Genotype: Gpr26)

This psychometric curve depicts the percent of trials the mouse got correct for each gap duration (ms). This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. This shows that the laser does not influence the percentage of trials that the mouse got correct.

**Data for PV cohort**

*Behavior*

| **PV Mouse Behavior Performance** | **Number of Mice (total=18)** |
|---|---|
| Performed gap detection | 0 |
| Right port bias<br>(Went to right port >50% trials regardless of stimulus) | 10 |
| Left port bias<br>(Went to left port >50% trials regardless of stimulus) | 1 |
| No port biases<br>(Equal interaction with each port regardless of stimulus) | 7 |

Table 4. Summary of PV Mouse Behavior Performance

As outlined in Table 4, out of the eighteen PV mice, there were none that demonstrated gap detection ability. Ten of them showed a strong bias towards the right port, meaning they went to the right port the same percentage of trials regardless of the stimulus, as seen in Figure 20 and 21. One mouse showed bias towards the left port, meaning it went to the left port the same percentage of trials regardless of stimulus. And seven mice had equal interactions with both ports regardless of the gap duration, as seen in Figure 22. Additionally, the percentage of trials the mice got correct was relatively the same for each gap duration, as seen in Figure 23. None of the eighteen mice met the gap detection criteria outlined in the methods section. This data suggests that the mice were not making their choices based on the stimulus. We could not calculate a MGT for this cohort due to the lack of gap detection ability.

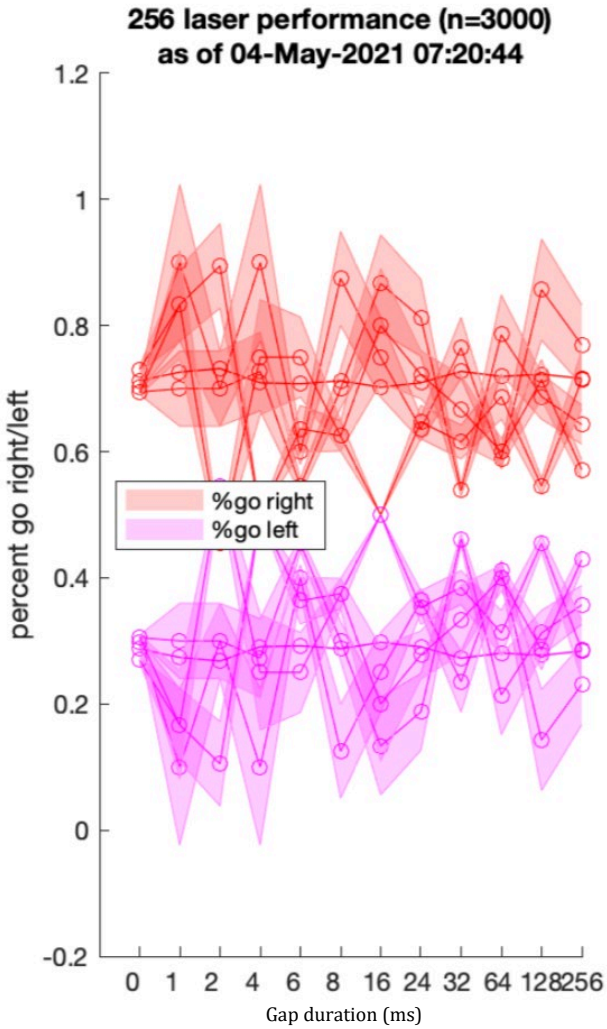


Figure 20. Gap detection performance for mouse 256 (Genotype: PV)

This psychometric curve depicts the percentage of times the mouse went to the right and left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. This mouse showed a strong bias towards the right port which is evident because it went to the right port consistently, regardless of the gap duration. This mouse did not display gap detection ability.

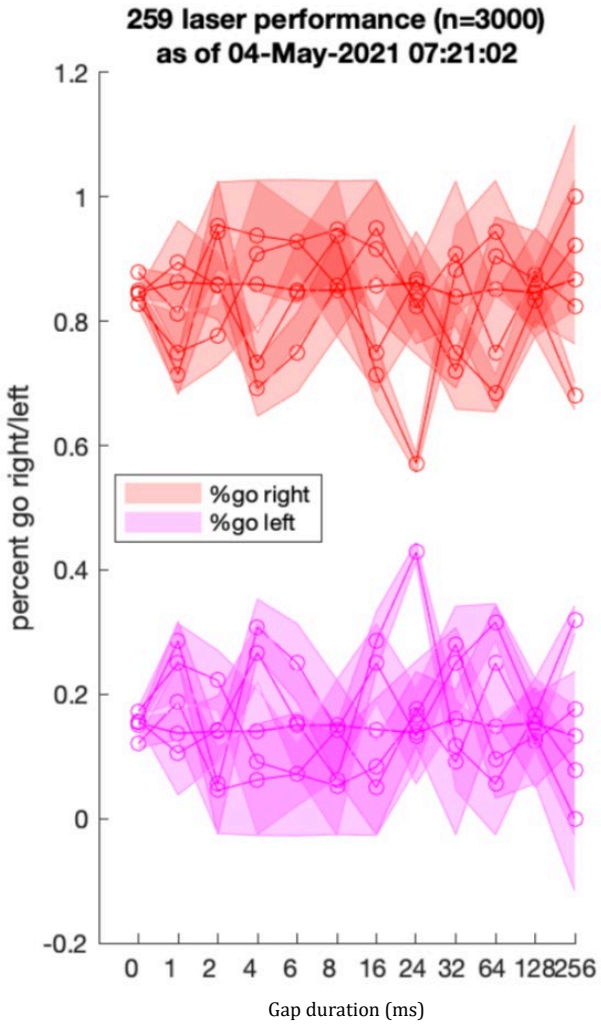


Figure 21. Gap detection performance for mouse 259 (Genotype: PV)

This psychometric curve depicts the percentage of times the mouse went to the right and left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. This mouse showed a strong bias towards the right port which is evident because it went to the right port consistently, regardless of the gap duration. This mouse did not display gap detection ability.

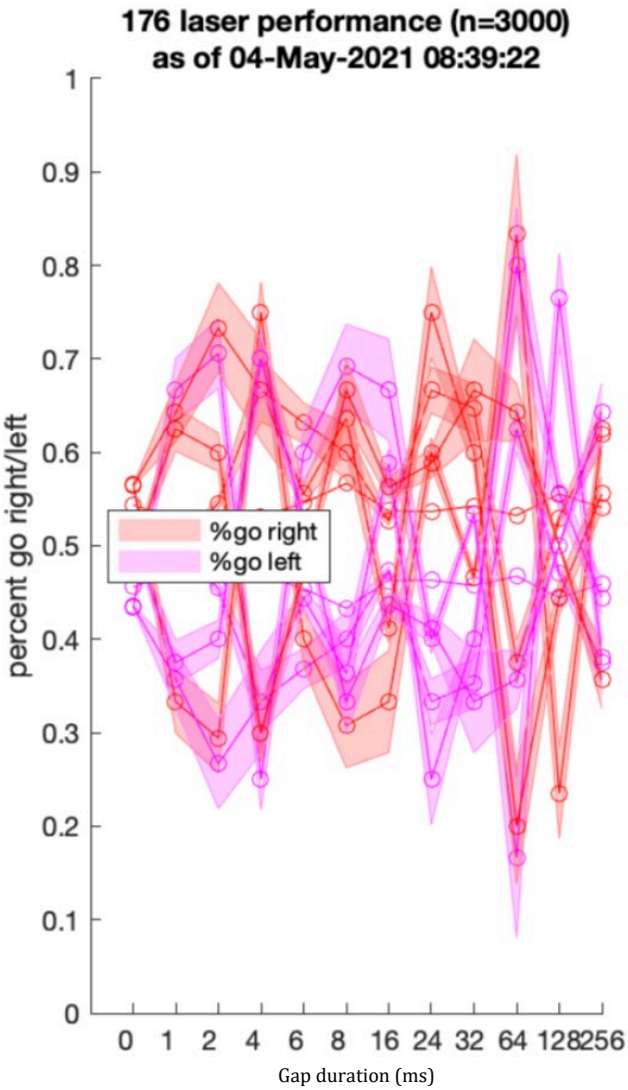


Figure 22. Gap detection performance of mouse 176 (Genotype: PV)

This psychometric curve depicts the percentage of times the mouse went to the right and left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. This mouse showed a strong bias towards the right port which is evident because it went to the right port consistently, regardless of the gap duration. This mouse did not display gap detection ability.

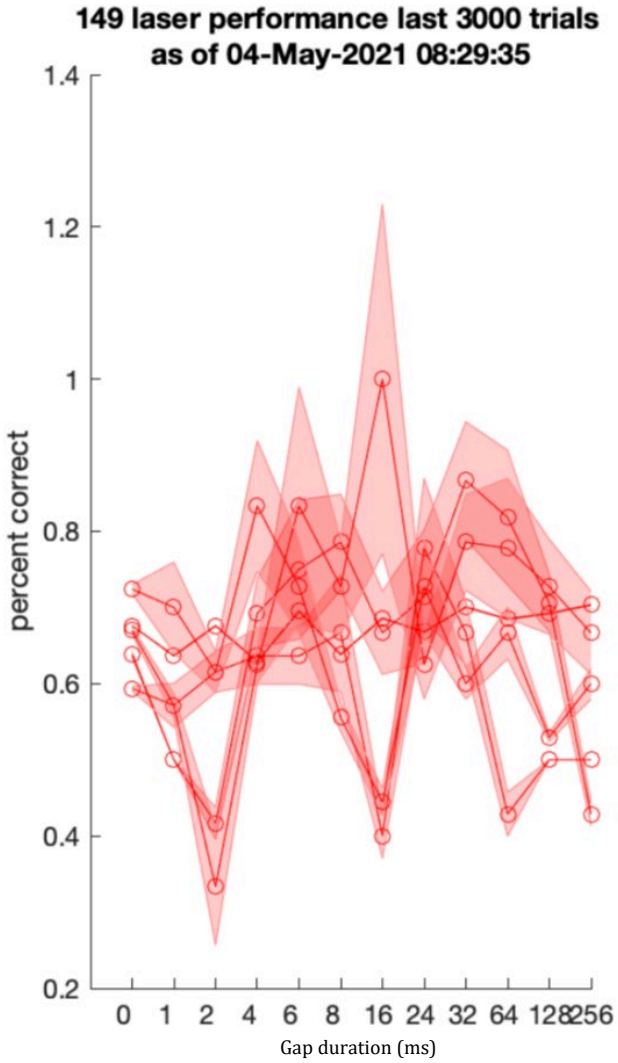


Figure 23. Percent of trials correct for mouse 149 (Genotype: PV)

This psychometric curve depicts the percent of trials the mouse got correct for each gap duration (ms). This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. This shows that the mouse got each gap duration correct to the same degree, there are no gap durations that were significantly more difficult to get correct. The shape of this graph is in stark contrast to the graph in Figure 2. This mouse did not display gap detection ability.

*Effect of lasers*

Similar to the Gpr26 mice, there was no clear effect of the laser on the PV mice's performance. This is evident because the laser did not shift the psychometric curves as expected in Figure 12. Instead, the curve for the gap detection ability for when the laser was off and the curves for the various laser intensities were all overlapping, as seen in Figures 24 and 25. These two graphs only represent two mice, but the data for each PV mouse followed this trend and did not show a clear laser effect.

Even though the PV mice did not exhibit gap detection ability, it is still evident that the laser did not have a clear effect on their behavior.

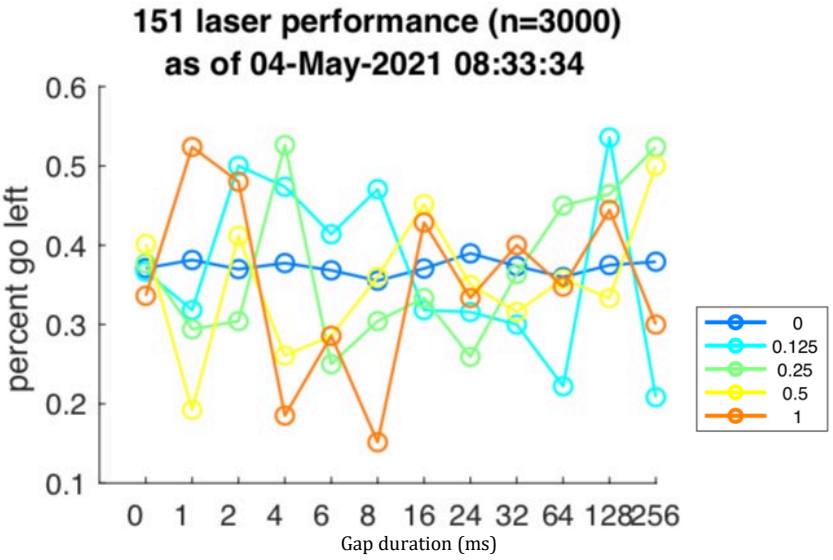


Figure 24. Gap detection for each laser strength in mouse 151 (Genotype: PV)

This psychometric curve depicts the percentage of times the mouse went to the left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The dark blue line shows the trials when the laser was off, and the orange line shows when the laser was at full strength.

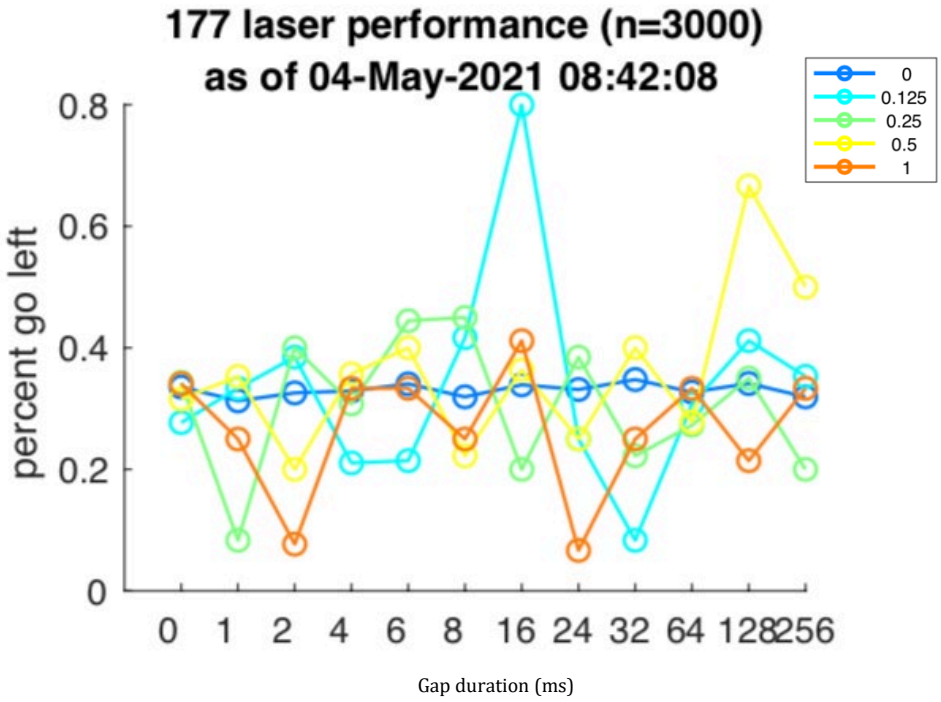


Figure 25. Gap detection for each laser strength in mouse 177 (Genotype: PV)

This psychometric curve depicts the percentage of times the mouse went to the left port for each gap duration. This graph shows the data from the last 3000 trials (n=3000) on the 2-AFC Laser task. The dark blue line shows the trials when the laser was off, and the orange line shows when the laser was at full strength.

# DISCUSSION

These results do not show the data we expected to see. Due to the both the lack of precision and accuracy in the Gpr26 mice's gap detection threshold and the lack of gap detection ability in the PV mice, these experiments did not find a precise or accurate minimum gap threshold for gap detection. Since the results for the Gpr26 and the PV mice's behavior was not affected by the presence of the laser, the experiment does not confirm or deny the hypothesis that GTR mediates gap detection.

## Lack of gap detection

20 out of the 28 mice did not show gap detection ability. While this could suggest that the mice are unable to detect gaps, and thus unable to perform gap detection, previous studies have already proven that mice can perform gap detection. Thus, the more likely scenario is that there is a problem with the data collection.

### *Sound stopping bug*

One issue that became apparent to us while collecting data is that there was a bug with the software that would cause the background white noise sound to randomly turn off during the "Laser 2-AFC" task. Without a background sound, there could be no gap in background sound. Without a stimulus, the mouse could not make a choice. Thus, this bug interfered significantly with the data collection. This experiment needs to be repeated without the sound stopping bug.

### *Bias towards one port*

When the sound stopped, the stimulus went away, and the mice lost any way to distinguish between the presence and absence of a gap. To be able to continue getting

their water reward, the mice learned that they could get rewarded if they chose one port and went to it repeatedly. Since they could not hear a stimulus, this was a simple way for mice to behave and get rewarded. Once we had addressed the sound stopping bug in the code, we attempted to reteach the mice how to use the boxes gap detection, by setting them back a few steps in Table 2. This bug did not occur until the "Laser 2-AFC" step, so the mice were able to initially learn gap detection well enough to graduate up to the laser step. Thus, we thought setting them back a few steps would force them to relearn the gap detection behavior. However, by the time we had fixed the bug, they had already been on the "Laser 2-AFC" step for a while, and they had unlearned gap detection behavior. Setting them back a few steps did not work. They were not able to quickly revert to performing gap detection, they stuck with having bias towards one port.

*Why did only Gpr26 mice show gap detection ability?*

While 8 out of 10 Gpr26 mice showed gap detection ability, none of the 18 PV mice showed gap detection ability. This was a surprising result. We think this may be because the Gpr26 mice started training first, so they had more time on the "Laser 2-AFC" step before the sound stopping bug became prevalent. Since the PV mice had their surgeries done and started training after the Gpr26 mice, by the time they got to the "Laser 2-AFC," the sound stopping bug had become quite prevalent and drastically impacted their gap detection ability.

**Problems with laser data collection**

There was no clear impact of the laser on behavior in any of the 28 mice. While this could indicate that optogenetics cannot manipulate neuronal activity in auditory cortex, previous studies have already shown that optogenetics are an effective tool to manipulate cortical activity. This suggests there was an error with the laser data collection.

*Laser timing*

One problem we discovered was that the laser pulse was not delivered at the correct timing with the gap. As discussed previously, for both cohorts of mice, the laser should turn on immediately following the gap (or following the absence of a gap). However, when testing the laser timing with an oscilloscope, it became evident that the timing of the laser pulse was not consistent for each gap duration. It was consistently delivered at an incorrect time. An example of this can be seen in Figure 26. This image shows an oscilloscope reading where the laser pulse is being delivered early. This is just one example; the laser pulse was not consistently delivered early. The timing of the laser varied, and different gap durations had different timings, but the laser pulse was consistently delivered at an incorrect timing. This incorrect timing caused an error with the laser's ability to manipulate neuronal activity during GTR. This helps explain why there is no clear effect of the laser in any of the mice. The experiment needs to be repeated when the laser is properly calibrated with the gap and the GTR.

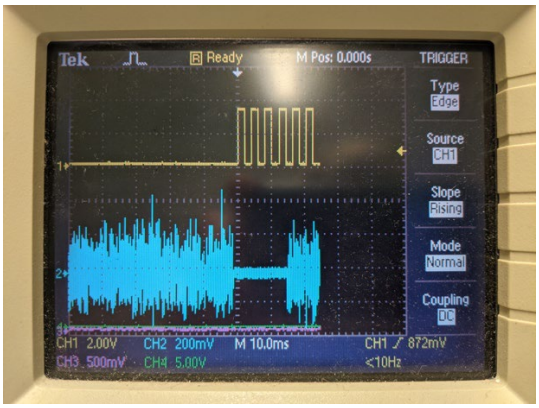


Figure 26. Example of incorrect laser timing seen on oscilloscope

In this image, the blue line represents the background sound and yellow line represents the laser pulse. This shows the laser pulse delivered early: it is delivered simultaneously with the gap, not delivered during the GTR. This is one example of how the laser pulse timing was incorrect, but the timing did vary with every gap duration, which is incorrect. This means the laser was not manipulating the neuronal activity we wanted it to.

*Is the light reaching auditory cortex?*

By the time the mice had been doing the "Laser 2-AFC" task for a while and we figured out there was a lack of laser effect, it had been a while since the mice had been implanted with optic fibers. Due to this, we thought another problem with the laser data could be that the optic fiber implants might be old and had acquired scabbing or other tissue from the healing process that prevented the laser from reaching the neurons in auditory cortex. To combat this, we increased the laser power output significantly, from 6.3mW to 30mW. After the mice did more trials at this laser setting, it became clear that there was still no laser effect on the behavior. Thus, this was not the problem. The lack of laser effect is most likely due to the incorrect timing of the laser pulse with the gap and the GTR.

## Future research

### *Repeat experiments*

To understand if GTR mediates gap detection through optogenetic manipulation and to find an accurate and precise MGT, these trials must be repeated with new mice. Since these data were collected, the code of the software has been debugged, the laser has been timed properly with gap and the GTR, and the hardware set up in the behavior room has been revamped to be as efficient as possible. As a result of this process, hopefully many of the issues that effected data collection with this thesis will be avoided. This repetition with new mice is currently underway.

### *Phoneme discrimination*

In future research, we plan to test the mice's ability to discriminate between phonemes that are distinguished by voice-onset time (a gap that characterizes different speech sounds). To accomplish this, the mice will do 2-AFC trials, but instead of distinguishing between the presence and absence of a gap as the mice did for this thesis, they will distinguish between the phonemes /b/ and /p/. This data will provide more insight on the pathways used for speech processing in the temporal lobe.

# Bibliography


Ashwood Z. C., et al. (2020). Mice alternate between discrete strategies during perceptual decision making. *bioRxiv*. 10.1101/2020.10.19.346353.

Burns E. G. (2020). The effect of fear conditioning on parvalbumin-expressing neurons in mice. *University of Oregon Scholars Bank.*

Fitzgibbons P. J., Gordon-Salant S. *(*1996*)* Auditory temporal processing in elderly listeners*. JAmAcadAudiol.* **7***,* 183–189*.*

Humes L., et al. (1991). Speech identification difficulties of hearing-impaired elderly persons. *Journal of Speech, Language, and Hearing Research.* 34: 3, 686-693. 10.1044/jshr.3403.686.

Kaylegian K., Stebritz A. J., Weible A. P., Wehr M. (2019). 5XFAD Mice Show Early Onset Gap Detection Deficits. *Frontiers in Aging Neuroscience*. 11: 66. 10.3389/fnagi.2019.00066.

Keller C. H., Kaylegian K., Wehr M. (2018). Gap encoding by parvalbumin-expressing interneurons in auditory cortex. *J. Neurophysiol.* 120, 105–114. 10.1152/jn.00911.2017.

Kim T. S., Chung J. W. (2013). Evaluation of Age-Related Hearing Loss. *Korean J Audiol.* 17: 2, 50-53. 10.787/kja.2013.17.2.50.

King A. J., Teki S., Willmore B. D. B. (2018). Recent advances in understanding the auditory cortex. *F1000Res*. 7. 10.12688/f1000research.15580.1.eCollection2018.

Levy R. B., et al. (2019). Circuit asymmetries underlie functional lateralization in the mouse auditory cortex. *Nature communications*. 10: 2783. 10.1038/s41467-019-10690-3.

Liu Y., et al. (2020). Hearing loss is an early biomarker in APP/PS1 Alzheimer's disease mice. *Neuroscience Letters*. 717. 10.1016/j.neulet.2019.134705.

Nahar L., Delacroix B. M., Nam H. W. (2021). The role of parvalbumin interneurons in neurotransmitter balance and neurological disease. *Frontiers in Psychiatry.* 10.3389/fpsyt.2021.679960.

O'Sullivan, C., Weible, A. P., & Wehr, M. (2019). Auditory cortex contributes to discrimination of pure tones. *Eneuro*, *6*(5).

O'Sullivan, C., Weible, A. P., & Wehr, M. (2020). Disruption of early or late epochs of auditory cortical activity impairs speech discrimination in mice. *Frontiers in Neuroscience*, 1394.

Saunders J., Wehr M. (2019). Autopilot: Automating behavioral experiments with lots of Raspberry Pis. bioRxiv 10.1.1101/807693.

Saunders J., Wehr M. (2019). Mice can learn phonetic categories. *The Journal of the Acoustical Society of America*. 145: 1168. 10.1121/1.5091776.

Shenoy P., Yu A. (2012). Strategic Impatience in Go/NoGo versus Forced-Choice Decision-Making. *Advances in Neural Information processing Systems.* 25.

Stuttgen M. C., Schwarz C., Jakel F. (2011). Mapping spikes to sensations. *Frontiers in Neuroscience*. 5: 125. 10.3389/fnins.2011.00125.

Tan, N. G. A., Wu, W., Seifalian, A. M. (2015). Optogenetics: Lights, camera, action! A ray of light, a shadow unmasked. In *Applications of Nanoscience in Photomedicine* (pp. 185–203). Elsevier Inc. 10.1533/9781908818782.185.

Tokuoka K., Kasia M., Kobayashi K., Isa T. (2020). Anatomical and electrophysiological analysis of cholinergic inputs from the parabigeminal nucleus to the superficial superior colliculus. *Journal of Neurophysiology*. 124. 10.1152/jn.00148.2020.

Urai A. E., et al. (2021). Citric acid water as an alternative to water restriction for high-yield mouse behavior. *eNeuro*. 230: 20. 10.1523/ENEURO.0230-20.2020.

Watkins L. R., Orlandi C. (2020). Orphan G Protein Coupled Receptors in Affective Disorders. *Genes.* 11: 694. 10.3390/genes11060694.

Weible A. P., Liu C., Niell C. M., Wehr M. (2014). Auditory Cortex Is Required for Fear Potentiation of Gap Detection. *Journal of Neuroscience*. 34: 46. 10.1523/JNEUROSCI.3408-14.2014.

Weible, A. P., et al. (2014). Perceptual gap detection is mediated by gap termination responses in auditory cortex. *Current biology: CB*, *24*(13), 1447–1455. 10.1016/j.cub.2014.05.031

Weible A. P., Yavorska I., Wehr M. (2020). A cortico-collicular amplification mechanism for gap detection. *Cerebral Cortex*. 30: 6. 10.1093/cercor/bhz328.