# THE ROLE OF CORTICAL INHIBITORY INTERNEURONS IN AUDITORY SIGNAL DETECTION

by

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A THESIS

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Santiago Jaramillo, PhD

For years, researchers have been trying to uncover how different auditory areas in the brain allow us to segregate signals from noise. The goal of this study was to use a mouse model to understand how two types of cortical inhibitory interneurons found in the auditory cortex, known as somatostatin-expressing (SOM) and parvalbumin-expressing (PV) interneurons, contribute to our perception of sound masked by noise. We hypothesized that inactivating auditory cortical SOM interneurons would decrease mice's ability to detect a tone masked by noise when the bandwidth of the background noise was broadband. We expected a similar decrease when PV interneurons were inactivated regardless of the bandwidth of the background noise. To test these hypotheses, we taught transgenic SOM-ArchT and PV-ArchT mice how to perform a behavioral task where they had to determine if a pure tone was present in background noise. We then implanted optical fibers over each mouse's auditory cortex and they performed the same task while having their respective interneurons inactivated, which was achieved through an interaction between the light-sensitive ArchT protein found in the interneurons and green light delivered by a laser output box. We found that inactivating SOM interneurons decreased the percentage of trials in which the mice

were able to detect a tone masked by noise in all bandwidths of background noise, while inactivating PV interneurons did not affect the mice's ability to do so, showing that signals from PV interneurons might not be needed for mice to perform this task. These findings allow us to better understand the roles that different sources of inhibition play when it comes to our ability to detect a sound masked by noise.

## Acknowledgements

I would first like to thank my lab Principal Investigator and Primary Thesis Advisor Professor Santiago Jaramillo for giving me the opportunity to be a part of his lab for the past two and a half years and for serving as my mentor during my entire thesis process and for always challenging me to learn new things and improve as a researcher.

I would also like to thank Anna Lakunina, my second reader, and another one of my mentors during this project, for always being there to answer any of my questions and concerns and for teaching me most of the methods involved with this study and how to train mice successfully. I can honestly say that without her, this thesis would not have been possible.

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# Introduction

Imagine you are at a party with your friends. Loud music is playing, and hundreds of conversations are happening at the same time. There are thousands of different auditory signals present that make up the noisy background you are in, yet, you are able to focus on the conversation you are having with the person next to you by extracting the signal related to your conversation from the noisy background. This phenomenon, known as the cocktail party effect, is one that we experience on a daily basis. Although this is a common experience, the neural basis for our ability to extract a sound masked by noise is still baffling scientists to this day, and it is this question that guides the rest of this study.

As researchers, one common way for us to understand the neural basis of some neural function is to study specific neurons that are believed to be important in that process. Neurons are cells found in our body that help communicate and transmit signals to other neurons. This is done through the use of action potentials, which are characterized by a change in voltage in the neuron that is communicating the information. An action potential triggers the release of neurotransmitters in a neuron. Neurotransmitters are small molecules that are released by one neuron and picked up by another neuron through the use of receptors. It is through these neurotransmitters that neurons are able to communicate with one another. The number of action potentials in a unit of time is the firing rate of a neuron, so by measuring the firing rate of a neuron, it is possible to measure its activity. By conducting experiments that focused on understanding the activity of our neurons of interest in our mouse model, we were able to better understand the process of detecting a sound masked by noise.

The auditory pathway involves a number of different brain regions that all play a role when it comes to our ability to perceive a sound (Appler, et al., 2011). Once a sound is detected by the ears, neurons in the brain carry information about the sound using neurotransmitters, eventually reaching the primary auditory cortex. The auditory cortex processes information about the sound and leads to an action. This region of the brain is the focus of our study, as the auditory cortex is one of the regions of the auditory pathway that directly influences whether or not an action will occur. For this reason, understanding how neurons in the auditory cortex function allows us to better understand the exact processing that occurs in the cortex that leads to an action.

There are two properties of sound that we are able to pick up and distinguish. The two properties are frequency, which is related to the pitch of a sound, and amplitude, which is interpreted by us as the loudness of a sound. For this study, we were particularly interested in frequency. Frequency is also defined as the number of wave cycles that pass a given point in one second (Hall, et al., 2006). A low frequency sound has a low number of wave cycles per second and is perceived as low-pitched, while a high frequency sound has a high number of wave cycles per second and is perceived as high-pitched. All sounds are made of different frequency components, and the further you get in the brain, the more complex the representations of sound become (Nelken, et al., 2014). The bandwidth of a sound is defined as the range of frequencies in that sound. Most sounds we hear in our daily lives have a broad bandwidth (broadband), meaning they are sounds that contain a large range of different frequencies. In comparison, pure tones, such as a single note on a guitar, have a narrow bandwidth (narrowband), meaning that they contain a small range of different

frequencies (Gladden, 2011). In our daily lives, such as when we are at a loud party, we want to filter out the broadband noise so we can detect and focus on a narrowband one, like a conversation we are having with a friend. This concept guided the design of this study.

One biological process that is potentially involved in our ability to filter out background noise is known as cortical inhibition, which is when specific inhibitory interneurons in the brain reduce the activity of other neurons in the brain. We expected cortical inhibition to be involved in this task, and for that reason, our neurons of interest in this study were two of the most commonly found cortical inhibitory interneurons in the auditory cortex, known as parvalbumin-expressing (PV) and somatostatin-expressing (SOM) interneurons, which together make up about 70% of the inhibitory neurons found in the auditory cortex (Rudy, et al., 2011). Cortical inhibitory interneurons are neurons that transmit signals between other neurons and can cause both feedforward inhibition (Schneider, et al., 2013) and lateral inhibition (Kato, et al., 2017).

Previous studies have discussed the differences between PV and SOM interneurons (Moore, et al., 2013 and Li, et al., 2014). PV cells are thought to form short, local connections in the brain (Li, et al., 2014). This means that PV cells inhibit auditory neurons that are close to them and do not pick up signals that are farther away. On the other hand, SOM cells form long-range connections in the brain (Li, et al., 2014), meaning that they carry information about frequencies outside of the receptive field of neurons they form connections with. Also, it has previously been shown that SOM cells play a role in producing network suppression that underlies lateral inhibition

(Kato, et al., 2017). These differences show that SOM and PV neurons respond to sound differently. This, along with their morphological differences, implies that SOM and PV interneurons might have different functions and that one, or both, of these specific interneurons might be important when it comes to our ability to detect a tone masked by noise.

Based on this background information, it was possible to construct a study that allowed us to study the role that these two cortical inhibitory interneurons play when it comes to our ability to detect a sound masked by noise. Our study revolved around teaching two different kinds of mice, SOM-ArchT and PV-ArchT, how to perform a behavioral task where they had to determine if a pure tone was present in background noise. Mice then underwent surgery to have optical fibers implanted over their auditory cortex and they then performed the same task while having their respective interneurons inactivated using optogenetics via an interaction between green light and the ArchT protein found in their interneurons. By comparing how well the mice were able detect a tone masked by noise when their respective interneurons were inactivated to when they were active, we were able to better understand the role that these specific neurons play when it comes to a mouse's ability to detect a tone masked by noise.

**Hypotheses**

The justification for our hypotheses came from a model established by Anna Lakunina, a graduate student in the Jaramillo lab, prior to the inception of this study. This model, and the study that it was based on, revolves around the idea of spectral surround suppression, which is described as a reduction in the activity of a neuron in response to a stimulus outside of its receptive field, the area of sensory space that elicits

a response in a neuron. This phenomenon has previously been observed in the primary auditory cortex (O'Connor, et al., 2010). Data collected in this preliminary study showed that SOM interneurons, but not PV interneurons, mediate spectral surround suppression in the auditory cortex (Lakunina, et al., in preparation). This was shown by inactivating SOM and PV interneurons and comparing the firing rates of excitatory neurons that receive signals from these interneurons when the interneurons were active and inactivated. We found that when SOM interneurons were inactivated, the firing rates of excitatory neurons increased (figure 1b and 1c) and the average suppression index of these neurons, which is a measure of the amount of surround suppression in a cell and is ratio between the peak response and white noise response, normalized to the peak, decreased as well (figure 1d). No decrease in suppression index was seen when PV interneurons were inactivated (figure 1d), showing that they do not mediate surround suppression. It was also found that SOM inactivation reduced surround suppression by increasing responses to high bandwidth stimuli, while PV inactivation does not affect surround suppression and affected all bandwidths of background noise equally

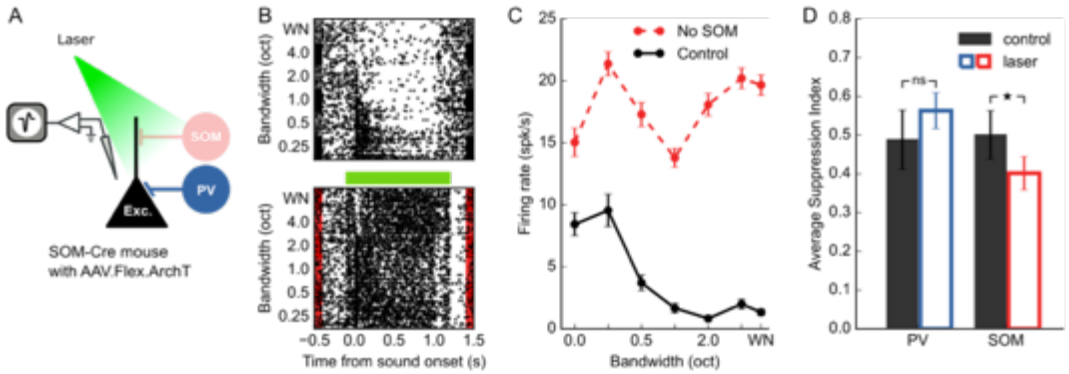


**Figure 1:** SOM/PV interneurons and surround suppression

**a)** SOM interneurons that synapse onto excitatory neurons in the auditory cortex were optogenetically inactivated in SOM-ArchT and PV-ArchT transgenic mice (not shown). **b)** Raster plot showing the activity of excitatory neurons when SOM interneurons were intact (top) or inactivated (bottom) with the bandwidth depicted on the y-axis and the time from sound onset on the x-axis. A dot represents a single action potential in the neuron. The green bar indicates laser presentation. **c)** Bandwidth tuning curve for the neuron in panel b. SOM inactivation lead to a decrease in suppression of responses to high-bandwidth stimuli. **d)** Change in suppression index, a ratio between the peak response and white noise response, normalized to the peak, when PV and SOM interneurons were inactivated. Inactivation of SOM interneurons lead to a significant decrease in surround suppression (N=48 cells, $p<0.01$, Wilcoxon signed-rank test) while PV inactivation did not (N=23 cells, $p<0.18$, Wilcoxon signed-rank test).

Based on these preliminary findings, a model of signal extraction using surround suppression was proposed. According to this model, when neurons are surround suppressed, they fire at a preferred bandwidth of sound that is narrowband (figure 2c). For this reason, surround suppressed neurons are able to detect a tone masked by noise because the tone lies in their range of preferred bandwidth (figure 2d). In comparison, neurons that are not surround suppressed have higher firing rates in response to broadband stimuli (figure 2a). As a result, non-surround suppressed neurons are unable to successfully detect a tone masked by noise because quiet noise and loud tone elicit similar responses in the neurons (figure 2b). We were thus able to design our model for

this study, which was that mice are able to detect a narrowband stimulus by setting a threshold on the activity of surround suppressed neurons. Our model thus predicted that if we make neurons not surround suppressed, the threshold will no longer work, and mice that previously relied on activity from that neuron to detect narrowband signals will no longer be able to do so. This allowed us to design our hypotheses for this study.

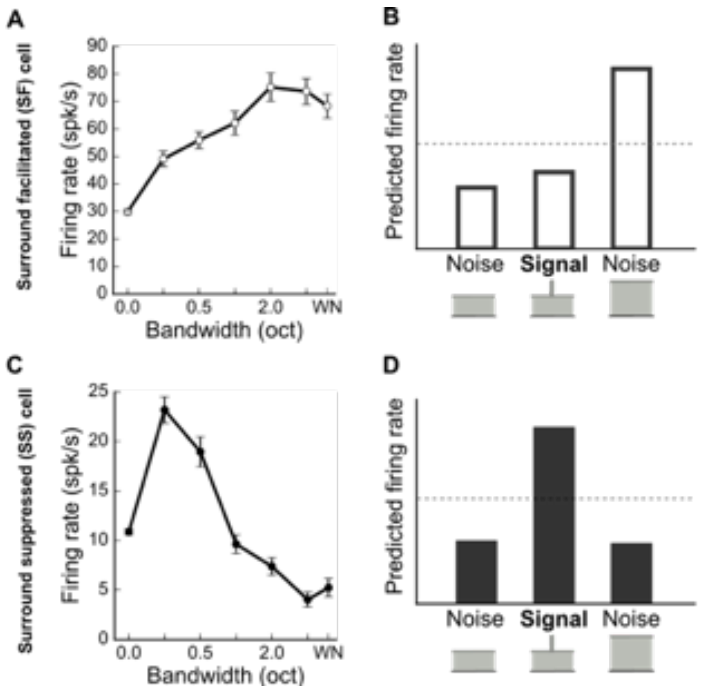


**Figure 2:** Model of signal extraction and surround suppression

This figure shows our proposed model of signal extraction that was developed using preliminary data collected by Anna Lakunina. **a)** Firing rate of an example neuron in the auditory cortex that is not surround suppressed. **b)** Predicted firing rates of non-surround suppressed neurons in the auditory cortex. The vertical dashed line represents an example threshold that when crossed, allows for identification of stimuli containing a narrowband signal. Three different stimuli were presented (left to right: quiet broadband noise, quiet broadband noise with loud narrowband tone, loud broadband noise). Based on these finding, no threshold will allow the subject to differentiate noise from signal **c)** Firing rate of an example neuron in the auditory cortex that is surround suppressed. **d)** Predicted firing rates of surround suppressed neurons in the auditory cortex to the same stimuli as panel b. Based on these finding, the threshold passes only for signal, meaning that the subject will be able to detect the tone masked by noise. Data from Lakunina, et al. (in preparation).

Two main hypotheses guided our research in this study. The first was that when SOM interneurons were inactivated in our SOM-ArchT mice, the mice's ability to detect a tone masked by noise would decrease when the bandwidth of the background noise was

broadband in terms of frequency. This hypothesis was supported by our preliminary findings and the model established by them. We previously found that SOM inactivation mediates surround suppression. For this reason, we predicted that SOM inactivation would decrease the mice's ability to detect a tone masked by noise because when SOM interneurons are inactivated, the amount of surround suppression decreases. We suspected that the effect would be more prevalent when the bandwidth of the background noise was broadband/high, as the effect of SOM inactivation was stronger for high bandwidths (figure 2b).

Our second hypothesis was that when PV interneurons were inactivated in our PV-ArchT mice, their ability to detect a tone masked by noise would decrease regardless of the bandwidth of the background noise because by inactivating PV interneurons, we are disrupting a major source of cortical inhibition in the auditory cortex. This hypothesis was based on the preliminary finding that PV inactivation had no effect on surround suppression and affects all responses to bandwidths of background sound equally

**Significance of This Study**

The findings of this study will add to the general understanding about the neural basis of perception and attention and how we are able to detect a tone masked by noise. Disruption of this basic task can result in hearing and communication disorders like auditory agnosia. These disorders cannot be treated by things such as a hearing aid, and, for that reason, it is important to find an alternative treatment for people who suffer from them. Through the increased understanding of this process that was gained by this

study, we might be able to implement our findings in ways that could potentially aid those living with these disorders in the future.

**My Role in the Jaramillo Lab**

I conducted this project under the direction of Professor Santiago Jaramillo in the University of Oregon's Institute of Neuroscience and Department of Biology. I first joined the Jaramillo lab in January of 2017, and after training mice in a variety of different tasks, I began working on this project in early 2018. Between April 2018 and March 2019, I collected data for this study and analyzed our findings. During the entire course of this project, I collaborated with Anna Lakunina, a graduate student in the Jaramillo lab. She assisted me by conducting all of the optical fiber implant surgeries in this study and by showing me how to properly teach our mice how to perform the behavioral task.

# Materials and Methods

## Overview

The goal of this study was to examine the role that two different cortical inhibitory interneurons play in auditory signal detection. In order to achieve this goal, multiple stages of experimentation were needed. Mice were first taught how to perform a two-alternative forced choice behavioral task according to a set training paradigm and procedure. After they were deemed to have learned the task, the mice had surgery conducted on them to place optical fiber implants on their auditory cortex bilaterally. The mice then performed the same behavioral task as before the surgery while having either their SOM or PV interneurons optogenetically inactivated using a laser. At the conclusion of the experiment, the mice were euthanized and had their brains perfused, sliced, and imaged. Finally, data from each phase of the experiment was analyzed using psychometric curves and accuracy plots to help determine the role of SOM and PV interneurons in auditory signal detection. This research was supported by the National Institute on Deafness and Other Communication Disorders (R01DC015531), and the Office of the Vice President for Research and Innovation at the University of Oregon.

## The Mouse Model

Mice have been used for decades as models in scientific research such as this for a multitude of reasons. Mice are easy to maintain and breed in a controlled laboratory environment, have many physiological and genetic similarities to humans (Waterston et al., 2002), and their genomes are quite easy to manipulate through the creation of

transgenic mice (Perlman, 2016). This makes it possible to translate most findings found in mice to humans. For these reasons, a mouse model was chosen for this study.

All mice in this study were obtained from Jackson Laboratory (JAX). A total of six mice (four SOM-ArchT and two PV-ArchT) were used in this study. These transgenic mice were produced via a cross of either a SOM-Cre or PV-Cre parent with a Cre dependent-ArchT parent. These crosses allowed for ArchT, a light sensitive molecule that was used during the optogenetics portion of the study, to be present only in the SOM or PV interneurons of each mouse. During the entire course of the study, the mice had continued access to food, but they were put under water restriction to increase their motivation to perform the behavioral task. Mice were only given access to water while they performed the task, and free water access was provided to the mice on days where no experimental sessions were conducted. All mice were weighed after each experimental session to ensure that they received enough water during the session. If it was determined that the mice did not receive enough water (according to the guidelines established by the Jaramillo lab and the University of Oregon Animal Care and Use Committee), they were given more water. All animal care and procedures were monitored by both the Jaramillo lab and the University of Oregon Animal Care and Use Committee.

**Explanation of the Behavioral Training Task**

Once the mice reached the age of four months, they were ready to begin training in the initial behavioral task (figure 3).

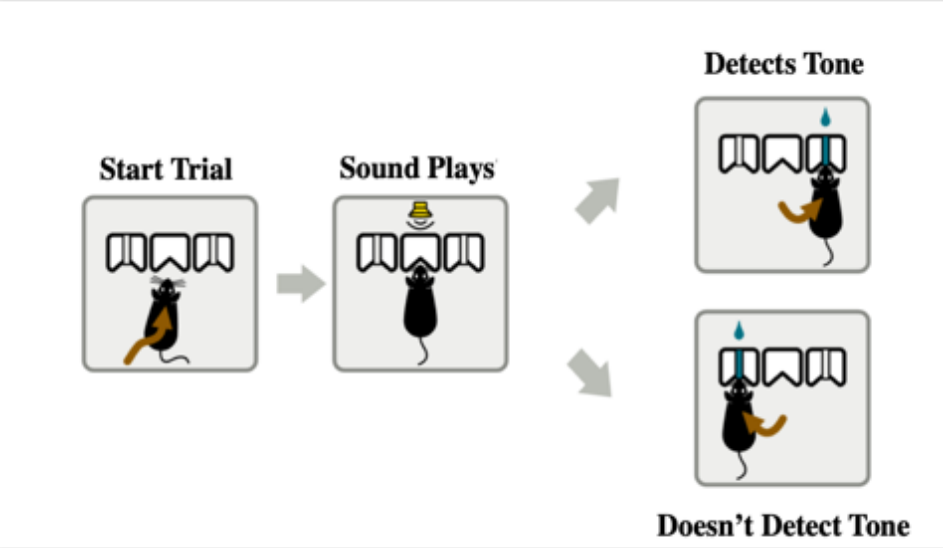


**Figure 3:** Overview of the two-alternative forced choice auditory detection task performed by the mice in the study

This figure describes the behavioral task that the mice in this study were taught. SOM-ArchT and PV ArchT mice were placed in a three-port behavioral training rig. Mice initiated a trial by poking their noise into the center port, at which time a sound would play. The sound would either be a pure tone masked by background noise or a sound that only contained the background noise. If the mice detected the pure tone, they poked their nose into the right port to collect a water reward. If mice did not detect the tone, they poked their noise into the left port to collect a water reward. Mice were given reward on trials where they chose the correct side. This process was repeated for sixty minutes each day the mice were trained.

In this task, mice were placed in a behavioral training rig with three ports (one on the left, right, and center of the rig) once a day. Mice were trained in a two-alternative forced-choice task, meaning that they were forced to choose which of the two alternatives presented to them was the correct option. Each mouse started a trial by poking its nose into the center port. An artificial sound then played in the soundproof behavior rig for up to 500ms after a randomized delay of 200 ms-300 ms. If the mice removed their nose from the center port, the sound turned off. There were two

possibilities for the types of sounds that could have played during each trial. The first option was a sound that only contained background noise consisting of either broadband white noise, a wide bandwidth sound according to a mouse's hearing threshold, or a narrowband noise. The other option was a sound that consisted of that same background noise as well as a pure tone overlaid on top of the background noise. If the sound that contained the pure tone was played, the mouse must go to the right port to receive a water reward of 2.25 microliters. If the sound that only contained the background noise was played, the mouse must go to the left port to receive the same water reward. The mouse only received a water reward if it chose the correct port. After either outcome, the mice initiated another trial by poking their nose into the center port again. This process was repeated for 60 minutes every day. The code for this task, and all its other iterations, were written in Python language by Santiago Jaramillo, Anna Lakunina, and myself.

**Different Stages of the Initial Behavioral Training Task**

Over the course of the initial behavioral training stage, the mice were trained in nine different stages, each of which introduced a different component to the task so the mice would be able to eventually learn how to do the final task prior to surgery. Each stage kept all of the parameters that were present in the previous stage. In the first task, called "sides direct mode", mice could poke their nose into either of the ports in the behavioral rig to get water. The goal of this task was to teach the mice how to use the water ports. In order for the mice to get a reward in the next stage, "direct mode", they had to poke their nose into the center port before going to either the right or the left port. In the third stage, called "next correct with no delay mode", the mice could go to

both ports during a single trial and were rewarded once they went to the correct port. The sound also played in the center port without a delay, meaning that it played immediately when the mouse poked its nose into it. The next two stages, “next correct with delay mode one and two”, are similar to the third stage except that after the mouse poked into the center port, the sound played after a delay of 0.1 and 0.2 seconds respectfully. The sixth stage, “outcome only if correct mode”, is the first stage that rewarded the mice only if they went to the correct port and did not give them multiple attempts per trial. In “outcome only if correct with on/off withdrawal mode”, the sound turned off when the mice left the center port. The goal of this stage was to teach the mice that they have to stay in the center port to hear the sound. The eight stage was “outcome only if correct with multiple bandwidth mode”, which was the first stage that introduced the possibility of having different bandwidths of background noise. Lastly, the final stage was known as “multiple SNR with multiple bandwidth mode”, which introduces multiple signal-to-noise ratios (SNRs) to the task. These ratios represented how loud the tone was in relation to the background noise such that as the SNR increased, the tone was louder and thus easier for the mice to detect. This was the final stage of the task that the mice were trained in. Each stage had an associated criterion associated with it that the mice had to reach before moving onto the next stage (table 1).

| **Stage of Training** | **Criteria to Move onto Next Stage** |
|---|---|
| Sides direct mode | One day of 200+ rewarded trials |
| Direct mode | One day of 200+ rewarded trials |

| | |
|---|---|
| Next correct with no delay mode | One day of 400+ rewarded trials |
| Next correct with delay mode | One day of 400+ rewarded trials |
| Next correct with delay mode 2 | One day of 400+ rewarded trials |
| Outcome only if correct mode | One day of 400+ rewarded trials and 70%+ correct on each side |
| Outcome only if correct with on/off withdrawal mode | One day of 400+ rewarded trials and 80%+ correct on each side |
| Outcome only if correct with multiple bandwidths mode | Three consecutive days of 400+ rewarded trials and 80%+ correct on each side |
| Multiple SNR with multiple bandwidths mode | Three consecutive days of 400+ rewarded trials and 80%+ correct on each side for both bandwidths |

**Table 1:** Stages of the initial behavioral training task

This table shows the nine different stages of training that each mouse in this study went through during the initial behavioral training task. Each change added a new element to the task while maintaining the settings established by the previous stage. The number of trials and the percentage of correct trials were monitored and recorded through the training paradigm written by Santiago Jaramillo, Anna Lakunina, and myself.

As soon as the mice reached the criterion for a given stage, they were moved onto the next stage of the task. On average, it took the mice approximately 61 days to proceed through all nine stages of initial behavioral training. After the mice reached the last stage and met the final criterion, they were given free access to food and water for one day in order to prepare them for surgery.

### Constructing Optical Fiber Implants

Before surgery could be conducted, optical fiber implants had to be constructed. Each mouse had two optical fibers implants placed on their auditory cortex bilaterally during surgery, one on each hemisphere of the brain. Each implant was constructed

using 15G stainless steel tubing and about 1cm of ThorLabs 100um core glass optical fibers. Each optical fiber was etched with concentrated hydrofluoric acid (HF) under a fume hood according to the standard lab procedures of the Jaramillo lab to sharpen the end to a tip, allowing for a consistent cone of light to exit from the implant.

**Laser Calibration**

The purpose of this stage was to calibrate our optical fiber implants so that the correct laser power, in terms of milliwatts, was delivered to the mice when they performed the laser behavioral task post-surgery. This was done using a photodiode machine (model number s120VC) from ThorLabs. Since each mouse had two different optical fiber implants, they were labeled "L" for left and "R" for right prior to calibration because their calibrations could have been different. Next, the photodiode was set up so that the sensor was plugged into the light meter and the optical fibers were suspended above it. The dial on the laser output box was then turned until the photodiode read that a laser power of 5.0 mW was being delivered. The dial number for this power and the output level displaced on the laser box were both recorded. This process was then repeated for a laser power of 10.0 mW, 15.0 mW, 20.0 mW, 25.0 mW, and 30.0 mW for both of the optical fibers.

**Surgery**

Anna Lakunina, a graduate student in Jaramillo lab, conducted all of the surgeries in this study. Mice were first anesthetized with isoflurane and shaved before being mounted onto a stereotax using ear bars and a mouth piece, which was connected to an oxygen source. Puralube was then applied to the mouse's eyes before it was

injected subcutaneously with a carprofen solution in order to hydrate the mouse and minimize pain during the surgery. An incision was then made from the front of the scalp to the back to allow us to easily peel the skin away and expose the skull. The mouse was then moved so that the bregma and lambda landmarks were at the same height and level. The location for where the implants were to be placed on the auditory cortex on each side of the brain were calculated to be -2.8mm from bregma in the anterior-posterior plane, +4.5mm in the medial-lateral plane, and -0.5mm in the dorsal-ventral plane (figure 4). Using these coordinates, the craniotomy sites were marked on the mouse's skull. Skull screws were also applied at this time, which were used to help stabilize the external structure of the implant later on. One screw was applied on the left and right side of the skull above bregma and on the bottom left quadrant below bregma and above lambda.

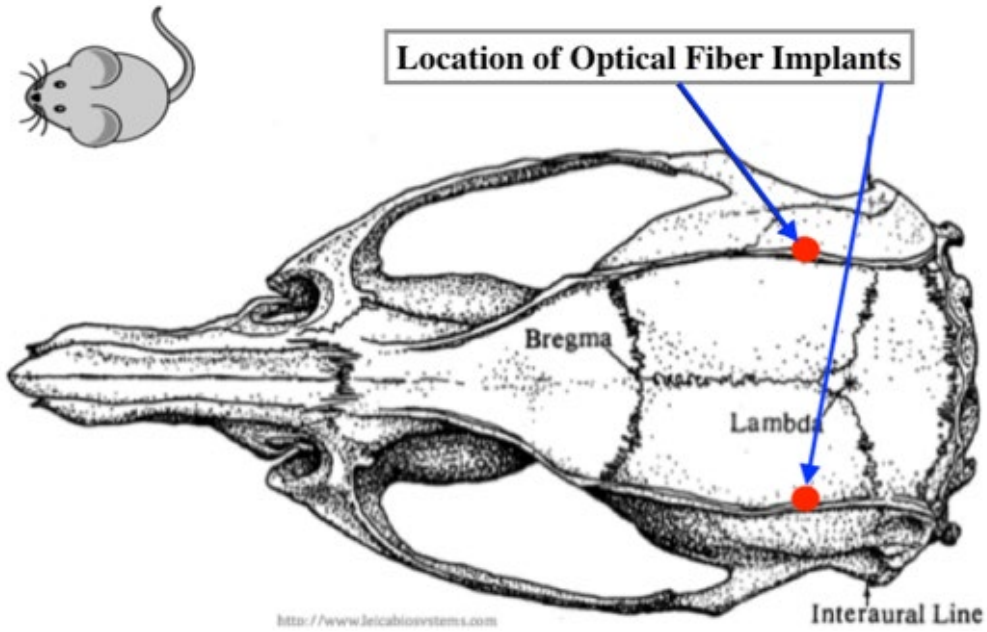


**Figure 4:** Location and placement of the optical fiber implants during surgery

This figure shows the location of the optical fiber implants that were placed during surgery. This shows the approximate location of the craniotomy according to the lambda and bregma skull landmarks when implanting the optical fiber implants onto the right and left auditory cortex of our mice. Previously determined coordinates based on the positions of lambda and bregma were used to find the location of the auditory cortex in each of side of the mouse's brain.

Using a scalpel, the skull surface was then marked with crosses so that the super glue was able to stick better. The skull was then drilled through at the marked craniotomy sites to expose the brain and remove the skull. The skull was also drilled at one of the skull screw sites and the skull was cleaned out. The optical fiber was then loaded into the micromanipulator arm and moved to the bregma line. X and Y coordinates were then zeroed out and the fiber was moved out of the way. The screw was then implanted, making sure that there was enough room for a dental acrylic and glue mixture to be placed over it later on, which acted as a protective layer over the fiber. The fiber was

moved to our XY coordinates and the micromanipulator was zeroed out in the Z direction. The fiber was then lowered into the target site. This process was repeated for the second optical fiber on the opposite side of the brain. A dental acrylic solution was formed and put around the optical fibers to form a protective layer. Finally, a protective box was placed around the implant using cut pieces of plastic weigh boat and covered with tape. A picture of a mouse with a completed implant is shown below (figure 5).


**Figure 5**: Picture of a post-surgery mouse with optical fiber implants

This figure shows what a post-surgery mouse from this study with optical fibers placed on its auditory cortex looks like. Two optical fibers were implanted during surgery, along with a dental acrylic solution on top to protect the fibers from getting damaged. A protective box made of plastic and covered with tape (red in this case) was placed around the implants for further protection. Later, when the mice were doing the laser behavioral task, the protective box was opened to allow for the laser-providing tethers to be plugged in. When the mice were not doing the behavioral task, the box was closed. The tape around the box was replaced as needed.

During the entire course of the surgery, safety precautions were put in place to ensure that the mice were safe and not in pain. After surgery was conducted, mice were placed in a warm chamber for three days to recover. They were weighed everyday over the course of these three days to ensure that they were not losing a considerable amount of weight. They were also monitored according to the standards of the University of Oregon Animal Care Facility to ensure that they were recovering properly.

**Laser Behavioral Task**

After surgery was conducted and the mice were allowed three days to recover, they were ready to be trained in the behavioral task again. For the first two days, the mice performed the same task they were trained in prior to undergoing surgery, known as the "multiple SNR with multiple bandwidths mode", while having a laser-providing tether plugged into their optical fiber implants. No laser was provided during these two days, as the only purpose of these trials was to ensure that the mice were still able to perform the behavioral task at the same ability they were able to prior to undergoing surgery and that the act of the tethers being plugged in alone was not changing their ability to perform the task. If the mice's ability to perform the task was stable after these two days, then they were ready to begin the laser behavioral task.

During the laser behavioral task, mice performed the task for a total of twelve days. During these twelve days, mice performed the "multiple SNR with multiple bandwidths mode" task while having tethers deliver laser from a laser output box. Gray-colored foam was also put in the box surrounding the two implants as an additional control to minimize the amount of light that could get out of the box and disturb the mouse. This allowed us to ensure that the effects seen were not being caused by the mice seeing the light coming from the laser. Laser was delivered via the tethers at a power of 10 mW at 520 nm to the mice during 25% of trials while the mice performed the behavioral task each day. Laser power was set by turning the dial on the laser output box to the number recorded during the calibration process for each fiber. Laser was not delivered continuously so that we could compare the mice's performance when the laser was on and off. Mice were trained with the same basic parameters discussed above in

the previous section and were water restricted during the duration of the twelve days. Mice were weighed after each training day in order to ensure that they were reaching their baseline weights.

During eight out of the twelve days (known as “laser days”), the tethers were plugged into the two optical fiber implants as the mice performed the behavioral task (figure 6). Laser delivered by the tethers would then interact with light-sensitive ArchT, which was found in the mouse’s respective auditory cortical inhibitory interneuron. This interaction lead to the inactivation of the mouse’s SOM/PV interneurons in the auditory cortex. By comparing the performance of each mouse during trials when the laser was and was not presented, we could determine how the inactivation of these interneurons impacted the mice’s ability to perform the behavioral task.

**Figure 6:** Laser-providing tethers plugged into optical fiber implants

This figure shows how the laser providing tethers were plugged into the optical fiber implants in our SOM-ArchT and PV-ArchT mice during “laser days.” The tethers were connected to a 520nm laser output box. One tether was marked as “number one” and was always plugged into the left optical fiber, while the other was marked as “number two” and was always plugged into the right optical fiber to provide consistency.

During the remaining four days (known as “control days”), instead of the tethers being plugged into the mice’s optical fibers, they were tapped onto the side of the inside of the

box surrounding the implants. For this reason, on these days, mice did not have their auditory cortical inhibitory interneurons inactivated by the laser. These days acted as an additional control to ensure that it was the inactivation of the interneuron that was causing an effect in the mice instead of the action of the mice reacting to the shining of laser light.

Mice were trained in a pattern of two "laser days" followed by one "control day" during the course of the twelve days. After the mice finished this stage, they were given free access to food and water in their cages and were considered to be done performing the behavioral tasks for this study. Mice were thus kept in our mouse room in LISB 142 until it was time for them to be euthanized.

**Perfusion, Brain Extraction, Slicing, and Imaging**

After the mice were euthanized via a 0.075 mL injection of euthasol, they were perfused with a paraformaldehyde and saline solution, and their brains were extracted. The brains were kept in paraformaldehyde (PFA) overnight to allow them to solidify as much as possible. The next day, the brains were removed from the PFA solution and were sliced coronally using a Leica VT1000 S vibratome machine at a thickness of 100 microns. A single brain produced about 20-40 complete slices. The slices were then placed in a container with phosphate-buffered saline (PBS) to allow them to solidify while they awaited imaging.

The purpose of imaging was to ensure that our optical fiber implants were placed in the intended region of the brain, which was on the auditory cortex. Brain images were captured using a Zeiss Axio imaging microscope and ZEN software. A total of five images were captured for each brain slice. The first was taken at a

magnification of 1.25x and showed the entire brain slice. The remaining four images were done at a magnification of 2.5x, two on each side of the brain. The first image on each side was taken with the "brightfield" setting turned on, with a purpose of getting an image of the indentation on the slice that showed where the optical fiber implant was placed. The second image was taken with the "fluorescent" setting turned on, with the purpose of getting an image that showed where ArchT was expressed in the brain. By looking at these two images and comparing them to the online Allen Brain Atlas for mice, it could be determined if our optical fiber implants were on the auditory cortex as intended (figure 7). This process was repeated for all the mice in the study.

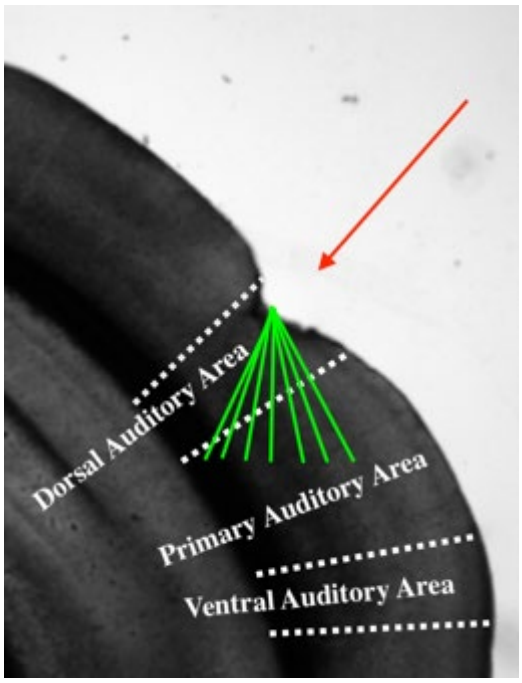


**Figure 7:** Labeled brightfield image of a SOM-ArchT mouse's (band065) right sided coronal brain slice showing the location of the optical fiber implant

This image was produced using a Zeiss Axio Imaging Microscope using ZEN software on a magnification of 2.5x with the brightfield setting turned on. The indentation, as highlighted by the red arrow, shows the location of the optical fiber implant in this mouse. Using this image and comparing it to the Allen Brain Atlas for a mouse's brain (image 78/132) allowed us to find and label different regions of the brain in the slice (as shown in white). The green lines depict how the green laser spread once it enters the brain via the optical fiber implant.

## Data Storage and Analysis

All behavioral data was stored on a python-based computer software database that was written by Santiago Jaramillo. Data analysis was conducted using this same program. All figures representing the data were made using scripts written in python language by Anna Lakunina and myself.

# Results

## Inactivating SOM interneurons Decreased the Mice's Ability to Detect a Tone Masked by Noise

By the conclusion of this study, we were able to teach a total of four SOM-ArchT mice how to perform the behavioral task. Two of the mice were female, while the other two were male. One of these mice, known as band066, was considered to be a control mouse, as due to an error, it did not end up expressing the ArchT protein in its SOM interneurons as intended. For this reason, this mouse was used as an additional control to show that the results we obtained were actually due to the inactivation of SOM interneurons in the mice instead of a response to laser flashing.

All four of the mice had optical fiber implants placed on their auditory cortex and were able to complete the laser behavioral task. Once each mouse completed the laser behavioral task, psychometric curves were produced for each mouse. These plots graphed the signal-to-noise ratio (SNR), which represents how loud the tone was in comparison to the background noise, from -inf to 20.0 dB on the x-axis. The percentage of trials that each mouse went to the right, correlating to the percentage of trials that each mouse detected the tone, was graphed on the y-axis, as the mice were taught to go to right port to collect a reward when they thought they heard the tone. A total of four lines were graphed on a single plot, two of which showed the performance of the mouse when the background bandwidth was 0.25 (narrowband) while the other two showed the performance when the background bandwidth was "infinity" or white noise (broadband). For each bandwidth, the performance of the mouse when the laser was on, representing when their respective interneurons were inactivated, and when the laser

was off, representing when they weren't inactivated, was graphed. Two psychometric curves were produced for each mouse, one of which showed the average performance of the mouse over the eight "laser days" while the other showed their average performance over the four "control days". By comparing the performance of the mice during trials when their SOM interneurons were inactivated to when they were active, we were able to determine if the inactivation had a significant effect on the mice's ability to detect a tone masked by noise.

Two of the SOM-ArchT mice in this study, one male and one female, showed a significant decrease in their ability to detect a tone masked in noise when their SOM interneurons were inactivated by a 10mW laser (figures 8 and 9). This is shown on each of these mouse's psychometric curves because during sessions when the laser was on, the percentage of trials that the mice detected a tone decreased for both bandwidths of background noise (figures 8a and 9a). The effect was seen more during trials with higher SNRs, which was expected as it is easier for the mice to detect the tone during these trials. This effect was not seen during "control days", (figures 8b and 9b) supporting the fact that it is the inactivation of SOM interneurons that is causing the effect in these mice.

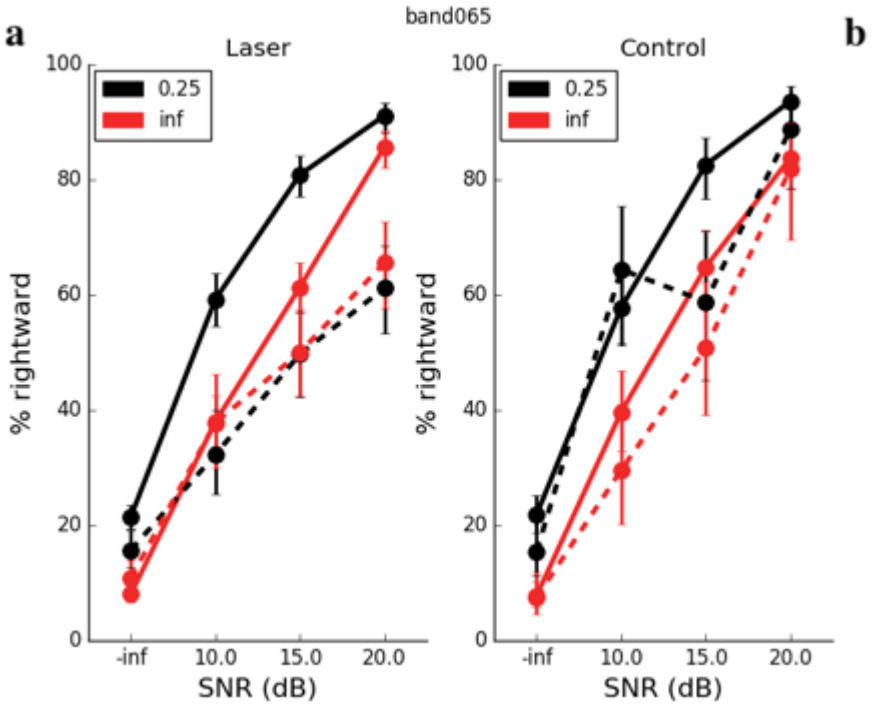


**Figure 8:** Psychometric curve for band065, a SOM-ArchT mouse, showing a decrease in its ability to detect a tone masked by noise when SOM interneurons were inactivated by a 10mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

These psychometric curves show the ability of band065, a SOM-ArchT mouse, to detect a tone masked by noise during the 10mW laser behavioral task during the eight "laser days" (panel a) and the four "control days" (panel b). SOM interneurons were not inactivated during "control days". "SNR" is graphed on the x-axis in decibels with "-inf" representing the trials where there was no tone present in the background noise. "% rightward" was graphed on the y-axis. The red lines depict the trials where the tone was being masked by a broadband bandwidth noise (white noise, depicted as "inf"), while the black lines depict the trials where the tone was being masked by a narrowband bandwidth noise (0.25). The solid lines represent trials where SOM interneurons were not being inactivated, while the dashed lines represent trials where they were being inactivated. Error bars on the graphs depict the standard deviation. No statistical tests were conducted for the data depicted on this figure. **a)** note that the % rightward is lower for the dashed lines than the solid lines for both bandwidths of background noise, showing an effect of SOM inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths of background noise.

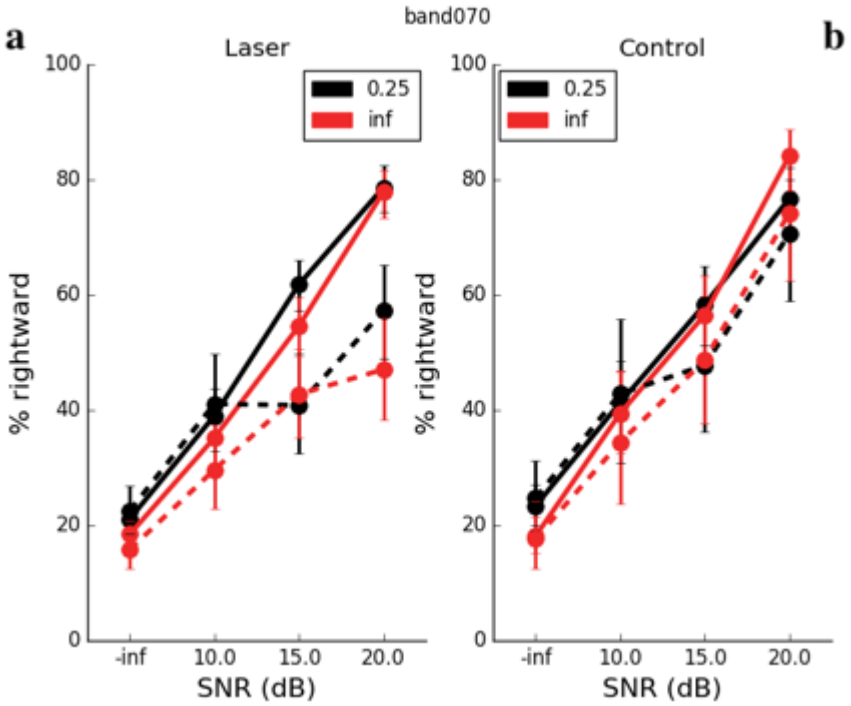


**Figure 9**: Psychometric curve for band070, a SOM-ArchT mouse, showing a decrease in its ability to detect a tone masked by noise when SOM interneurons were inactivated by a 10mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

These psychometric curves show the ability of band070, a SOM-ArchT mouse, to detect a tone masked by noise during the 10mW laser behavioral task during the eight "laser days" (panel a) and the four "control days" (panel b). SOM interneurons were not inactivated during "control days". "SNR" is graphed on the x-axis in decibels with "-inf" representing the trials where there was no tone present in the background noise. "% rightward" was graphed on the y-axis. The red lines depict the trials where the tone was being masked by a broadband bandwidth noise (white noise, depicted as "inf"), while the black lines depict the trials where the tone was being masked by a narrowband bandwidth noise (0.25). The solid lines represent trials where SOM interneurons were not being inactivated, while the dashed lines represent trials where they were being inactivated. Error bars on the graphs depict the standard deviation. No statistical tests were conducted for the data depicted on this figure. **a)** note that the % rightward is lower for the dashed lines than the solid lines for both bandwidths of background noise, showing an effect of SOM inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths of background noise.

It is important to note that this effect was seen when the tone was being masked in both narrowband and broadband background noises. This does not support our hypothesis, which was that the effect would only be seen when the bandwidth of the background noise was broadband. This result might lead us to reconsider our original hypothesis and design, and this will be addressed later in the discussion section

One experimental mouse in this study, band069, did not show a similar decrease in its ability to detect a tone masked by noise when its SOM interneurons were inactivated using the 10mW laser. This is seen on its psychometric curve because during "laser days", there was no decrease seen in its ability to detect the tone when its SOM interneurons were inactivated for either bandwidth of background noise (figure 10a). No effect was seen during "control days" as well (figure 10b).

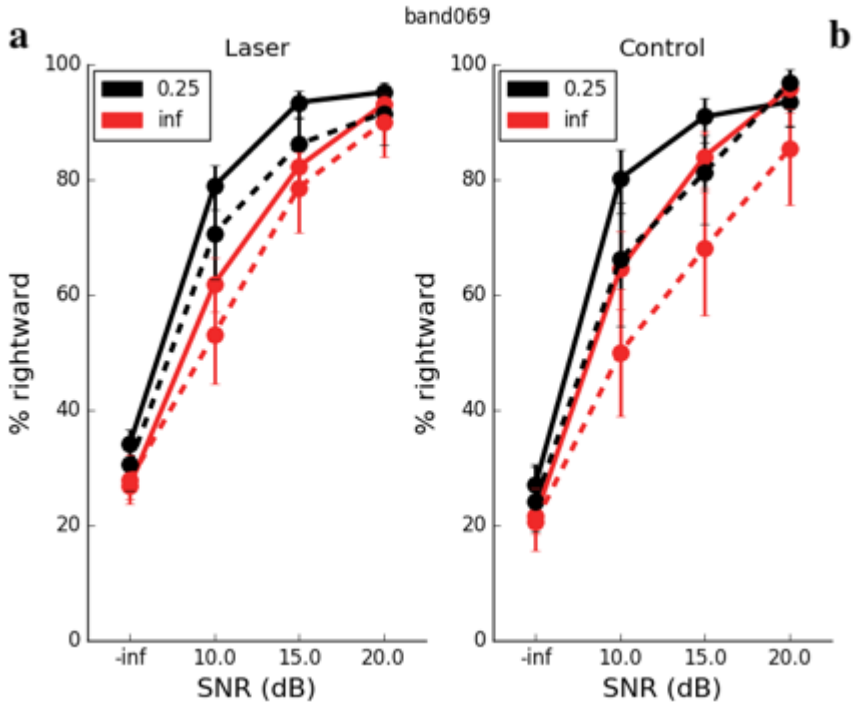


**Figure 10:** Psychometric curve for band069, a SOM-ArchT mouse, showing no change in its ability to detect a tone masked by noise when SOM interneurons were inactivated by a 10mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

These psychometric curves show the ability of band069, a SOM-ArchT mouse, to detect a tone masked by noise during the 10mW laser behavioral task during the eight "laser days" (panel a) and the four "control days" (panel b). SOM interneurons were not inactivated during "control days". "SNR" is graphed on the x-axis in decibels with "-inf" representing the trials where there was no tone present in the background noise. "% rightward" was graphed on the y-axis. The red lines depict the trials where the tone was being masked by a broadband bandwidth noise (white noise, depicted as "inf"), while the black lines depict the trials where the tone was being masked by a narrowband bandwidth noise (0.25). The solid lines represent trials where SOM interneurons were not being inactivated while the dashed lines represent trials where they were being inactivated. Error bars on the graphs depict the standard deviation. No statistical tests were conducted for the data depicted on this figure. **a)** note that the % rightward is not lower for the dashed lines when compared to solid lines for both bandwidths of background noise, showing no effect of SOM inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths of background noise.

Based on these results, we hypothesized that this mouse might need a stronger laser power delivered through the optical fiber implants from the laser output box in order for an effect to be seen. For this reason, this mouse was trained in the laser behavioral task a second time using the same protocol described earlier but with the laser power delivered turned up to 15 mW instead of the original 10 mW. A psychometric curve was produced for this second running of the laser behavioral task and a similar result was obtained (figure 11). For this reason, we concluded that this mouse did not show an effect in its ability to detect a tone when its SOM interneurons were inactivated.

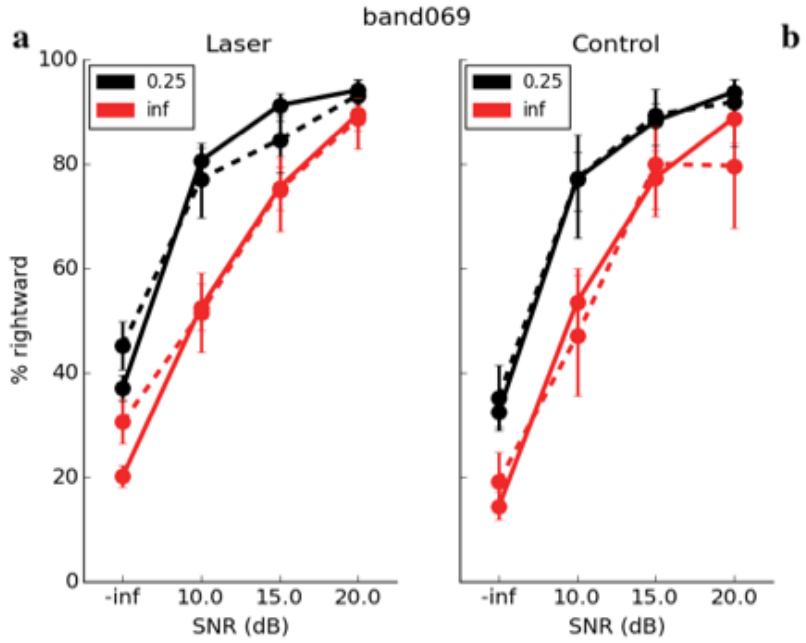


**Figure 11:** Psychometric curve for band069, a SOM-ArchT mouse, showing no change in its ability to detect a tone masked by noise when SOM interneurons were inactivated by a 15mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

This figure uses the same graphing methods as figure 10 except the laser power was changed to 15mW. **a)** note that the % rightward is not lower for the dashed lines when compared to solid lines for both bandwidths of background noise, showing no effect of SOM inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths.

Based on these results, we concluded that it might be possible that this mouse had its optical fiber implants placed in the incorrect location during surgery and not on the auditory cortex as we intended. If this was the case, then no effect would be seen because the laser would not be interacting with the ArchT protein, meaning that SOM interneurons would not be inactivated. After extracting and imaging the brain of this mouse, we determined that the optical fiber implants were indeed placed in the correct location in band069's brain. For this reason, there are two different explanations for why no effect was seen in this mouse. The first is that it was not expressing ArchT in its

SOM interneurons as we intended, and the other is that although no experimental error was made, this mouse was just not impacted by SOM inactivation. Both explanations are likely and explain why we observed the results we did in this mouse.

Lastly, our control mouse in this study, band066, did not show an effect in its ability to detect a tone masked in noise when its SOM interneurons were inactivated during “laser days” (figure 12a) or “control days” (figure 12b). This was expected and further supports the fact that the effect was due to the inactivation of SOM interneurons in the auditory cortex and not due to an effect of the laser shining in the mouse’s eyes.

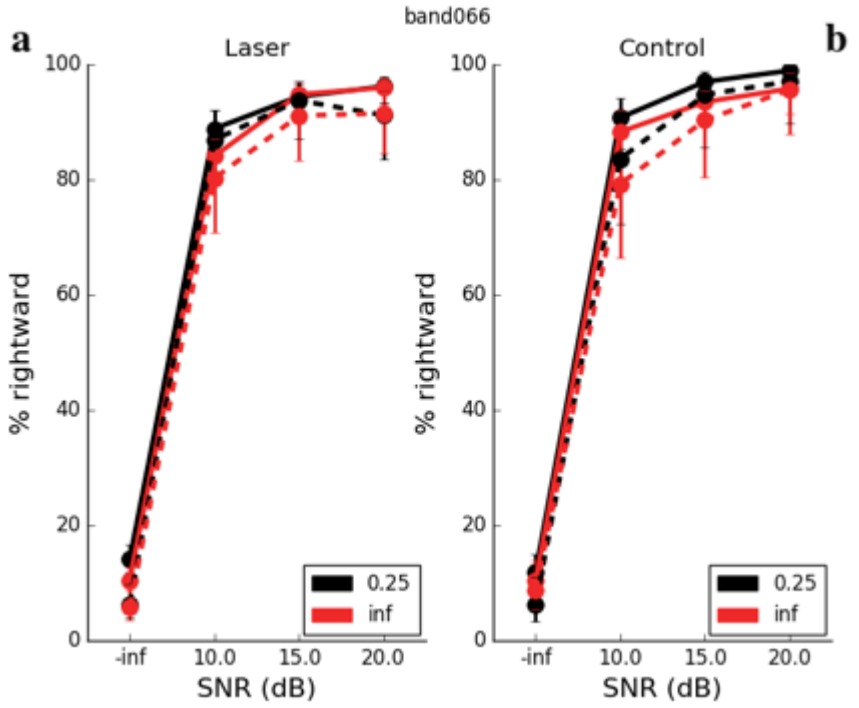


**Figure 12:** Psychometric curve for band066, a SOM-ArchT control mouse, showing no change in its ability to detect a tone masked by noise when SOM interneurons were inactivated by a 10mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

These psychometric curves show the ability of band066, a SOM-ArchT control mouse, to detect a tone masked by noise during the 10mW laser behavioral task during the eight "laser days" (panel a) and the four "control days" (panel b). SOM interneurons were not inactivated during "control days". "SNR" is graphed on the x-axis in decibels with "-inf" representing the trials where there was no tone present in the background noise. "% rightward" was graphed on the y-axis. The red lines depict the trials where the tone was being masked by a broadband bandwidth noise (white noise, depicted as "inf"), while the black lines depict the trials where the tone was being masked by a narrowband bandwidth noise (0.25). The solid lines represent trials where SOM interneurons were not being inactivated, while the dashed lines represent trials where they were being inactivated. Error bars on the graphs depict the standard deviation. No statistical tests were conducted for the data depicted on this figure**. a)** note that the % rightward is not lower for the dashed lines when compared to solid lines for both bandwidths of background noise, showing no effect of SOM inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths of background noise.

**Inactivating SOM interneurons Decreased the Mice's Accuracy in the Behavioral Task**

After analyzing how inactivating SOM interneurons impacted our mice's ability to detect a tone masked by noise, we next wanted to see how it affected their accuracy in the behavioral task. This was done by constructing an accuracy plot that showed the results for our four SOM-ArchT mice on a single plot. The trial type was plotted on the x-axis, which showed the trials where the laser was turned on, representing when SOM interneurons were inactivated, and trials where the laser was turned off, representing when SOM interneurons were active. Accuracy was plotted on the y-axis and was defined as the percentage of trials where the mouse went to the correct side to obtain a reward during the laser behavioral task.

This meant that it went to the left side when no tone was present and to the right side when a tone masked by noise was present. By comparing the accuracy of our mice during the two trial types, the effect of SOM inactivation on accuracy was understood. Two accuracy plots were created for our SOM-ArchT mouse, one showing the average data for the eight "laser days" and another showing the average data for the four "control days". Data for all four mice were shown on a single plot in different colors.

By looking at the accuracy plot for the "laser days" it was determined that inactivating SOM interneurons decreased the mice's accuracy in the behavioral task (figure 13). This is shown in our three experimental mice because their accuracy (as shown on the y-axis) decreased during trials when the laser was on, correlating to when

their SOM interneurons were inactivated. A decrease of this magnitude was not seen in our control mouse, band066, or the plot depicting the four “control days” (figure 14), further supporting that this effect was caused by the inactivation of SOM interneurons.

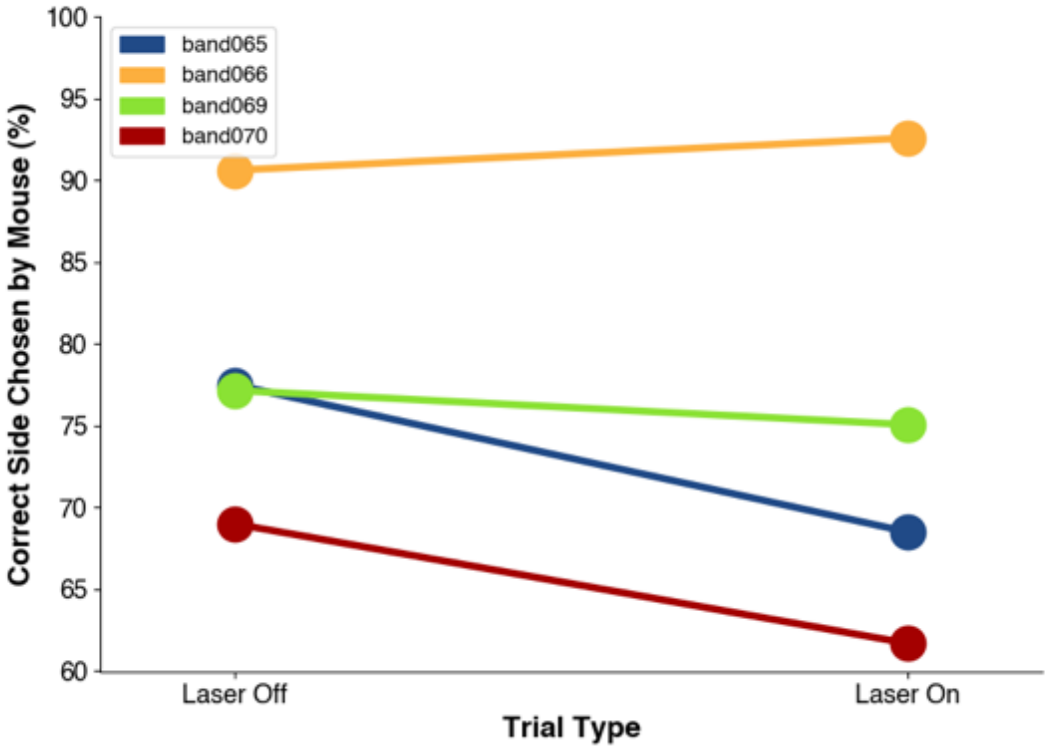


**Figure 13:** Accuracy plot showing that during "laser days", inactivating SOM interneurons with a 10mW laser in our three experimental SOM-ArchT mice decreased the percentage of trials that they chose the correct side.

This figure depicts the accuracy for each of our four SOM-ArchT mice during "laser days" of the laser behavioral task when the laser power was set to 10mW. The x-axis represents the trial type. "Laser off" trials depict trials where SOM interneurons were not being inactivated, while "laser on" trials depict trials where they were inactivated. The y-axis depicts the percentage of trials in which each mouse went to the correct side to collect a water reward and is thus a measure of accuracy. No statistical tests were conducted for the data depicted on this figure. Note the decrease in accuracy during trials where the laser was turned on compared to when it was turned off, showing an effect of SOM inactivation

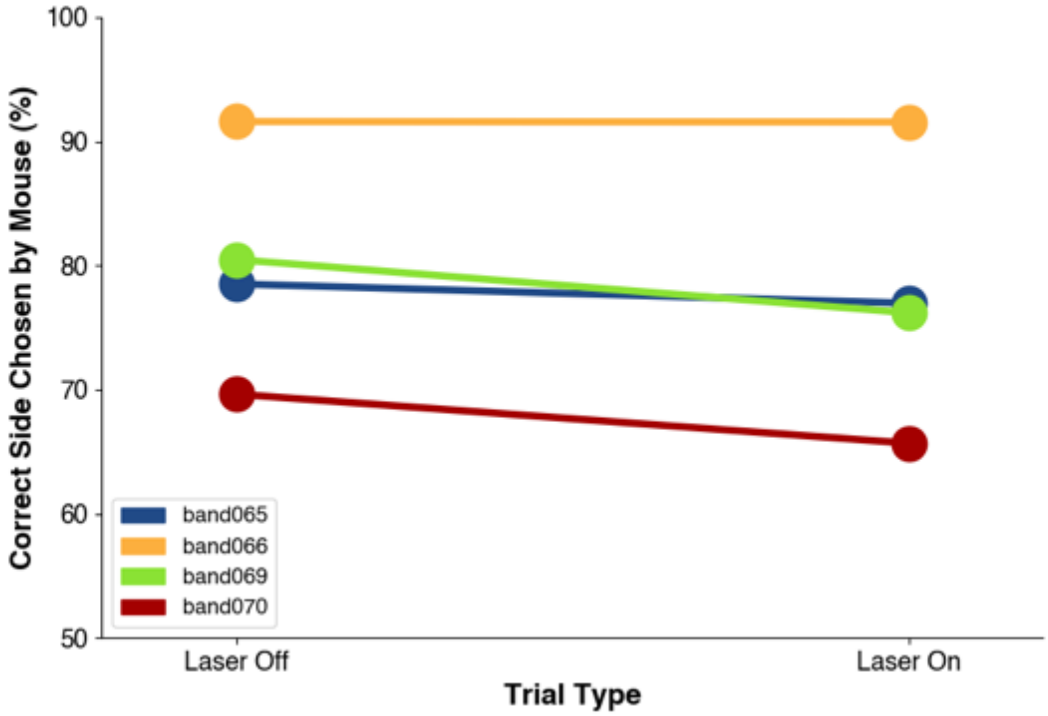


**Figure 14:** Accuracy plot showing that during "control days", inactivating SOM interneurons with a 10mW laser in our three experimental SOM-ArchT mice did not change the percentage of trials that they chose the correct side.

This figure depicts the accuracy for each of our four SOM-ArchT mice during "control days" of the laser behavioral task when the laser power was set to 10mW. SOM interneurons were not inactivated during "control days". The x-axis represents the trial type. "Laser off" trials depict trials where SOM interneurons were not being inactivated, while "laser on" trials depict trials where they were inactivated. The y-axis depicts the percentage of trials in which each mouse went to the correct side to collect a water reward and is thus a measure of accuracy. No statistical tests were conducted for the data depicted on this figure. Note the minimal decrease in accuracy during trials where the laser was turned on compared to when it was turned off.

**Inactivating PV interneurons had no Effect on the Mice's Ability to Detect a Tone Masked by Noise**

By the end of this study, we were able to teach a total of two PV-ArchT mice, one male and one female, how to perform the behavioral task. Both of the mice had optical fiber implants placed on their auditory cortex and were able to complete the laser behavioral task. Once the mice completed the laser behavioral task, psychometric curves were produced for each mouse, using the same methods described for the SOM-ArchT mice. Both of the PV-ArchT mice in this study, known as band081 and band087, showed no change in their ability to detect a tone masked by noise when their PV interneurons were inactivated using a 10mW laser for both bandwidths of background noise (figures 15 and 16). This is shown on their psychometric curves because during trials where the laser was on during "laser days", the percentage of trials that the mice detected a tone did not significantly change compared to the trials where the laser was off (figures 15a and 16a). No effect was seen during "control days" as well (figures 15b and 16b). This result shows that the activity of PV interneurons might not be important when it comes to mice's ability to detect a tone masked by noise

It is important to note that this finding was seen trials where the tone was being masked by both the narrowband and broadband backgrounds. This does not support our hypothesis, which was that there would be a decrease in the mice's ability to detect a tone masked by noise when PV interneurons were inactivated regardless of the bandwidth of the background noise, as no such decrease was observed. This result might lead us to reconsider our original hypothesis and design, and this will be addressed later in the discussion section.

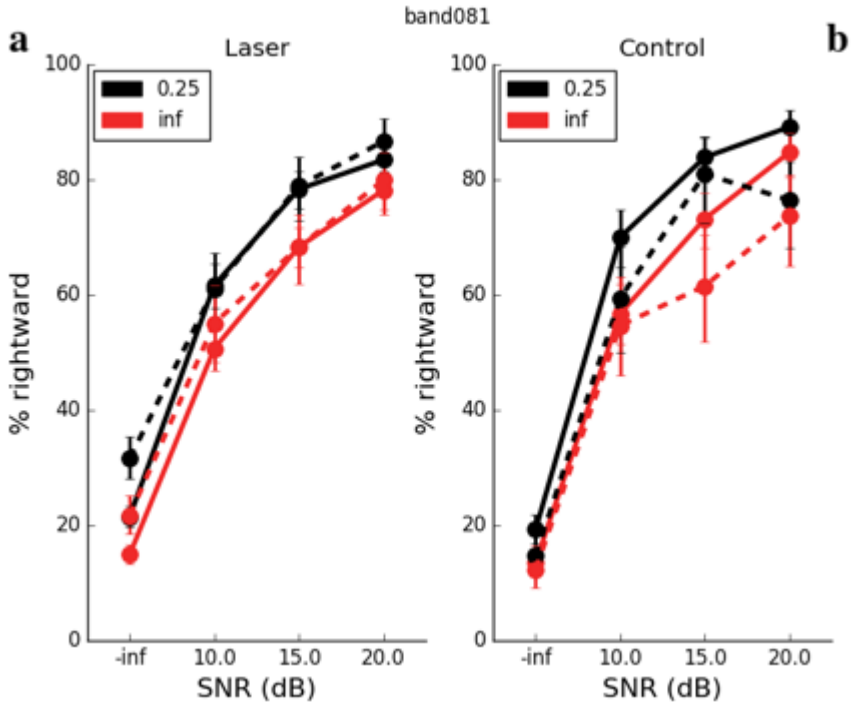


**Figure 15:** Psychometric curve for band081, a PV-ArchT mouse, showing no change in its ability to detect a tone masked by noise when PV interneurons were inactivated by a 10mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

These psychometric curves show the ability of band081, a PV-ArchT mouse, to detect a tone masked by noise during the 10mW laser behavioral task during the eight "laser days" (panel a) and the four "control days" (panel b). PV interneurons were not inactivated during "control days". "SNR" is graphed on the x-axis in decibels with "-inf" representing the trials where there was no tone present in the background noise. "% rightward" was graphed on the y-axis. The red lines depict the trials where the tone was being masked by a broadband bandwidth noise (white noise, depicted as "inf"), while the black lines depict the trials where the tone was being masked by a narrowband bandwidth noise (0.25). The solid lines represent trials where PV interneurons were not being inactivated, while the dashed lines represent trials where they were being inactivated. Error bars on the graphs depict the standard deviation. No statistical tests were conducted for the data depicted on this figure. **a)** note that the % rightward is not lower for the dashed lines when compared to solid lines for both bandwidths of background noise, showing no effect of PV inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths

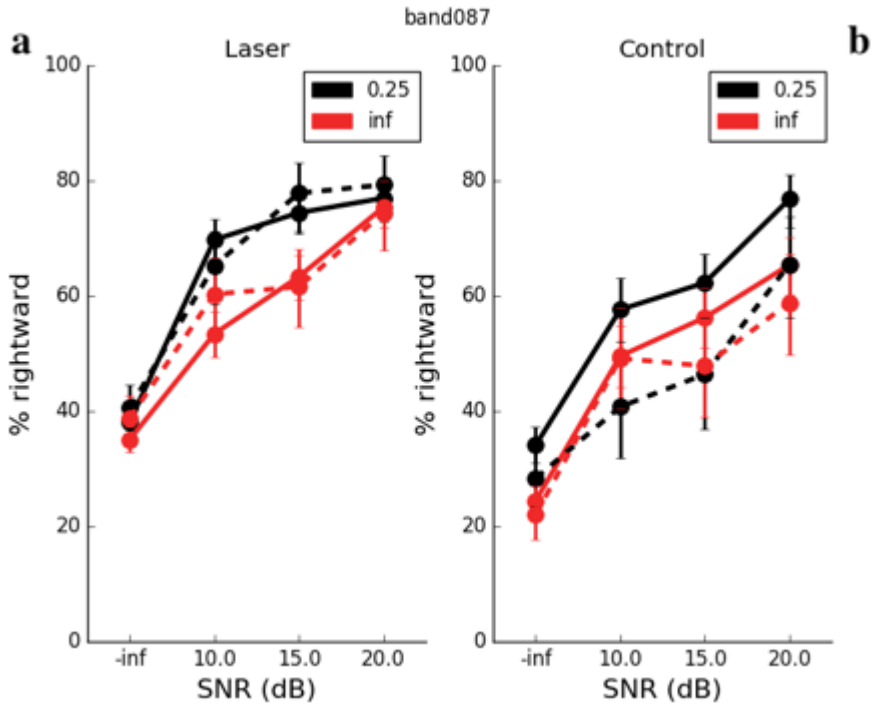


**Figure 16:** Psychometric curve for band087, a PV-ArchT mouse, showing no change in its ability to detect a tone masked by noise when PV interneurons were inactivated by a 10mW laser in both narrowband (0.25) and broadband (inf) bandwidths of background noise

These psychometric curves show the ability of band087, a PV-ArchT mouse, to detect a tone masked by noise during the 10mW laser behavioral task during the eight "laser days" (panel a) and the four "control days" (panel b). PV interneurons were not inactivated during "control days". "SNR" is graphed on the x-axis in decibels with "-inf" representing the trials where there was no tone present in the background noise. "% rightward" was graphed on the y-axis. The red lines depict the trials where the tone was being masked by a broadband bandwidth noise (white noise, depicted as "inf"), while the black lines depict the trials where the tone was being masked by a narrowband bandwidth noise (0.25). The solid lines represent trials where PV interneurons were not being inactivated, while the dashed lines represent trials where they were being inactivated. Error bars on the graphs depict the standard deviation. No statistical tests were conducted for the data depicted on this figure**. a)** note that the % rightward is not lower for the dashed lines when compared to solid lines for both bandwidths of background noise, showing no effect of PV inactivation. **b)** note that during control trials, there is no difference in the % rightward between the solid and dashed line for both bandwidths

## Inactivating PV interneurons had no Effect on the Mice's Accuracy in the Behavioral Task

After analyzing how inactivating PV interneurons impacted our mice's ability to detect a tone masked in noise, we next wanted to see how it affected their accuracy in the behavioral task. This was done by constructing accuracy plots that showed the results for our two PV-ArchT mice on a single plot in a similar manner that was done for our SOM-ArchT mice.

By analyzing the accuracy plot for the "laser days" it was determined that inactivating PV interneurons had no effect on the mice's accuracy in the behavioral task (figure 17). This is shown in our two mice because their accuracy (as shown on the y-axis) did not significantly change during trials where the laser was on, meaning that

their PV interneurons were inactivated, when compared to trials where the laser was off, meaning that PV interneurons were active. Interestingly, a larger decrease in accuracy was seen in the accuracy plot for the "control days" (figure 18). This further supports the fact that inactivating PV interneurons had no effect on the mice's accuracy, showing that PV interneuron activity might not be important when it comes to mice's ability to detect a tone masked by noise accurately, and could also show that the action of shining the laser itself might have had an impact on the mice in some way.

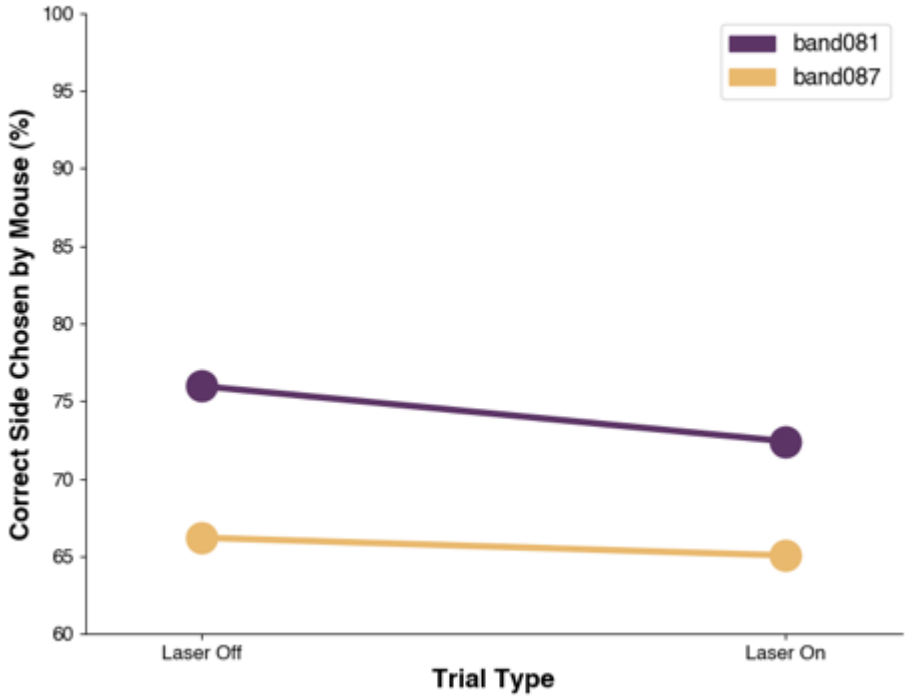


**Figure 17**: Accuracy plot showing that during "laser days", inactivating PV interneurons with a 10mW laser in our two experimental PV-ArchT mice did not change the percentage of trials that they chose the correct side.

This figure depicts the accuracy for each of our four PV-ArchT mice during "laser days" of the laser behavioral task when the laser power was set to 10mW. The x-axis represents the trial type. "Laser off" trials depict trials where PV interneurons were not being inactivated, while "laser on" trials depict trials where they were inactivated. The y-axis depicts the percentage of trials in which each mouse went to the correct side to collect a water reward and is thus a measure of accuracy. No statistical tests were conducted for the data depicted on this figure. Note the minimal decrease in accuracy during trials where the laser was turned on compared to when it was turned off, showing no effect of PV inactivation.

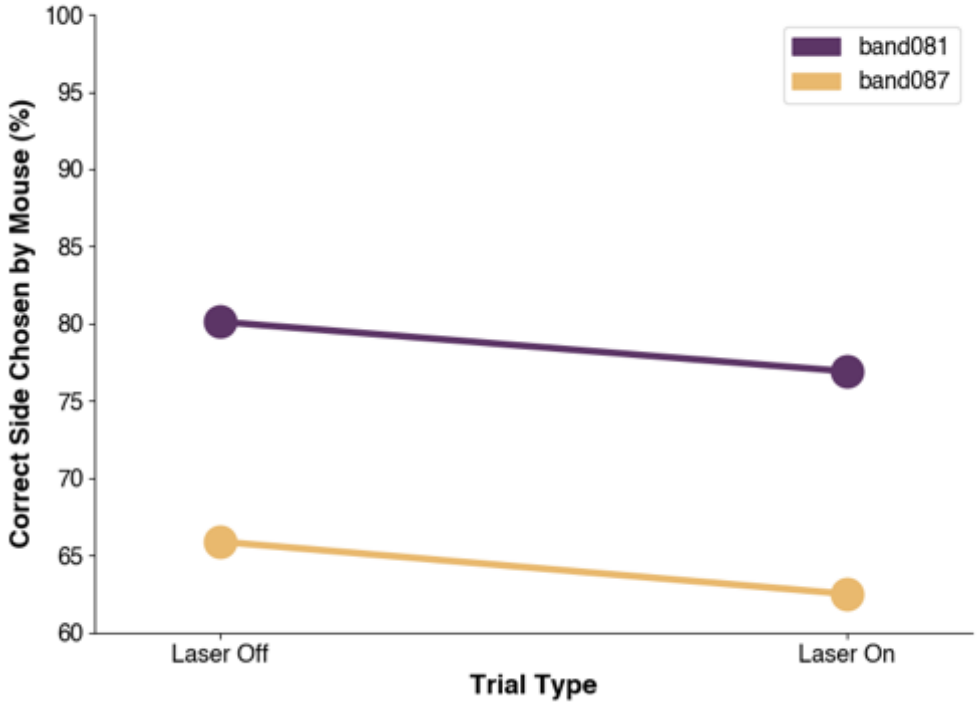


**Figure 18:** Accuracy plot showing that during "control days", inactivating PV interneurons with a 10mW laser in our two experimental PV-ArchT mice decreased the percentage of trials that they chose the correct side.

This figure depicts the accuracy for each of our four PV-ArchT mice during "control days" of the laser behavioral task when the laser power was set to 10mW. PV interneurons were not inactivated during "control days". The x-axis represents the trial type. "Laser off" trials depict trials where PV interneurons were not being inactivated, while "laser on" trials depict trials where they were inactivated. The y-axis depicts the percentage of trials in which each mouse went to the correct side to collect a water reward and is thus a measure of accuracy. No statistical tests were conducted for the data depicted on this figure. Note the large decrease in accuracy during trials where the laser was turned on compared to when it was turned off.

# Discussion

The purpose of this study was to use a mouse model to uncover how two different types of cortical inhibitory interneurons found in the auditory cortex, known as somatostatin-expressing (SOM) and parvalbumin-expressing (PV) interneurons, contribute to our perception of sound masked by noise. This purpose was achieved by teaching a total of four SOM-ArchT and two PV-ArchT mice how to perform a complex behavioral task that required them to detect a tone masked by background noise.

At the conclusion of this study, we were able to reach a number of conclusions. The first was that inactivating SOM interneurons decreased the mice's ability to detect a tone masked by noise regardless of which bandwidth of background noise the tone was in (figures 8 and 9). This conclusion was made by looking at the psychometric curves of our three experimental SOM-ArchT mice and comparing them to the curve of our control mouse and the curves of our experimental mice during "control days". In two of our three mice, we found that the percentage of trials that the mouse went to the right to obtain a reward, showing the percentage of trials that it detected the tone, decreased when their SOM interneurons were inactivated (figures 8a and 9a). This result was not seen in our control mouse (figure 12) or during "control days" for our experimental mice (figures 8b and 9b), supporting the fact that this finding is due to the inactivation of SOM interneurons in these mice. Although this is a fascinating finding, it does not support our original hypothesis, which was that when SOM interneurons were inactivated, mice would show a decrease in their ability to detect a tone masked by noise only when the tone was being masked in a background noise was broadband. Our

finding does not support this hypothesis because the effect was seen in both broad and narrow bandwidths of background noise. Our original hypothesis was constructed based on our model of surround suppression and signal detection that was previously developed using preliminary data collected by Anna Lakunina. After the findings of this study, it might be necessary to reconsider our original model of surround suppression and how it relates to signal detection. Our new model would have to incorporate the idea that SOM interneurons play a role in signal detection in all bandwidths of background noise, and the specifics of this new model will be formed in the near future.

Our next conclusion was that inactivating SOM interneurons decreased our mice's accuracy in the behavioral task. This was found by analyzing the accuracy plots of our three experimental SOM-ArchT mice during "laser days" (figure 13) and comparing them to our control mouse and the accuracy plot during "control days" (figure 14). These plots showed that during trials where the laser was on, meaning SOM interneurons were inactivated, the percentage of trials that the mice went to the correct side decreased. This finding was not related to our main hypothesis about how SOM inactivation would change the mice's behavior, but it is still a fascinating finding to report, as it showed that inactivation of SOM interneurons not only impacted the mice's ability to detect a tone masked by noise, but also their accuracy in the task

When it came to the PV-ArchT mice, our first conclusion was that inactivating PV interneurons had no effect on the mice's ability to detect a tone masked by noise (figures 15 and 16). This conclusion was reached by looking at the psychometric curves for our two experimental PV-ArchT mice during "laser days" and comparing them to the curves during "control days". In both mice, we found that the percentage of trials

that the mouse went to the right to obtain a reward, correlating to the percentage of trials that they detected the tone, did not significantly change when their PV interneurons were inactivated compared to when they were active (figures 15a and 16a). No effect was seen during our "control days" trials as well (figures 15b and 16b), showing that the shining of the laser itself did not cause an effect in the mice. This finding does not support our original hypothesis, which was that when PV interneurons are inactivated, mice would show a decrease in their ability to detect a tone masked by noise regardless of the bandwidth of the background noise. Our finding does not support this hypothesis because even though a similar effect was seen in both bandwidths of background noise, there was no decrease seen in our mice's ability to detect a tone masked by noise. Similar to our hypothesis about SOM interneurons, this hypothesis was constructed using our model of surround suppression and signal detection. Based on the results of this study, our original model has to be modified to better understand the role that PV interneurons play in auditory signal detection. Interestingly, based on our findings, it is possible that PV interneurons do not play a role in mice being able to detect a tone masked by noise, as their inactivation did not change our mice's ability to do so. This means that PV interneurons might be excluded from our new model entirely. This is an interesting concept that will be worked on in the future.

Finally, our final conclusion was that inactivating PV interneurons had no effect on our mice's accuracy in the behavioral task. This conclusion was drawn by analyzing the accuracy plot for our two experimental PV-ArchT mice during "laser days" (figure 17) and comparing them to the accuracy plot during "control days" (figure 18). These plots showed that during trials where the laser was on, meaning PV interneurons were

inactivated, the percentage of trials that the mice went to the correct side did not change compared to trials in which PV interneurons were active. Curiously, it looks like a larger decrease in percentage was seen during "control days", further supporting the finding that PV inactivation had no effect on accuracy. This finding was not related to our main hypothesis about how PV inactivation would change the mice's behavior, but it is still a compelling finding to report as it showed that inactivation of PV interneurons not only had no impact on the mice's ability to detect a tone masked by noise, but also had no impact on their accuracy in the task as well.

Based on the results of this study, we found that our proposed model of signal detection and surround suppression (figure 2) was incorrect. According to our proposed model, when SOM interneurons were inactivated, mice should have shown an increase in their false detection rate of a tone masked by background noise. Instead, in this study, we found the exact opposite, as mice showed a decrease in their tendency to detect a tone masked by background noise when SOM interneurons were inactivated in our SOM-ArchT mice. Due to this, our proposed model needs to be modified to accommodate these new findings. There are multiple possibilities for why our model was inaccurate, such as there being other brain regions and/or neurons that are involved in signal detection that were not accounted for in our original model or the possibility that other neurons in the brain were inadvertently inactivated during the laser behavioral task. These questions will be addressed in a future study.

There were a number of limitations with our experimental design. The first was that it is possible that the mice were being impacted by the action of the laser shining in their eyes instead of their respective interneurons being inactivated. We attempted to

control for this by covering the implant box with gray foam during the laser behavioral task to prevent the amount of light that came out of it and by having "control days" where the laser was not inactivating the interneurons directly. Although these methods did minimize the possibility of this limitation becoming a real problem and impacting our findings, some light was still able to escape the box during the laser behavioral task, so it is possible that the laser shining had some impact that could not be entirely minimized or controlled for. Another limitation was that although we received our transgenic SOM-ArchT and PV-ArchT mice from a reliable source, one of our mice, band069, potentially ended up not expressing ArchT in its SOM interneurons in the auditory cortex as was intended. This meant that we spent months training a mouse from which we could not obtain reliable data from instead of training a different mouse. Although this was unfortunate, it only happened once, so it did not have a severe impact on the conclusions of the study.

Through this study, we were able to obtain a better understanding of the neural processes that allow us to detect a tone masked by noise. The significance of this study is that although it was conducted in a mouse model, most of the findings can be translated to humans. For this reason, through the findings of this study, we can better understand the roles that different sources of inhibition play when it comes to our ability to detect a sound masked by noise, allowing us to better understand how humans are able to, for example, hear their name in a loud room or focus on one conversation while tuning out all of the others happening around them. These findings are compelling and supplement what we already know about the neural basis of attention and perception.

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