ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE AUDITORY STRIATUM DURING BEHAVIOR

by

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A THESIS

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The mechanisms by which animals form flexible associations between sounds and behavioral responses are not well understood. Understanding how associations between sounds and behavioral responses change dependent on contextual information requires identifying where in the brain these changes take place. The primary aim of this study was to determine whether the portion of the dorsal striatum receiving projections from the auditory cortex and auditory thalamus could be a center for associating sounds to actions that are known to bring reward. We refer to auditory neurons in this area as the auditory striatum. To investigate the role of the auditory striatum in forming flexible associations between sounds and learned behavioral responses, we examined the activity of auditory striatal neurons in male C57BL/6 mice, via chronically implanted electrodes, while the mice performed a sound categorization task in which the action associated with one of the sounds periodically changed.

Our recordings show that many neurons in the auditory striatum respond to sounds and are selective to sound frequency. We compared the average firing rate of auditory striatal neurons under conditions in which the same sound was presented, but

the rewarded action associated with it was different. We found that about 12% of sound responsive auditory striatal neurons respond differently during sound presentation, depending on the sound-action association. However, the majority of these neurons show no significant difference in activity between conditions. Our results suggest that the auditory striatum is not the sole mediator of flexibility in sound-action associations.

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Introduction

Human reactions to sound often depend on context. For instance, imagine the kinds of sounds produced in a typical Fourth of July celebration. Now imagine it is the middle of the night in the month of March. How would you react to hearing the same sounds? How is it that in one case loud sounds are interpreted as celebration, while at a different time the same sounds could be cause for great alarm? The idea that animals react differently to the same stimuli, based on changes in the context in which they are presented, is referred to as behavioral flexibility. Flexibility is present in a variety of behaviors, including reactions to sounds. For many behaviors, the mechanisms by which animals react according to context remain unknown.

Neuroscientists, medical doctors, and the general public all have interest in understanding how humans make flexible decisions. Context-informed decision-making is central to everyday life. Integrating context in decision-making allows us to interpret the same sounds differently depending on other cues that indicate to us what frame of mind we should use in deciding how to act. The decisions used in this research can be made quickly and automatically, once the appropriate associations have been learned. Behavioral flexibility, or the ability to make decisions informed by contextual information, allows us not to reach for car keys left in the ignition when we hear an elevator bell signaling that we've reached the requested floor; it allows us not to be hyperaware of innocuous background noise, like leaf blowers and chainsaws, or explosive sounds like gunshots and fireworks, when they can be reasonably attributed to a harmless source. The brain's ability to interpret sound according to context is critical to what most people consider normal behavior and responses to environmental sounds.

Normal responses to innocuous sounds like leaf blowers, chainsaws, and expected explosions can be impaired in post-traumatic stress disorder (Carr et al., 2011). In people with post-traumatic stress disorder, strong associations have often been created between traumatic events and sounds or other sensory details that occurred in conjunction with these events (Bensimon, Amir, & Wolf, 2012). Lack of flexibility in interpreting sounds and other sensory information can trigger flashbacks, which can be very disruptive to a person's everyday life.

Behavioral flexibility, more generally, is impaired in conditions such as schizophrenia and autism (Floresco, Zhang, & Enomoto, 2009; Hill, 2004). In humans, complex forms of behavioral flexibility depend on subcortical systems, including the dorsal striatum (Floresco et al., 2009), which is the area of the brain examined by this research. Abnormalities in subcortical systems, including the dorsal striatum, are thought to be potential causes of schizophrenia (Floresco et al., 2009). Improvements in treating disorders related to flexibility require better understanding of its neural mechanisms. Research that expands knowledge of subcortical systems, and their roles in behavioral flexibility will reveal the neural mechanisms behind disorders in which flexibility is impaired, which may contribute to improvements in the prevention and treatment of disorders of flexibility. Understanding the role of the auditory striatum in mediating flexible sound-action associations will be key in directing future research into the neural mechanisms behind this phenomenon.

A comprehensive understanding of behavioral flexibility requires knowing how behavior changes, what part of the brain causes the change, and how differences in brain activity create that change. Many areas of the brain are involved in auditory

processes, but previous research has identified areas that are most likely to confer the ability to make different decisions depending on contextual information. The striatum is connected to many major regions of the brain, especially those carrying auditory information (McGeorge & Faull, 1989). Auditory areas of other brain regions connect to the dorsomedial striatum specifically (McGeorge & Faull, 1989). Research on the striatum, as well as other brain areas that are connected to it, has suggested that it may be one step in the pathway between hearing a sound and acting based on the sound (Gimenez, Lorenc, & Jaramillo, 2015; Xiong, Znamenskiy, & Zador, 2015; Znamenskiy & Zador, 2013). In this study, we refer to the region of interest as the auditory striatum, which we define as the part of the dorsal striatum that receives auditory information from the auditory cortex (AC) and auditory thalamus (ATh). Neurons in the auditory striatum are activated when sounds are presented to subjects, and show preference to specific sound frequencies (Bordi & LeDoux, 1992) by increasing their activity when preferred frequencies are presented.

In addition to its role in processing auditory information, the dorsal striatum has also been known to contribute to motor control (Balleine & Ostlund, 2007). More recently, it has been shown to contribute directly to decision-making, especially action selection and initiation (Balleine & Ostlund, 2007; Yin, Ostlund, Knowlton, & Balleine, 2005). It also associates action and reward, and motivates decision-making in reward-related behavior (Balleine, Delgado, & Hikosaka, 2007). Integration of reward into behavior occurs where reward-related neural signals connect with motor circuits (Wickens, Reynolds, & Hyland, 2003). Much of the research in this area has focused on changes during learning, but not during choice selection. In this study, we have

investigated striatal activity after the behavior has been learned, and during choice selection.

The brain area controlling context-dependent decision-making is likely an output of the auditory cortex or auditory thalamus

Previous research has investigated the possibility that AC or ATh could mediate the flexibility of sound-action associations. Only 15% and 16% of neurons in AC and Ath, respectively, change their **firing rates** in response to changes in stimulus meaning (Jaramillo, Borges, & Zador, 2014), suggesting that AC and ATh are not prominent in controlling flexibility for this behavior. In rats, AC is not necessary for flexible decision-making in the behavioral task used in this study, but ATh is required. Rats were trained to do the same task used in this study, and AC was **lesioned**, after which they could still do the task with no significant change in accuracy. Rats with lesions to ATh were no longer able to perform the task (Gimenez et al., 2015). Thus, when AC is not available, flexibility in this task must be controlled at some output of ATh. (Gimenez et al., 2015). The auditory striatum is one such output, making it an interesting possibility to investigate in terms of this role; however, when AC remains intact, it is unclear whether its outputs are involved.

In the system examined in this study, sound travels through the ears, and is transduced into electrical signals by the hair cells of the cochlea; these signals eventually reach the auditory thalamus, auditory cortex, and other regions, including the striatum, before an action can be decided upon. The process proposed by this study is depicted in Figure 1.

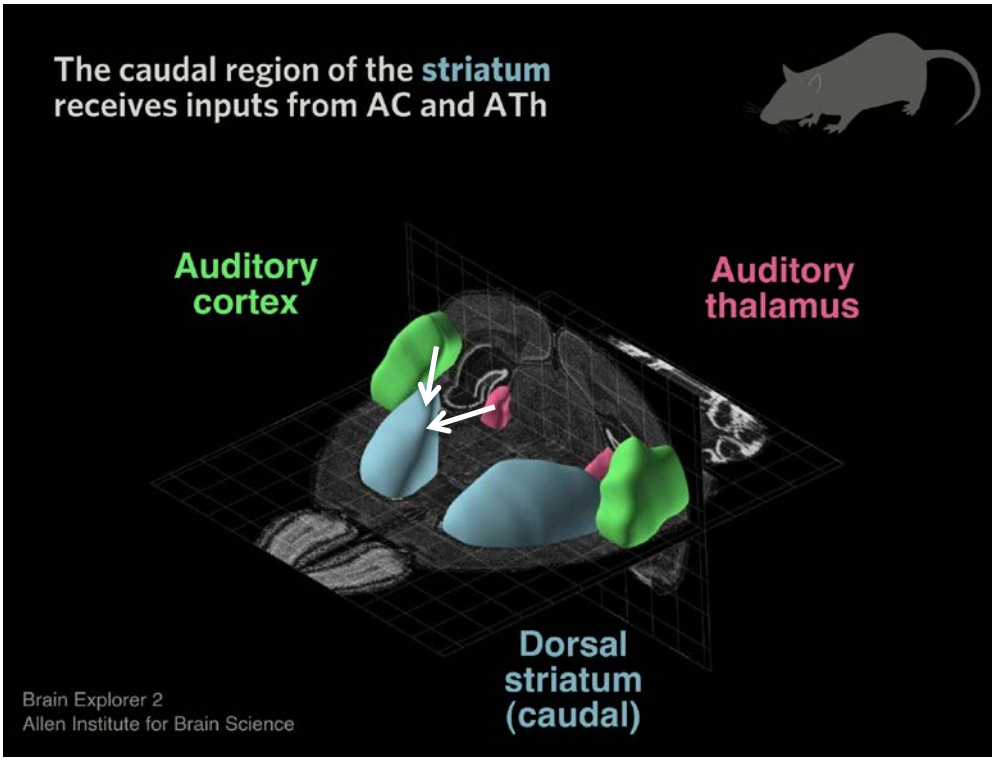


Figure 1: Auditory decision circuit through the striatum

This model circuit depicts how sound might influence behavior. Sound information travels through the ears where it is transduced into electrical signals, which are eventually transmitted to the auditory striatum via the auditory cortex and auditory thalamus.

Whether the activity of the auditory striatum is modulated by the context in which sounds are presented has been unknown. The mechanisms by which the brain mediates the flexibility of **stimulus**-action associations can be best investigated through use of a model system with fewer neurons and greater experimental access than is available in humans. This research used mice as a model organism. I explored the possibility that the auditory striatum mediates the flexibility of sound-action associations by recording from neurons in the auditory striatum in mice performing a flexible **auditory discrimination** behavior, using surgically implanted microelectrode

arrays. I compared the firing rates of neurons between two conditions in which the same sound stimulus had different actions associated with it. We hypothesized that the auditory striatum would show different activity depending on the action associated with the sound with which the mouse was presented.

Project development and research

This research was conducted under the direction of Dr. Santiago Jaramillo, who designed this study. I began training mice to do the behavioral task used in these experiments, and others, in June 2014. I became familiar with data collection procedures for this project throughout spring 2015, and worked full time on the project through summer 2015, recording neural activity, daily, in four mice that already had brain implants placed. I entered the parameters necessary for data recording into the computer, took notes on my observations, and monitored the mice throughout each experiment. I have also assisted with data analysis by categorizing the patterns of neural activity we recorded from each neuron according to a set of criteria I helped establish. Additionally, I contributed to building new brain implants as necessary. I have worked on this project in partnership with William Walker, a research assistant in the lab. William did all implant surgeries and managed data analysis, as well as recording data.

Methods

Experimental approach

We sought to determine whether activity in the auditory striatum changes during sound presentation when a different action is associated with the same sound. We recorded electrical signals, extracellularly, from multiple single cells in the auditory striatum in mice while they performed a two-alternative, self-initiated sound categorization task with a category boundary that changed so that one of the sounds would periodically change meaning throughout each experimental session.

We compared firing rates of neurons during sound presentation between conditions in which the sound-action association was different. We hypothesized that when behaving mice are presented with sounds during a flexible decision-making task, the firing rate of neurons in the auditory striatum differs, depending on the meaning of the sound at that time. We found some neurons that increased their firing significantly during the sound presentation, between the different sound-action association conditions; however, the majority of sound responsive neurons recorded did not show a significant change in activity related to stimulus meaning.

Mouse Model

Using a model organism is the most experimentally practical way to locate the area that links sounds with rewarded actions and provide a framework for future investigation of the mechanisms behind this link. In this study we used male C57BL/6J mice. These mice are suitable for this type of study in that they are able to learn and perform well at the auditory discrimination task that was used (Jaramillo & Zador,

2014). A few key considerations inform the selection of this model. One consideration is the technology available for genetic manipulation in these mice. Genetic manipulation was not important to this project specifically, but it will be for future studies. Other research tools, including the Allen Mouse Brain Atlas, have also been constructed based on C57BL/6J mice, which is another benefit to selecting this strain. All animal procedures were overseen by the University of Oregon Animal Care and Use Committee.

Behavioral paradigm

Mice performed a task in which they discriminated between three different sounds. Sounds were chords, each composed of 12 different frequencies, evenly spaced on a logarithmic scale around a specified center frequency. Center frequencies used were 6 kHz, 11 kHz and 19.2 kHz. During each behavioral session, mice were placed in an acoustic chamber with three reward ports. As shown in Figure 2, each trial was initiated when the mouse poked into the center port and waited through a 15-20-millisecond period of silence, until a sound was presented. Each sound played for 100 milliseconds. After the sound ended, the mouse indicated whether the sound was higher or lower in frequency by poking his head in the port right or left of the center to receive 2-3 microliters of water as a reward if correct. Following a choice, or if no choice was made within 4 seconds, a new trial could be initiated.

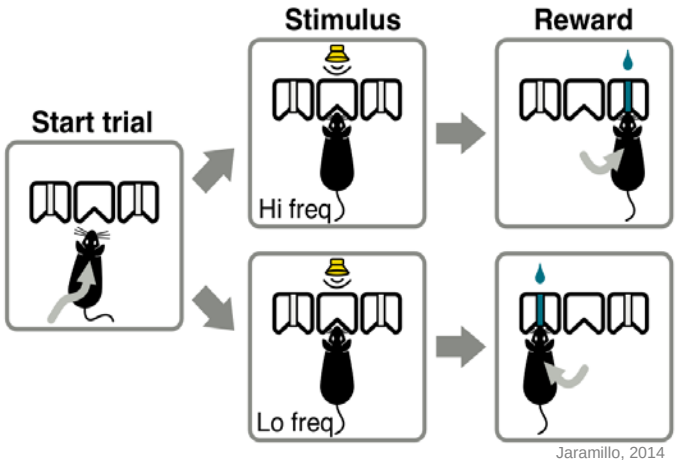


Figure 2: Mice performed a sound categorization task

Male C57BL/6J mice were trained to do a sound categorization task in which they initiated each trial by poking in the center port and then poked in a reward port right or left of the center, depending on whether they believed the sound to be of high or low frequency.

Throughout each one-hour session, we changed which sounds were associated with which reward ports every 200 trials, and the animals learned the new associations through trial and error.

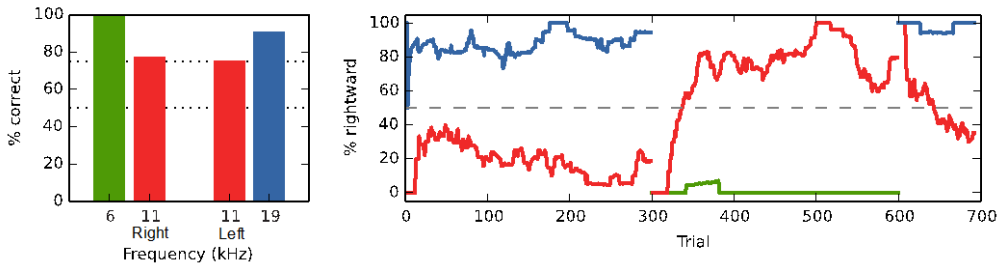


Figure 3: Mice switched between blocks of trials with different sounds

Within the behavioral task, there were three sounds: one of each in low, medium and high frequencies. Each behavioral session was grouped into blocks of trials in which either the low and medium or medium and high sounds were presented. Depending on the block, the medium sound would be categorized as either high or low depending on which other sound was in that block. In comparison to the lowest sound, the medium sound can be categorized as high, so the mouse would move to the direction associated with high sounds, the right. The opposite was true for the block with the medium and high sound.

During training and testing periods, water intake was restricted. Mice were examined and weighed daily to ensure that they remained adequately hydrated, and their health was monitored daily by the veterinary staff of the UO animal care facility. After mice could perform a minimum of 60% of attempted trials correct in both choice directions and complete at least 600 trials per session, they received an implant with eight **tetrodes** to the right auditory striatum.

Mouse Training Timeline

In the course of this study, I recorded data from four mice. According to our previously described training procedure (Jaramillo & Zador, 2014), it takes most mice between 5-10 training sessions to learn to make correct choices, and an average of 28 sessions to become proficient in switching between contingencies. This process starts with habituating the mice to the behavior box and helping them learn that water comes

out of the reward ports on the sides. Then they learn that sounds are associated with particular choices, by being rewarded only when they go to the correct reward port, but retaining the option to be rewarded on correct port if they go there after initially choosing incorrectly. Eventually, they are able to make correct choices most of the time, and are rewarded only for correct choices (Jaramillo & Zador, 2014). Once mice reached stable behavior, we conducted implantation surgeries and began electrophysiological recordings. On average, mice were 9.4 months old at the start of data collection. Implanted mice stayed on the study an average of 3.7 months following implantation, with a range from 1.5-7.1 months, the longest period of time corresponding to the mouse from which came most of the data in this study.

Surgical Procedure

William Walker conducted all tetrode implantation surgeries for this study. Implantation of electrodes into the brain is accomplished using a kind of coordinate system, based on landmarks on the skull of the mouse. During surgery, the skull was exposed, and the mouse's head angled in the stereotax so that **bregma and lambda** were level in the horizontal plane. The location for the craniotomy above the right auditory striatum was determined using the following measurements: 1.73mm from bregma in the anterior-posterior plane, 3.52mm right in the medial-lateral plane, and -2.0mm in the dorsal-ventral plane.

After the location for the craniotomy was marked, skull screws were applied, in order to help the external structure of the implant stay in place. One screw was applied in each of the following locations: the left side and right side of the skull above bregma, and the bottom left quadrant, below bregma but above lambda, but not over the location

of the left auditory striatum. Then, a craniotomy for the ground wire was made in the right side of the skull, as far back as possible, and the ground wire hooked in and secured with tissue adhesive. Tissue adhesive was also applied to the skull, except where the final craniotomy was to be made. The tips of the tetrodes in the implant were covered with **DiI**, and the implant aligned over the location for the craniotomy, then moved once the alignment was determined. The craniotomy was then made, all skull fragments cleared from the area, and the dura cut, in order to allow a smooth entrance for the electrodes. The implant was moved back to its position over the surface of the craniotomy and the electrodes lowered 1 millimeter into the brain. Finally, the outer parts of the implant were cemented to the skull, and skull screws, using dental acrylic. After the acrylic dried and the mouse was removed from the stereotax, a cover was taped over the outer part of the implant in order to protect the wires.

Implant

Implants consisted of an EIB (EIB-36-PTB from Neuralynx), eight tetrodes made of 0.007 inch diameter tungsten wire, and a device with which to move the tetrodes. This device was suspended on stainless steel hypodermic tubing. Each tetrode was encased in polyamide tubing, and all eight tetrodes and polyamide tubes were placed within a stainless steel tube, ensuring stability.

Electrophysiology

After recovering from surgery, mice resumed practicing the behavior until their performance returned to at least 60% of attempted trials correct for both choice directions, and at least 600 trials completed per session. Starting three days after

surgery, the implanted tetrodes were moved down into the brain approximately 80 micrometers at a time. Between tetrode movements, sound response tests were conducted. 80micrometer movements were performed up to four times per 24 hours, until sound responsive neurons were detected. The brain does not have pain receptors, so anesthesia is not necessary for tetrode movement once electrodes have been surgically implanted.

During each experimental session, an amplifier/accelerometer headstage, (RHD2132 by Intan technologies with RHD 2000 6-foot SPI interface cable, and RHD 2000 USB interface board), was plugged into the electro-interface board (EIB) of the implant, and the mouse placed into the behavior box with the reward ports covered. A depiction of a mouse connected to this setup can be found in Figure 4.

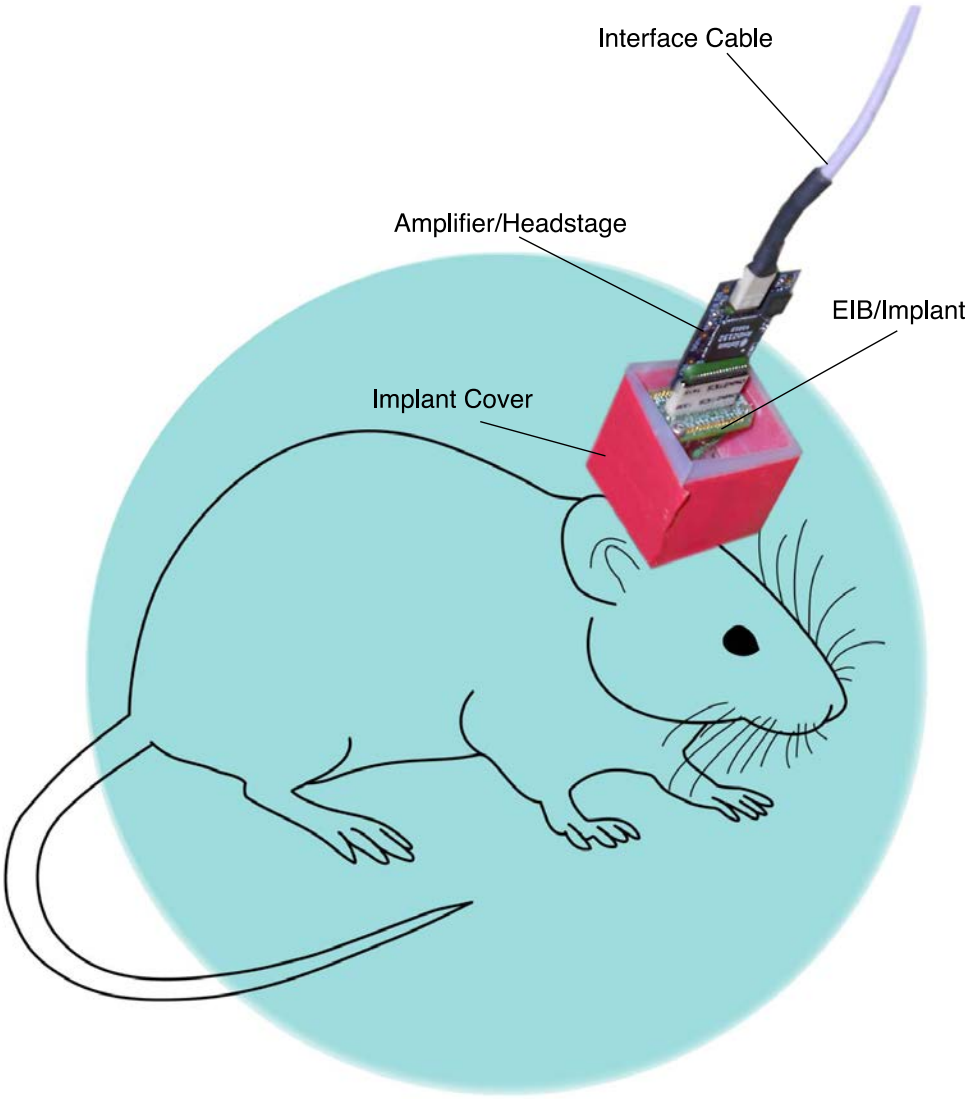


Figure 4: Experimental setup with implanted mouse

The top of the protective implant cover was removed and the headstage plugged into the EIB on the implant at the start of each experimental session.

After plugging the implant in to the rest of the setup, the experimenter looked at a **spike** trace for each tetrode and determined which tetrode should be the reference for comparison of the voltage changes picked up by the other tetrodes. Once a reference

tetrode was selected, the experimenter tested the sound responsiveness and frequency selectiveness of the neurons by recording activity during the presentation of 100 millisecond bursts of white noise, and thirty repetitions each of 16 chords centered between 2 and 40kHz. If neurons showed responses to sound, and frequency selectivity, neural activity was recorded while the mouse performed the behavioral task. The experimenter started a new recording, started the behavioral paradigm, uncovered the reward ports, and closed the acoustic chamber. The mouse was monitored through a video camera throughout each session.

After a successful recording in which the mouse behavior met the minimum criteria mentioned above, tetrodes were advanced about 40 micrometers at a time. Tetrodes were advanced at the end of a behavioral session, approximately 24 hours in advance of the next recording, in order to reduce tetrode movement during recording sessions. This minimized the movement of the tetrodes throughout each recording session. In looking at the clustered spike patterns, it appears that neurons often stayed in the same location relative to the tetrodes on days in between tetrodes movement.

Lesions for Measuring Accuracy of Implantation Surgery

Recording from an animal was typically finished when the signals ceased to be sound responsive, or the animal's behavior was no longer able to be maintained at a level suitable for the study. At the completion of recording for each animal, it was anesthetized with Isoflurane, and electrolytic lesions were created at the end of the longest and shortest electrodes; wires were plugged into a 12V DC source, and the power wire was placed in the animal's mouth, while the ground of the source was touched to the gold pin on the EIB corresponding to the longest and shortest tetrodes.

The mouse was then injected with Ketamine, perfused, and its brain extracted and sliced to be imaged. The slices were checked to see that the tetrodes had been in the target location of the striatum. Due to the nature of the surgery in which the electrodes were placed, it is necessary to complete this kind of verification in order to ascertain the validity of the data collected. In some mice, the electrodes went too far to one side of the target location, so data from those mice cannot be fairly compared with data from mice where data was recorded in the auditory striatum.

Data Collection/Analysis

Software

Electrophysiological recordings were conducted using the Open Ephys GUI ("OpenEphys GUI,") and the custom behavioral paradigm developed within the lab. Within Open Ephys, experimenters selected a reference channel for each session, depending on which electrode appeared to be detecting the least spiking. Experimenters also set thresholds from which to record action potentials, based on the visual representation of the spikes provided in the Open Ephys interface. Thresholds were set which appeared to record the majority of spikes, but excluded as much noise as possible. A bandpass filter from 300-6000Hz was applied during recording, and the sampling rate was 30.0kilosamples/second.

Clustering

The program Klustakwik was used to sort spike data, by calculating certain features of each spike. For each spike, the peak and valley were calculated, and Klustakwik sorted the spikes into 12 clusters, at most. The interspike interval (ISI) was

calculated for each cluster. Clusters sorted using Klustakwik were checked by hand for consistency with patterns characteristic of neural activity. Each cluster was categorized based on the number of spikes, spike shapes, separability of clusters, and firing rate throughout the session. Separate categories were formed for regular and inverted spikes. For each, there was one category including clusters of top quality—number of spikes above 200, consistent spike shapes with each tetrode having a slightly different size, and a relatively constant firing rate. Additional categories were created for clusters with good spike shape, but inconsistent firing rate, or exceptionally low numbers of spikes. Small spikes, of 40microvolts or less were placed in a separate category, as were clusters with an incomprehensible "spike" shape, or other indicators of noise. The determined cluster quality ratings were used to sort data clusters, based on their quality, throughout the process of examining and analyzing the data.

To be included in analysis, neuronal data had to meet minimum criteria for both recording quality and animal behavior during the session: The mouse's behavior during the recording needed to include at least three blocks of 200 trials, and have a minimum of 60% of trials completed correctly for the middle frequency in each block. Each cluster examined had a percentage of ISI violations less than 2%, and was sound responsive. Sound responsive neurons had spike counts that differed significantly from baseline (counted during the period -120ms to -20ms, with sound onset being 0ms) during the time window 0ms-100ms, as indicated by a maximum Z-score with an absolute value greater than 3.0 Significance was calculated using sound response during all valid trials.

Z-scores were calculated using the time bin -0.05 to -0.025 seconds as the base. Then, the maximum from time bins of 0.025 seconds from -0.01 to 0.1 seconds was calculated; 0 seconds is when the sound stimulus was presented for a duration of 0.1 seconds. Only valid trials were included in Z-score calculation, and each different frequency was considered separately.

Results

Though striatal neurons are recognized for their role in auditory discrimination learning (Xiong et al., 2015), and can be driven to influence behavioral choices in auditory tasks via cortical stimulation (Znamenskiy & Zador, 2013), it has been unknown what role striatal neurons play during flexible auditory discrimination behaviors. This study aimed to describe the activity of neurons in the auditory striatum during a flexible discrimination task. We recorded the activity of 123 sound responsive auditory striatal neurons in behaving mice, and compared the patterns of activation when the stimulus changed meaning. Only 15 of the sound responsive neurons showed a significant difference in firing during sound presentation between the different sound meanings.

We were able to train mice to switch between blocks in the flexible categorization task, and maintain their performance following electrode implantation. We detected auditory striatal neurons that respond to sounds and are frequency selective, including some that were selective to the medium frequency (11kHz) of the three sounds in our auditory discrimination task. We found that most auditory striatal neurons did not differ significantly in their activity when the sound stimulus presented was associated with a different action; however, a small number of neurons responded differently to the same sound during sound presentation, depending on action selection. Additionally, some neurons also showed greater differences in activity as mice were making choices (i.e. moving to the right or moving to the left).

Mice switched between blocks in the flexible categorization task

Following surgery, mice were still able to perform the sound categorization behavior at acceptable levels of accuracy. Figure 5, below, shows a few examples of behavioral performance at various lengths of time after implantation surgery.

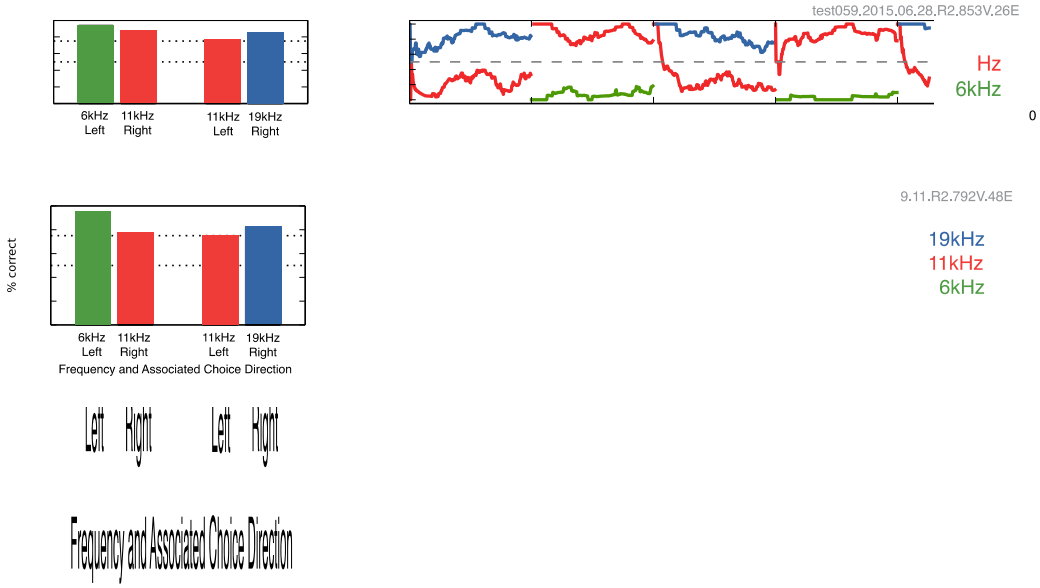


Figure 5: Mice continue to perform flexible sound categorization at acceptable levels of accuracy post-surgery

Mice continued to perform greater than 60% of attempted trials correctly, and to be able to switch between frequency categories throughout the task.

Neurons in the auditory striatum respond to sounds and are frequency selective

Of 724 auditory striatal neurons recorded that fit the quality standards we set, 123 (about 17%) were sound responsive. Many neurons in the auditory striatum respond to sounds with an increase in activity at sound onset. Others show an inhibition response at sound onset. Sound responsive neurons also showed frequency selectivity for varying frequency ranges.

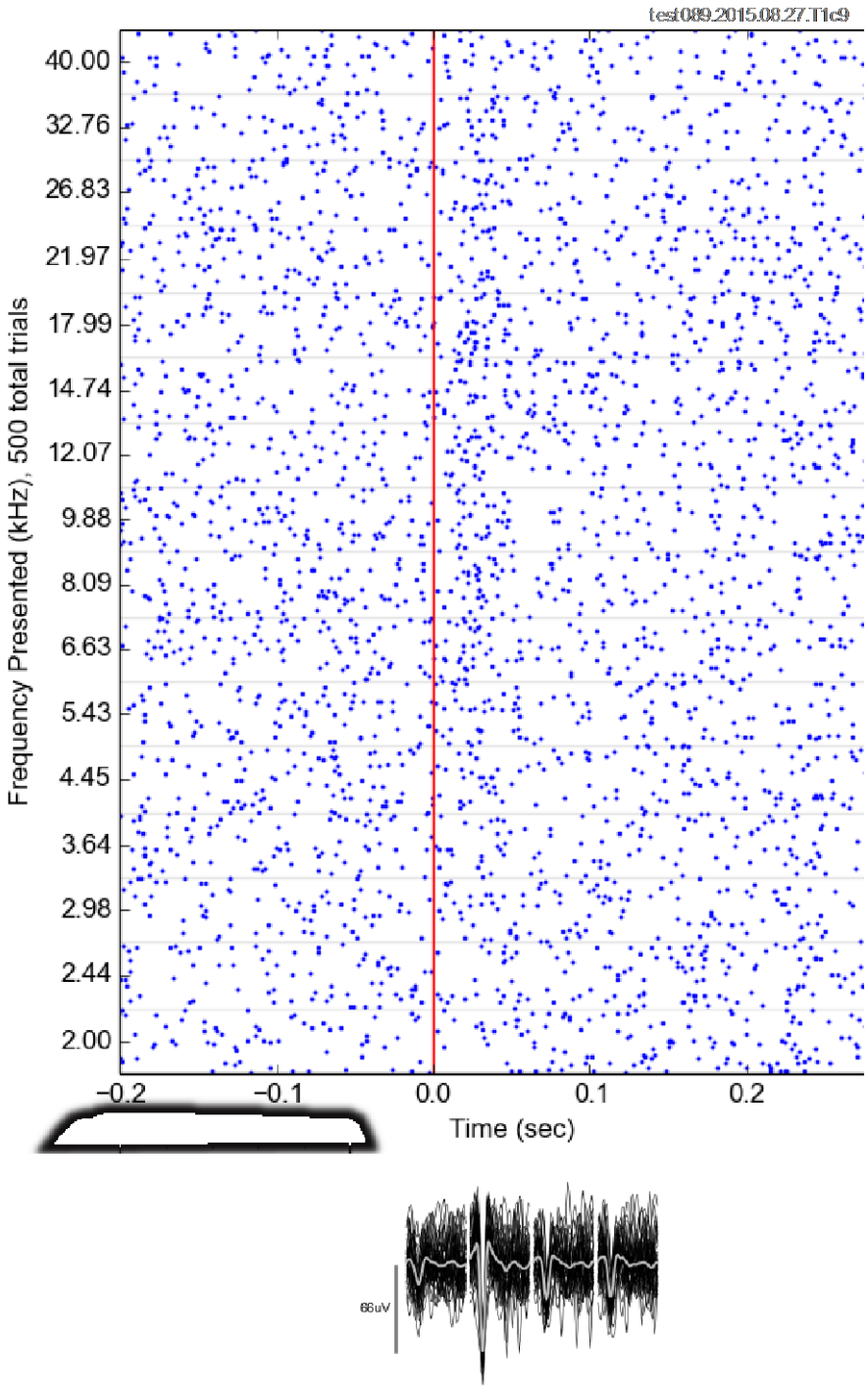


Figure 6: Auditory striatal neurons respond to sounds of various frequencies

Sound presentation is from 0.0-0.1s. Each blue dot represents one action potential. This neuron has frequency selectivity between 6-18kHz, meaning that its firing rate is greater when sounds of those frequencies are presented. The spike shape of the neuron this was recorded from appears beneath the graph.

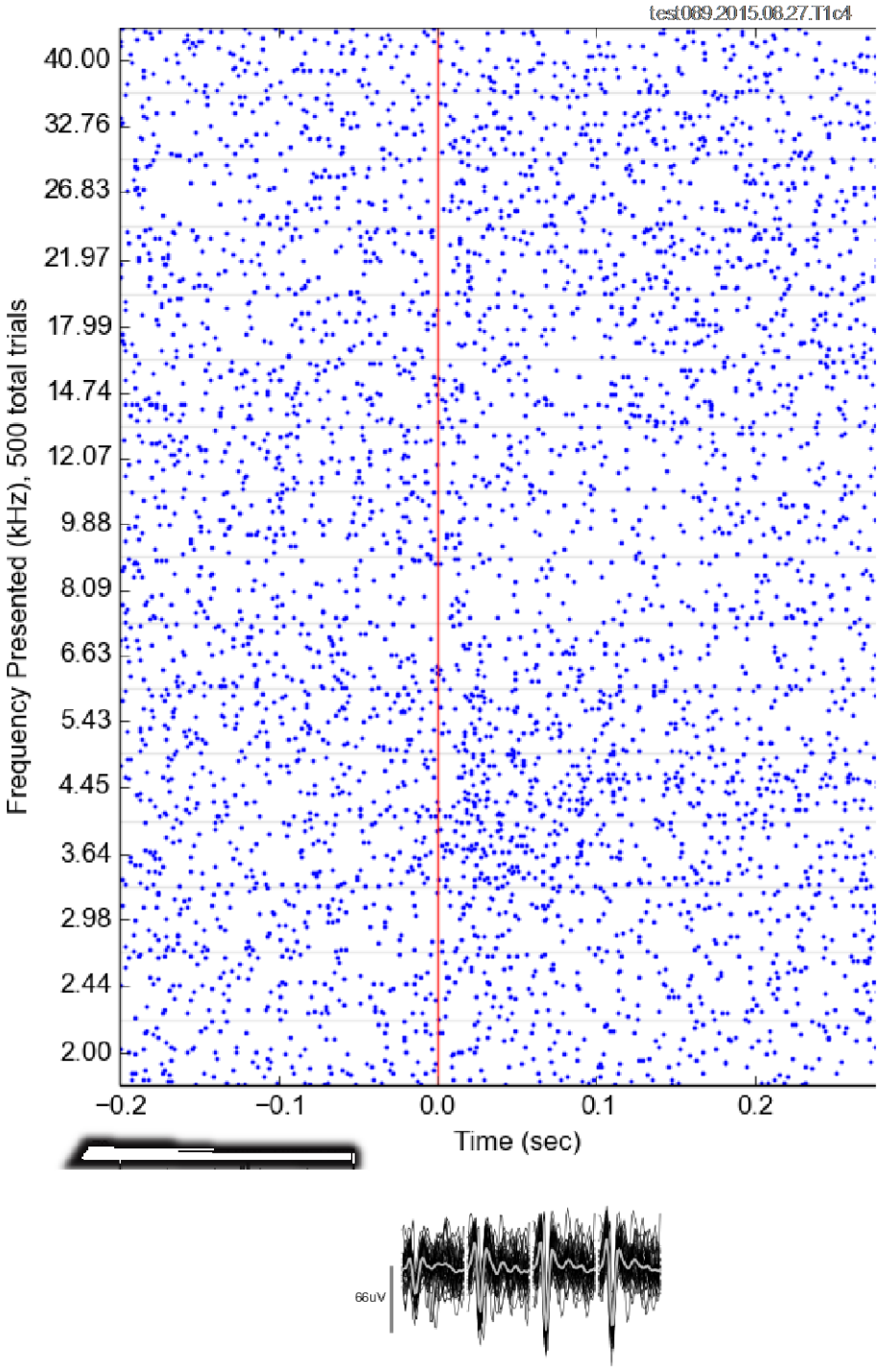


Figure 7: Some auditory striatal neurons are inhibited during sound presentation

Sound presentation is from 0.0-0.1s. This neuron has an inhibition response from about 6-14kHz, and an excitation response between about 3-5 kHz. The spike shape of this neuron appears beneath the graph.

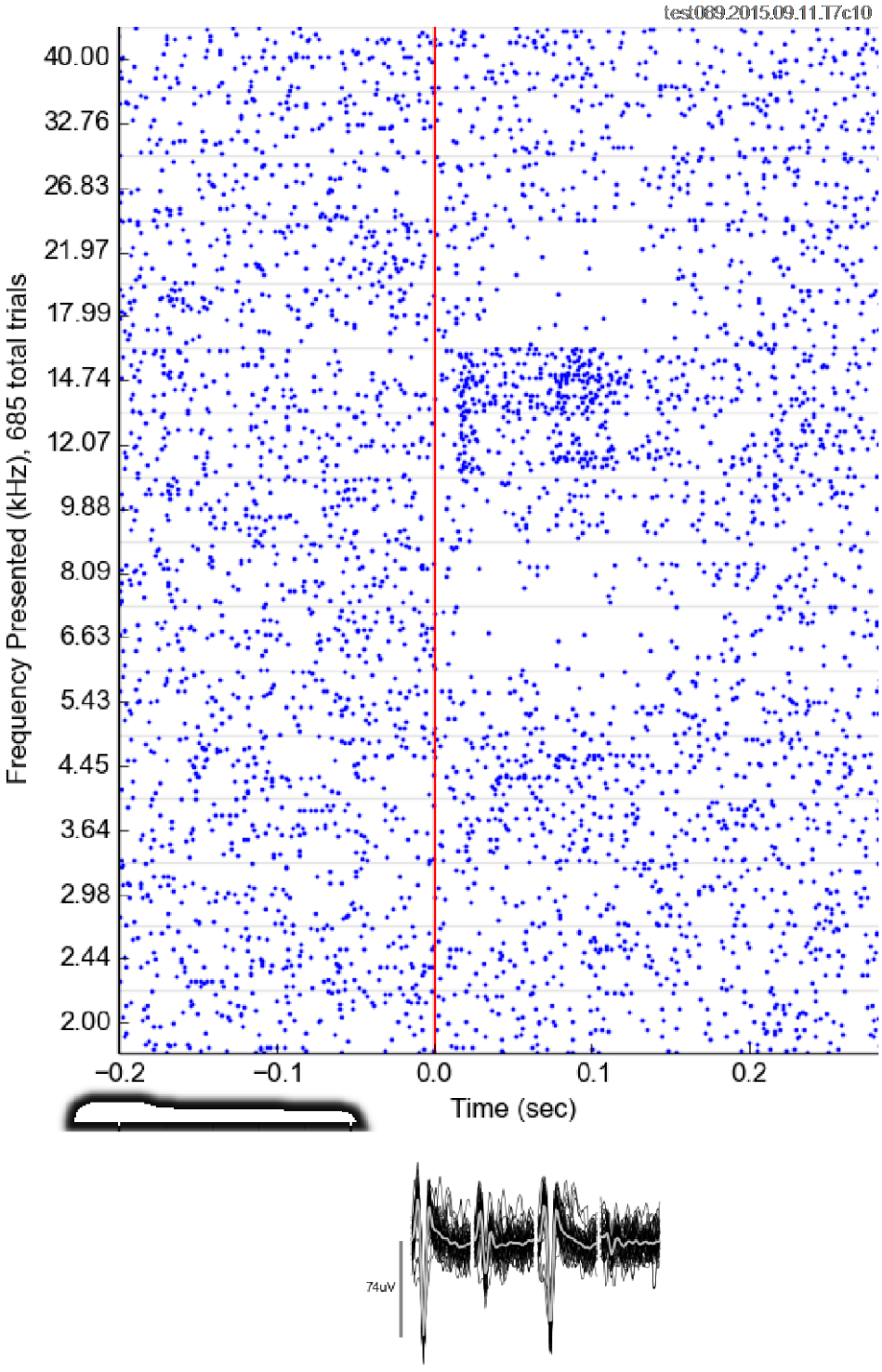


Figure 8: Some auditory striatal neurons have strong sound responses and frequency selectivity at different frequency ranges

Sound presentation is from 0.0-0.1s. This neuron has a strong excitatory response in the 12-14kHz range. Its spike shape is noted beneath the frequency-tuning plot.

Some sound responsive striatal neurons responds differently to the same sound, during sound presentation, depending on sound-action association

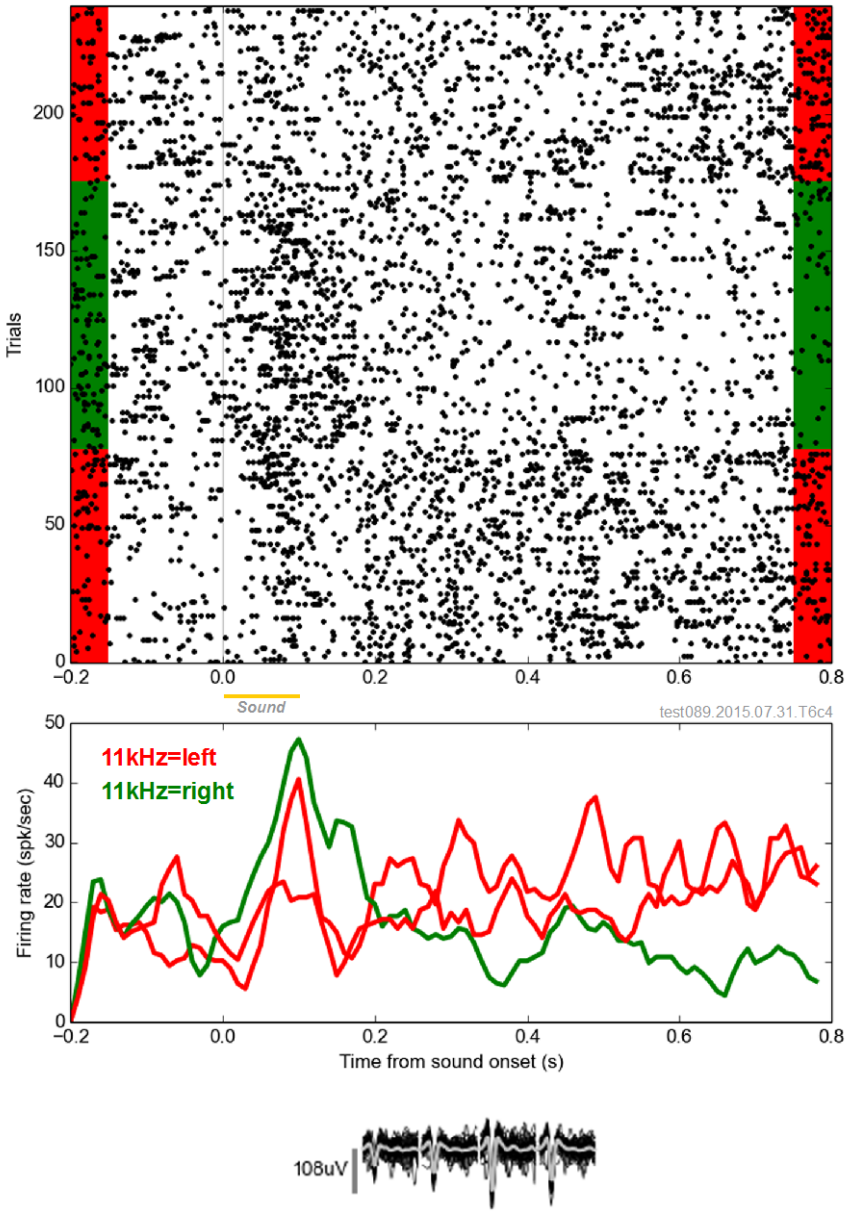


Figure 9: Some auditory striatal neurons respond differently during sound presentation depending on sound-action association

During sound presentation, the firing rate of this neuron for trials in which the rewarded direction for an 11kHz sound was right is significantly greater than for trials in which the rewarded direction was left.

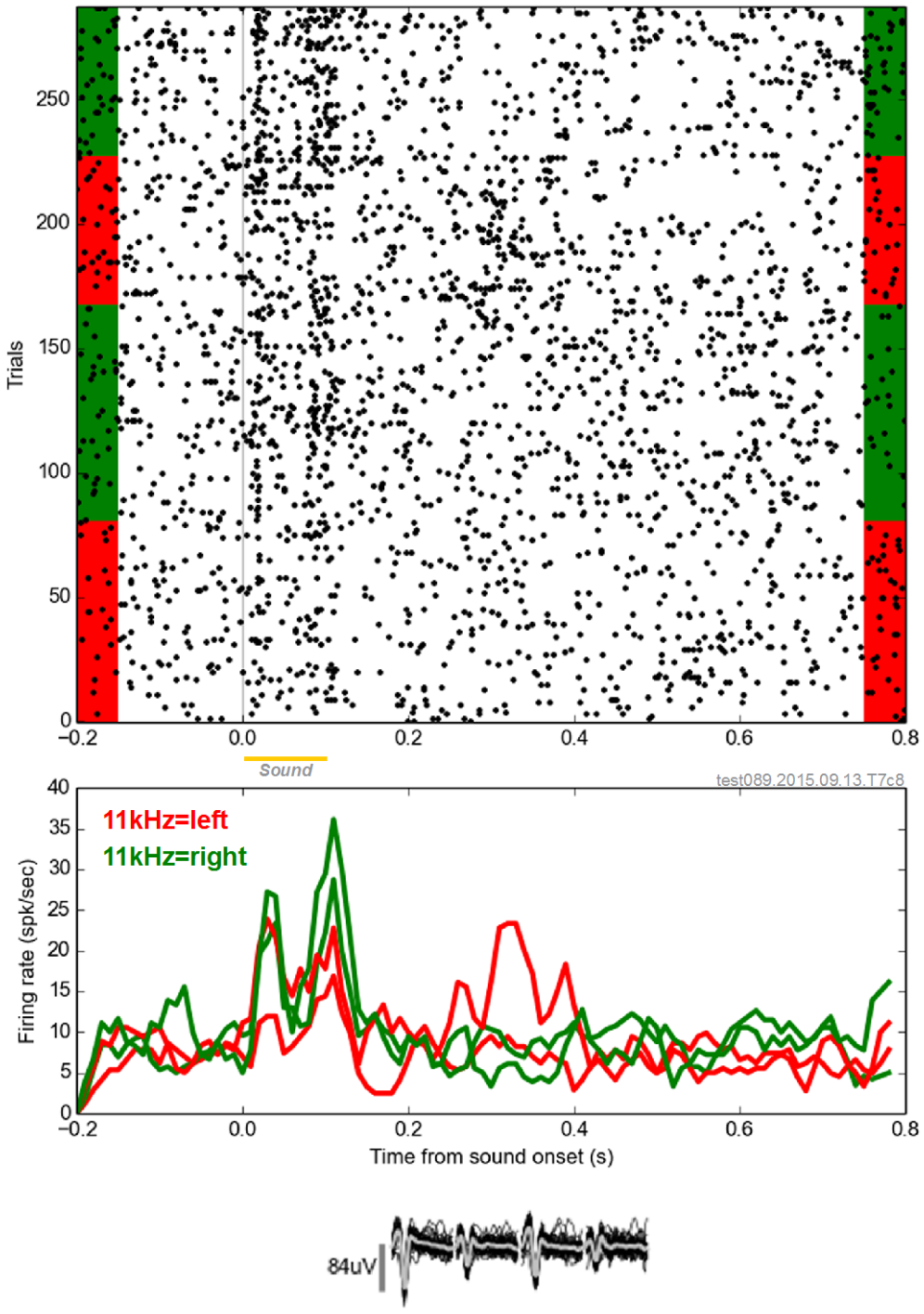


Figure 10: Some auditory striatal neurons respond differently during sound presentation depending on sound-action association

During the second part of the sound response, this neuron responded more to an 11kHz sound when the rewarded action associated with it was movement to the right.

Most sound responsive striatal neurons show no significant difference in firing during sound presentation when sound-action associations are different

We found that the activity of 15 out of 123 sound responsive neurons measured (12.2%) was significantly modulated by change in sound meaning (Figure 11).

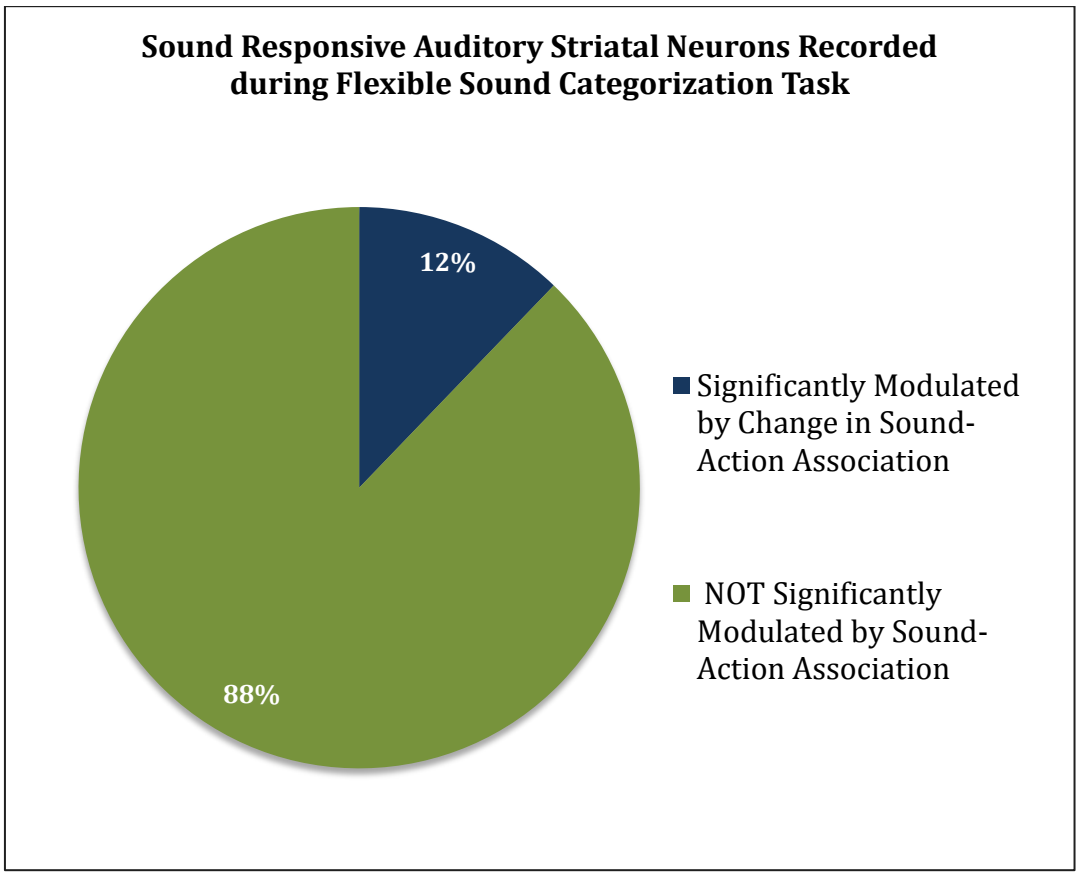


Figure 11: 12% of auditory striatal neurons have activity modulated by differences in sound-action association during sound presentation

Neurons with significant modulation are represented in blue; others are represented with green.

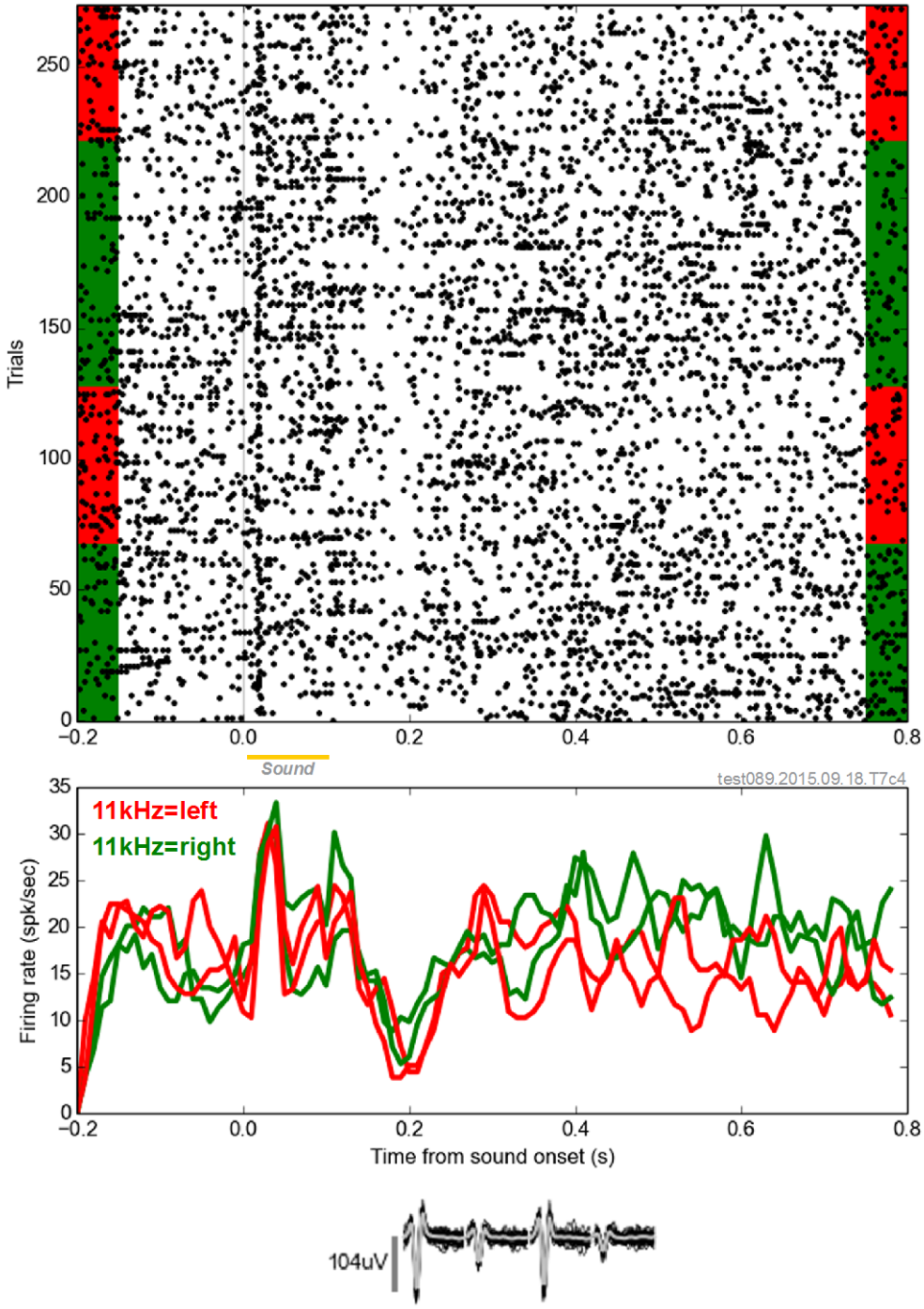


Figure 12: Sound responsive neurons often fire similarly during sound presentation, regardless of sound-action association

This neuron responds strongly at the beginning of the sound presentation, for trials in which 11kHz means to go to the right for reward, and when it means to go to the left.

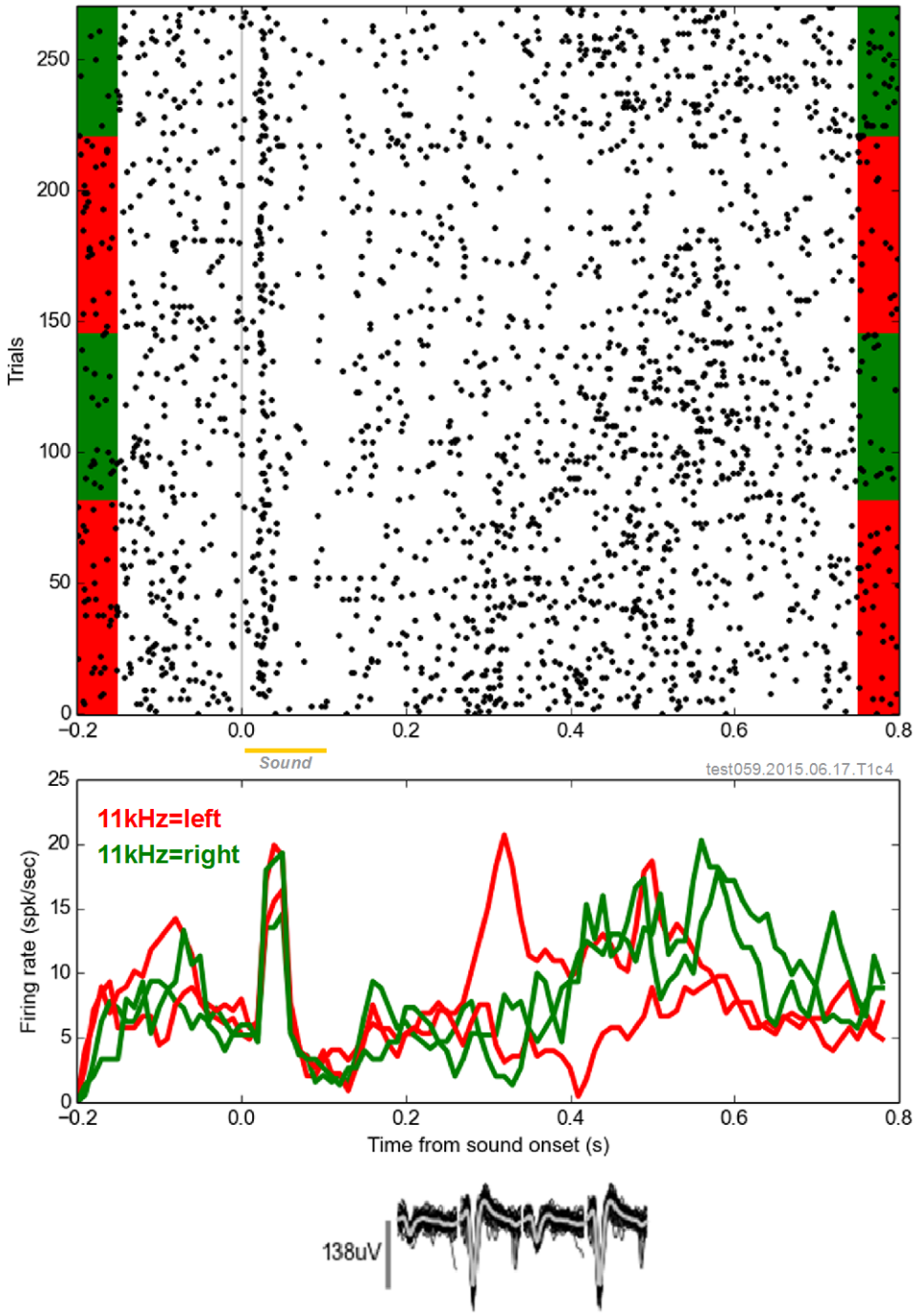


Figure 13: Sound responsive neuron firing similarly during sound presentation, regardless of sound-action association

This neuron also responds strongly at the beginning of the sound presentation in both sound-action associations. Its firing throughout the rest of each trial is not significantly different between the two conditions.

Some sound responsive striatal neurons have differences in activity after sound presentation when mice moved to the right or left

We analyzed data from sound responsive neurons during the 100-millisecond period immediately after sound presentation, and found that the activity of 32.5% of these neurons was significantly modulated by change in sound meaning (see Figure 14).

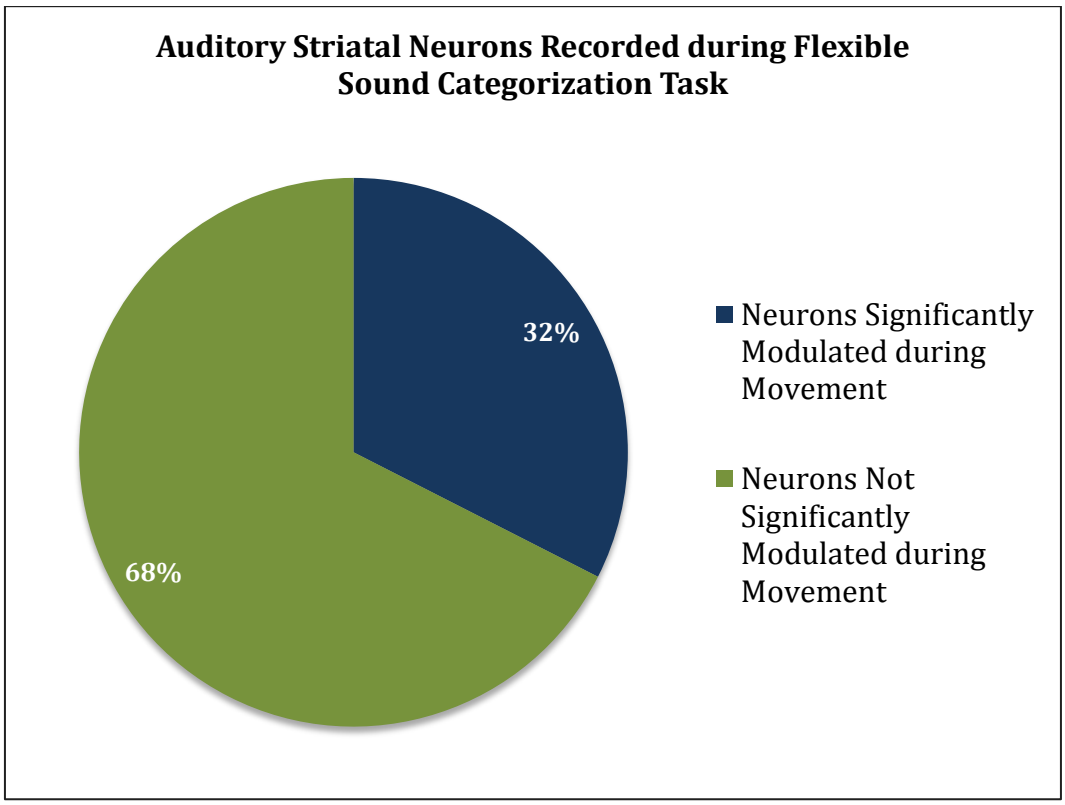


Figure 14: 32.5% of sound responsive striatal neurons recorded had activity modulated by movement direction during the period 100 milliseconds after sound presentation.

In this chart, neurons significantly modulated by movement direction during the period 100 milliseconds after sound presentation are represented by blue, and neurons without significant modulation are represented by green.

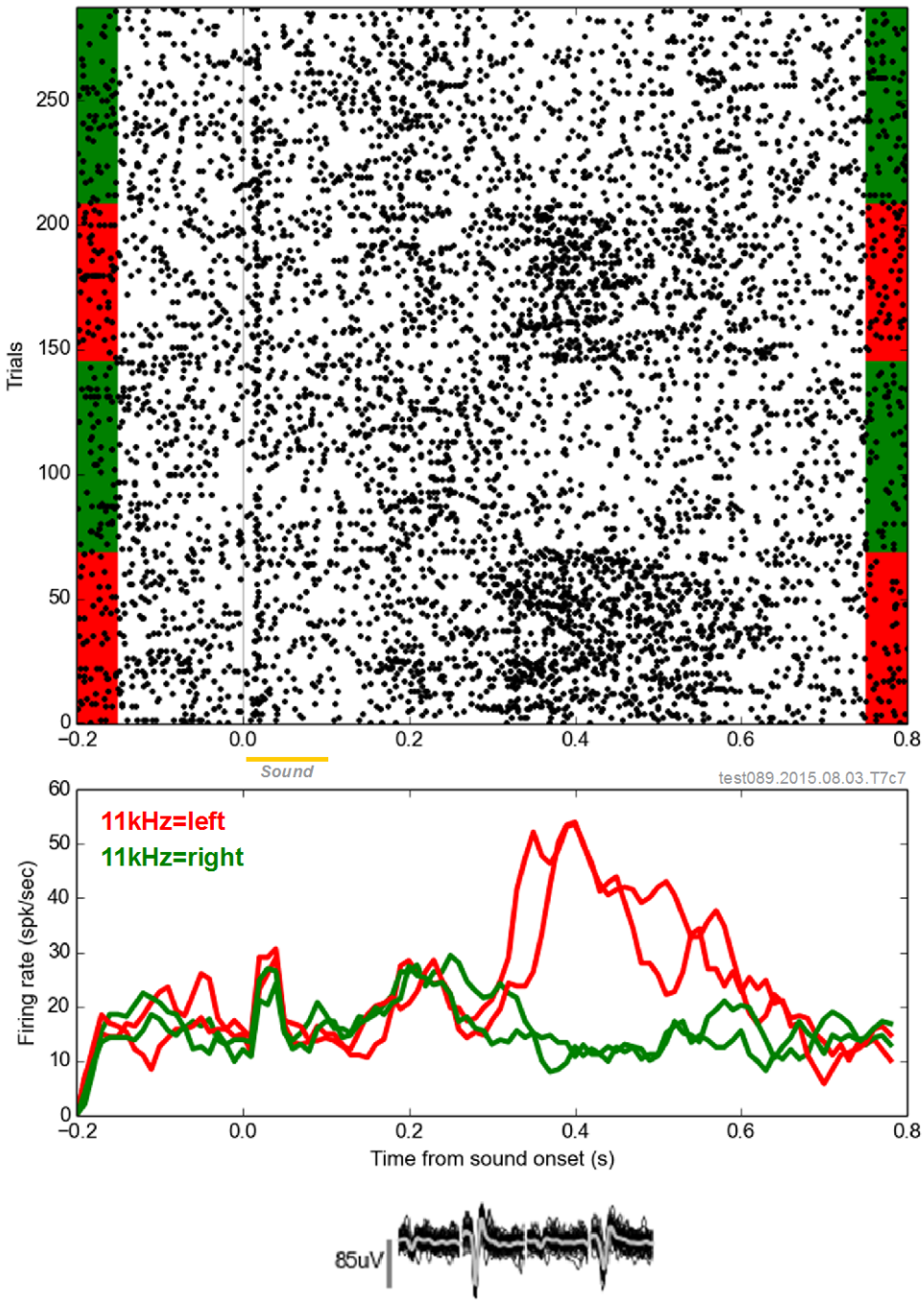


Figure 15: Some sound responsive striatal neurons have differences in activity after sound presentation when mice moved to the right or left

This neuron does not respond differently between the different conditions during the sound presentation; however, afterward, in the 0.3-0.6 second range, its response is greater for trials in which the mouse moved to the left.

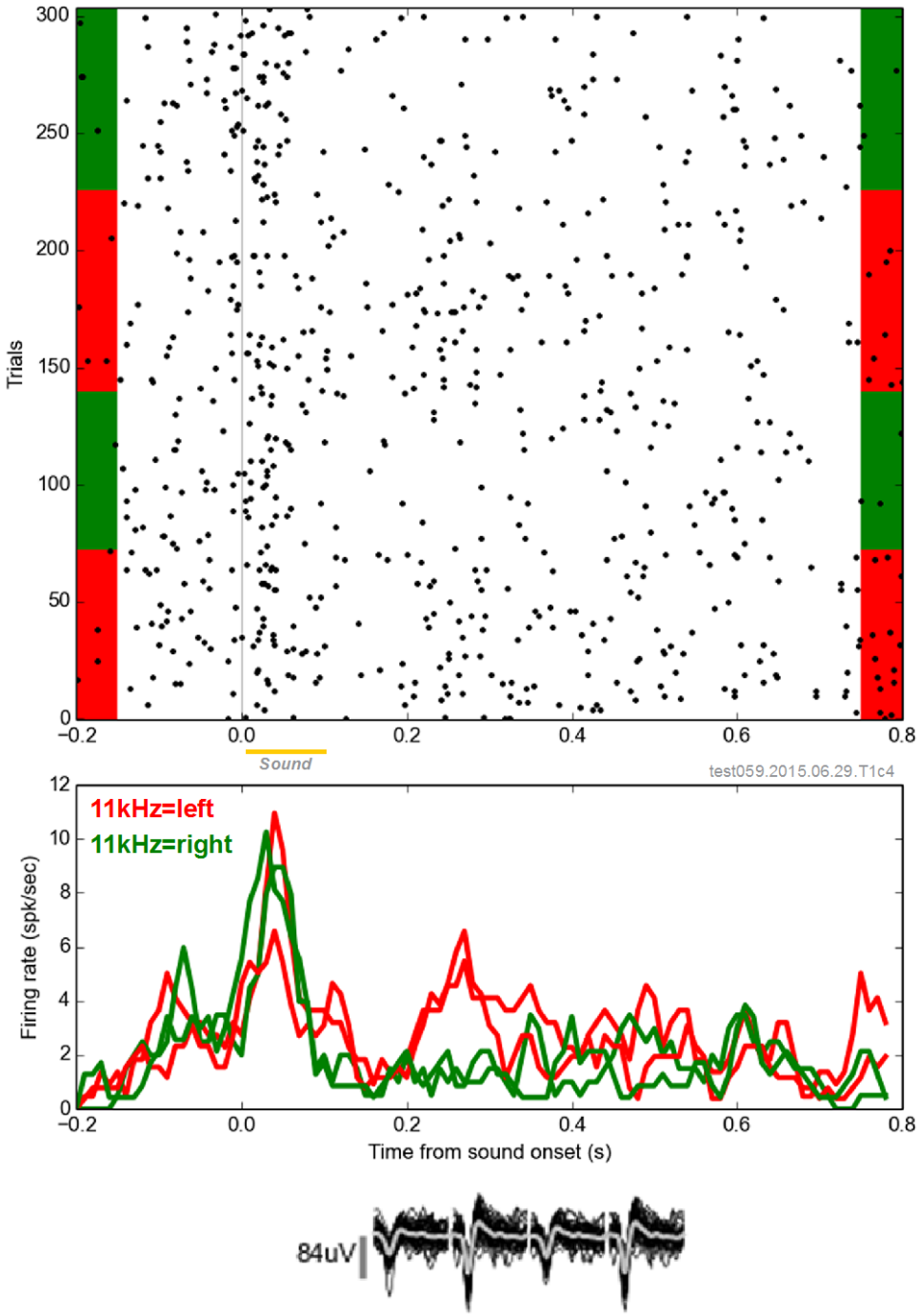


Figure 16: Some sound responsive striatal neurons have differences in activity after sound presentation depending on whether mice moved to the right or left

This neuron has similar firing during the sound presentation for both sound-action associations, but fires more during the 0.2-0.3s period when the mouse would have been moving to one side or the other.

Some neurons with weak sound response, or no sound response, have differences in activity after sound presentation when mice moved to the right or left

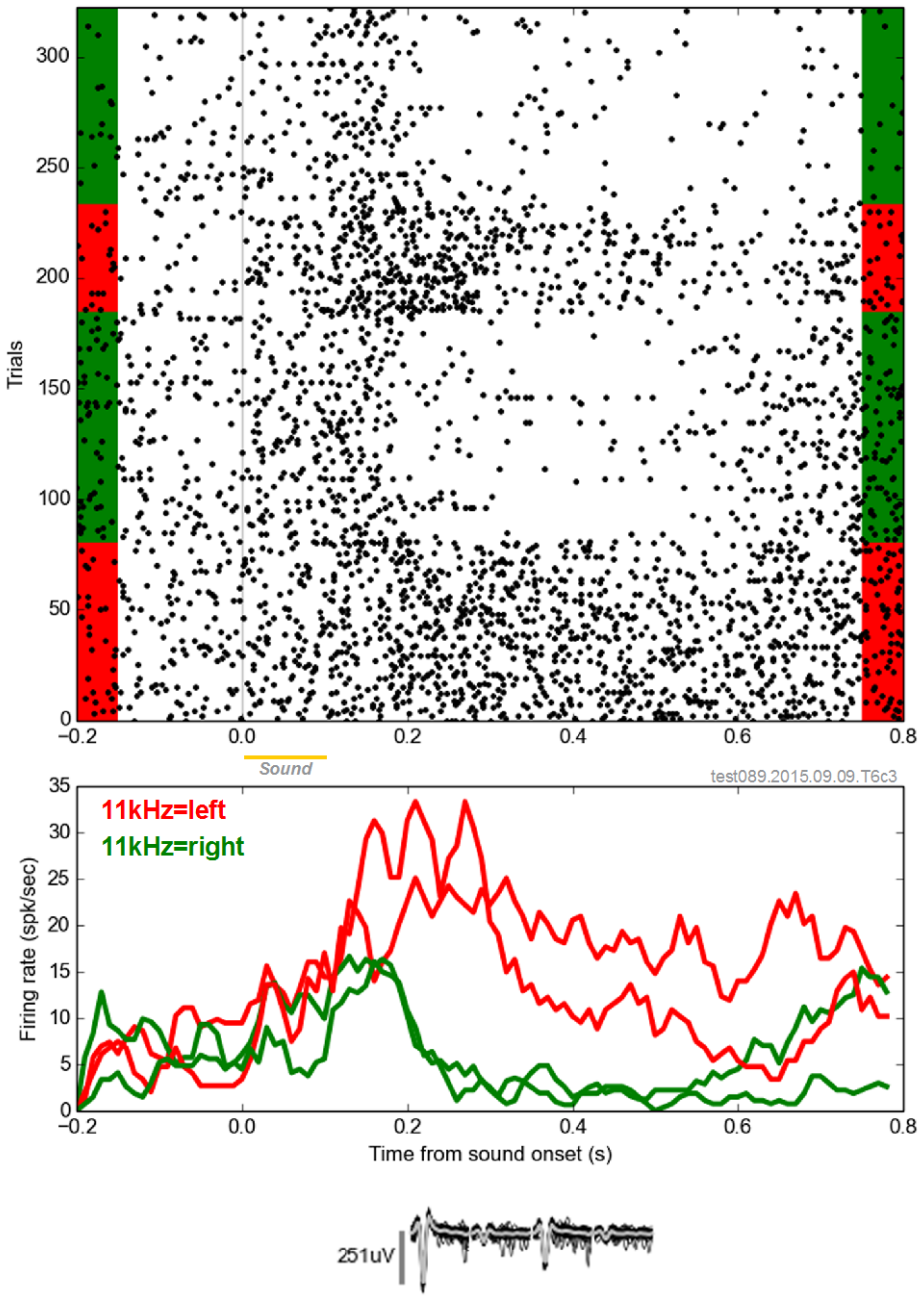


Figure 17: Auditory striatal neuron with modulation after sound presentation.

After sound presentation, this neuron fired more during trials in which the mouse moved to the left than those in which it moved to the right.

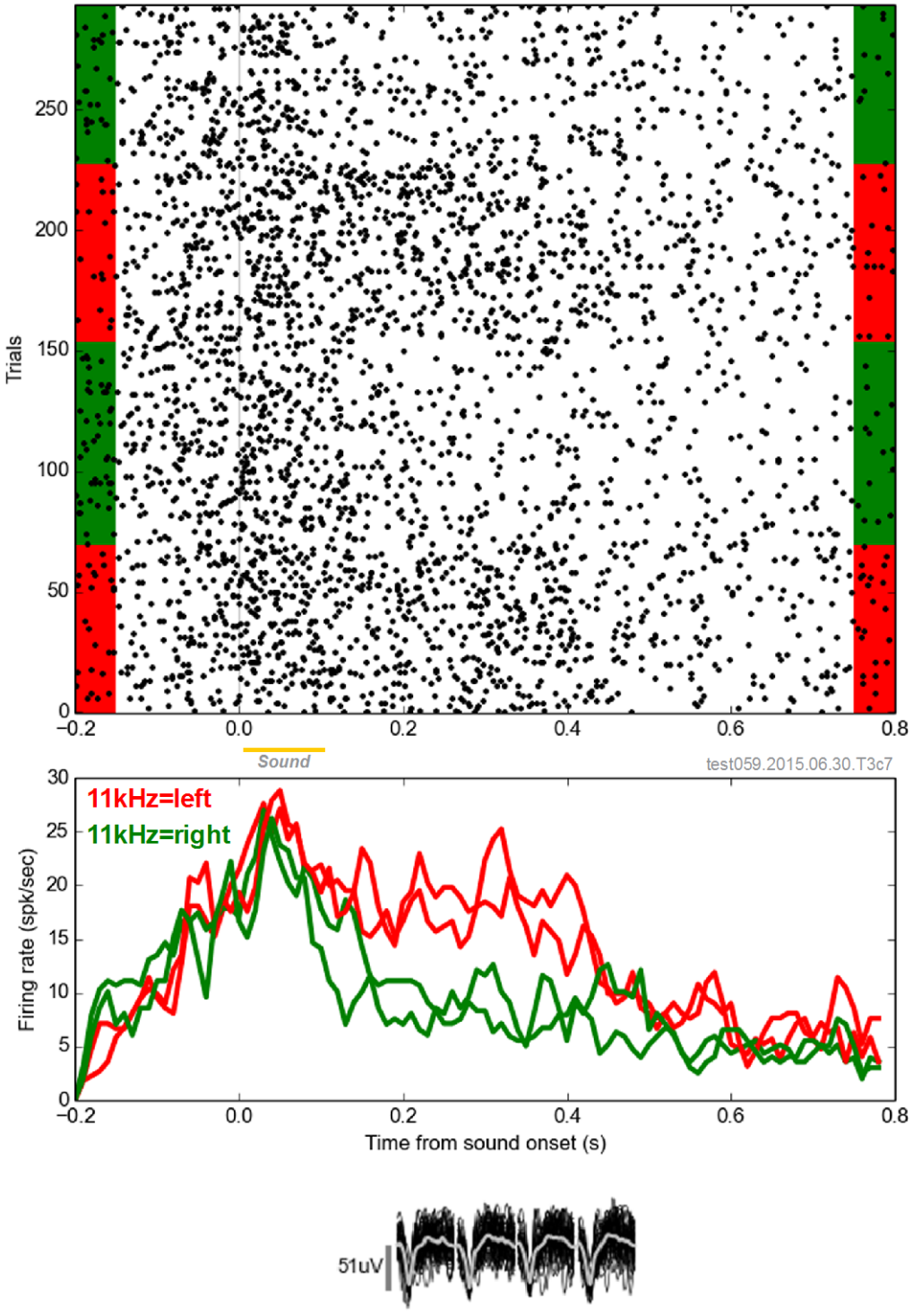


Figure 18: Auditory striatal neuron with modulation after sound presentation

After the sound ended, this neuron fired more when the mouse was moving to the left than when it moved to the right.

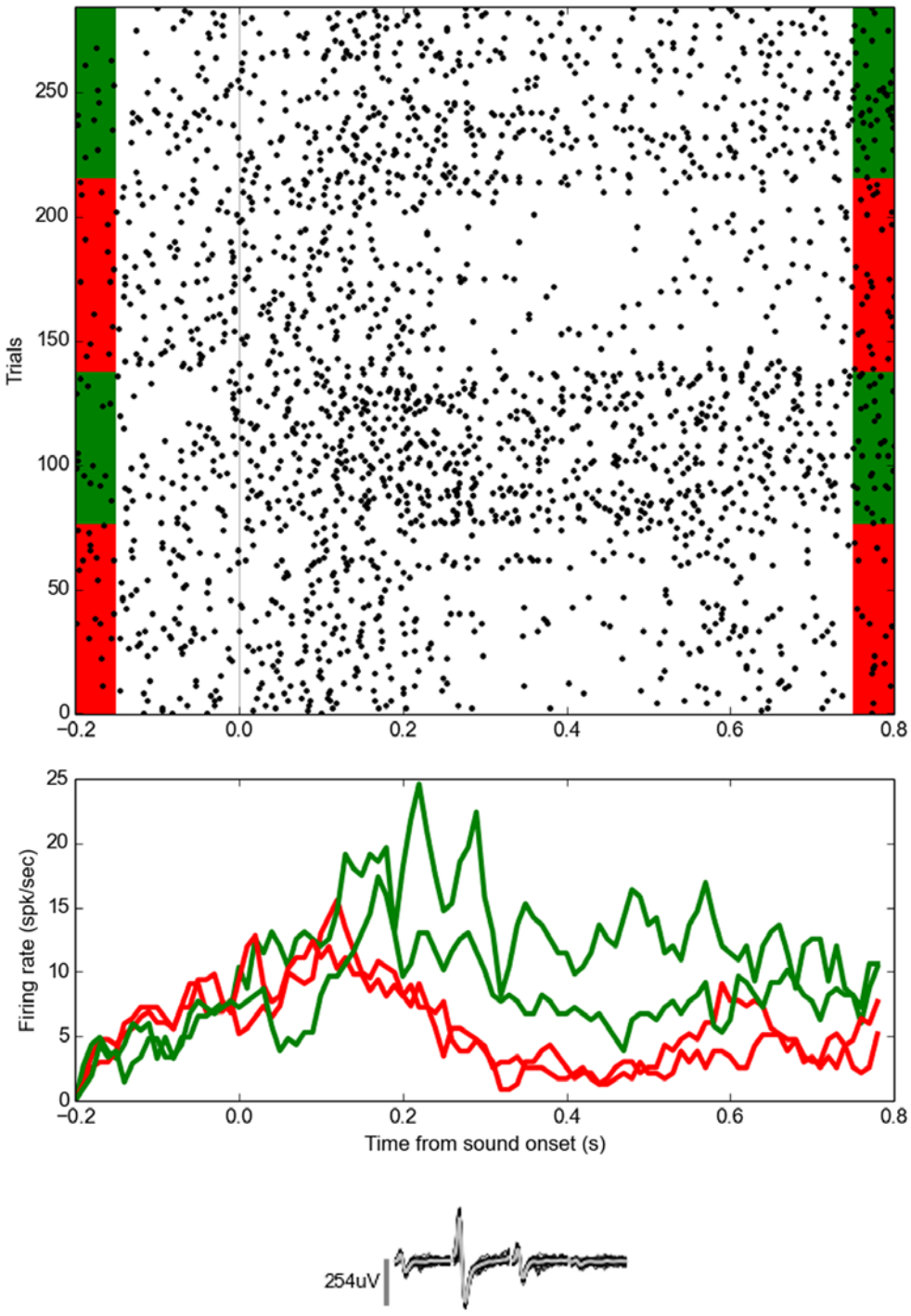


Figure 19: Auditory striatal neuron with modulation after sound presentation.

Some striatal neurons have activity that is modulated by differences in movement direction. For this neuron, after the sound ends, activity for left trials is greater than for right trials.

Discussion

The key aims of this research were to understand whether the auditory striatum is involved in processing information about changes in sound meaning, and to gain a framework for further investigation of dorsal striatum function. To investigate the role of the auditory striatum in mediating flexible associations between sounds and learned behavioral responses, we examined the activity of auditory striatal neurons in male C57BL/6 mice, via chronically implanted electrodes, while they performed a sound categorization task in which one of the sounds periodically changed meaning. We compared the average firing rates of neurons in the auditory striatum under different contextual conditions, and found that, during sound presentation, few neurons in this population produce activity that is modulated by difference in sound-action associations.

Our recordings show that some neurons in the auditory striatum are sound responsive, as well as frequency selective. In the future, others in the lab will look for trends in the sound responsiveness and frequency tuning of auditory striatal neurons. Since the auditory inputs to the striatum are organized by sound frequency, it is expected that the striatum also organizes sounds by frequency. One possibility is that different depths from the brain surface, or different points on some other spatial plane, correspond to different frequency ranges.

12.2% of sound responsive neurons recorded showed significant modulation according to sound-action association (Figure 11). This suggests that the activity of some neurons in the dorsal striatum represent not only the acoustic features of a stimulus but also its meaning. However, since these neurons comprise only a small

portion of the auditory striatal population, it is likely that their role in mediating changes in sound-action associations is limited.

Many of the neurons we recorded from had activity changes correlated with movement, rather than with sound presentation. Modulation scores were calculated $\left(\frac{\text{firing rate for right trials} - \text{firing rate for left trials}}{\text{total firing rate}}\right)$ for activity differences during the time when mice would have moved to make a choice (Figure 14). 32.5% of neurons had activity significantly modulated with respect to choice direction. In the future, characteristics of the neurons recorded in these experiments can be examined for patterns in their spike shapes and firing rates. If there were a correlation between spike shape, or firing rate, and modulation during sound presentation or movement, it could contribute to identification of the types of neurons being modulated.

One factor that limits these conclusions is that the majority of data collected came from a single mouse. Recordings were conducted in four mice, but technical issues, including electrodes missing the area of interest during surgery, electrical problems during recordings, and declining behavioral performance in older mice, contributed to very few usable data points coming from two of the mice. In order to be more certain in our determination of the percentages of auditory striatal neurons modulated during this behavior, it will be necessary to gather data from at least one more mouse. Additional data will also improve our ability to determine what patterns exist in the frequency selectivity and spike shapes of neurons in the auditory striatum.

Currently, other lab members are working on experiments that continue to address the role of the auditory striatum in flexible decision-making. In one set of experiments, the auditory striatum is temporarily inactivated by injection of muscimol,

and behavior during a sound categorization task is observed. Preliminary results suggest that temporary inactivation of the auditory striatum, using muscimol, does impair discrimination between the same kinds of sounds used in this study. Another, similar experiment would be to lesion auditory striatum permanently, and observe how behavior during the flexible frequency discrimination task is affected; however, temporary inactivation through pharmacological methods like muscimol injection allows experimenters to rule out the possibility that the brain is building new connections, or repurposing connections to reroute information to compensate for the loss of the lesioned area.

Since the striatum has been related to reward, the lab is also investigating whether activity in the auditory striatum during sound presentation is modulated by the amount of reward associated with the sound or its associated location. Preliminary data is showing that in the period of 100 milliseconds after the sound ends, about 15% of sound responsive neurons have differences in activity depending on reward amount. In the future, more data will be collected during behavior with changes in reward, and it will be analyzed during different time periods after the sound presentation.

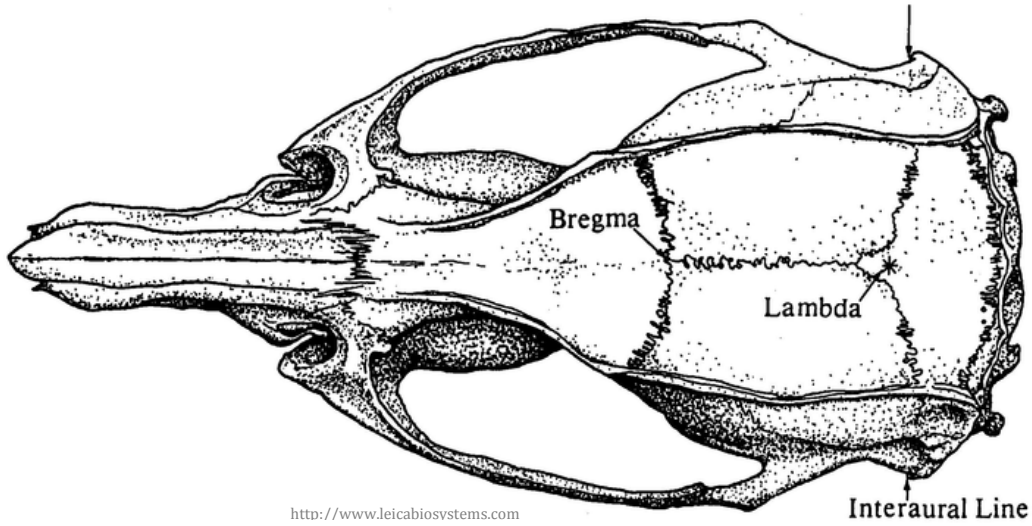
Continued improvement of the understanding of the neural mechanisms behind behavioral flexibility can be achieved in the future by investigating the potential role of other brain regions in mediating flexible associations. A likely target, aside from the auditory striatum, would be the amygdala, as it also receives input from the thalamus. It may also make sense to investigate outputs of the auditory cortex as well.

Glossary

Action potential (spike): a mechanism for transmitting information throughout the brain through electrical impulses, which can be measured as changes in electrical potential (voltage) that occur in the membrane of neurons as information passes from one neuron to the next.

Auditory discrimination: Differentiating between sounds or doing an action that shows one can differentiate between two sounds.

Brain landmarks (bregma/lambda): points of reference used in stereotactic surgery in order to approximate the location of a brain region that is a target of some procedure (in this case, electrode implantation).



DiI: (pronounced: dye-eye) a fluorescent dye used for staining brain tissue. In these experiments, electrodes were coated with DiI before implantation, so that they would stain the parts of the brain they passed through as they were lowered throughout experimental sessions. After the mouse is dead and its brain has been processed for imaging, this allows us to visualize whether the electrodes were in the auditory striatum throughout the study.

Firing rate: action potentials occurring over a given time unit (usually seconds).

Lesion: to damage or remove part of the brain in order to determine its function**.** If a behavioral or cognitive task can not be done when the brain area has been lesioned, then that area must be necessary for the function under investigation.

Stimulus: Something that causes a specific reaction. For these experiments, the stimuli are the sounds mice are presented with during the auditory discrimination task.

Tetrode: Four pieces of tungsten wire twisted together and heated slightly so that the outer coating of the wire melts and the group of wires keeps its shape. Using tetrodes is one way that we can group spike data according to the neurons they came from. Different neurons will be slightly different distances away from each wire in the tetrode, and signals that are further away will be weaker (lower voltage). Cutting the 8 tetrodes in an implant so that each is a slightly different length also helps us determine which signals can be attributed to the same neurons. The ability to differentiate between neurons is important for making conclusions about the population of neurons that we are examining. It allows us to come up with percentage counts of neurons in the area of interest that have different types of activity.

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