EFFECT OF REWARD SIZE ON THE ACTIVITY OF AUDITORY CORTICAL NEURONS

by

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A THESIS

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Santiago Jaramillo, PhD

The neural pathways that allow the brain to select the actions an animal should take in response to a sound in order to get a reward are not well understood. Previous studies have demonstrated that a region of the brain called the auditory striatum (AStr) receives information about sounds from the auditory cortex (AC) and uses this information to help drive actions. Additionally, the striatum may incorporate information about reward into decision-making. Recent work in our lab indicates that neurons in the AStr fire differently in response to a sound when the sound is paired with a large reward versus a small reward. These data suggest that the striatum may be integrating information about sound and reward size in a way that could support sound-action association learning. The primary aim of this study was to determine if neurons in the AC fire differently in response to a sound when the sound is paired with a large reward versus a small reward. These data will ultimately be used to discern whether modulation of sound responses by amount of reward arises in the striatum, or if it is already present in the inputs to this region arriving from the AC. To investigate the role of the AC in incorporating information about reward size during decision-making, we examined the activity of AC neurons in male C57BL/6J mice, via chronically implanted

electrodes, while the mice performed an auditory reward-change task in which the same sound and same action was paired with different amounts of reward.

Our recordings show that many neurons in the AC respond to sounds and are selective to sound frequency. We compared the average firing rate of AC neurons under conditions in which the same sound was presented and the mice traveled in the same direction, but received different amounts of reward. We found that 7.5% of sound responsive AC neurons were modulated by the amount of reward during the decision-making task. However, the majority of these neurons showed no significant difference in activity between the varied reward conditions. In addition, we found a number of neurons in the AC that responded to movement rather than sound, 21.8% of which were modulated by reward size. Together, our results suggest that the AC may play a role in incorporating information about reward size into auditory based decision-making, but likely it is not the sole brain region to do so.

Acknowledgements

I would like to thank my lab Principal Investigator and Primary Thesis Advisor Professor Santiago Jaramillo for giving me the opportunity to be apart of his lab, and for all of his guidance in not only the thesis process, but throughout my participation in lab activities.

I would also like to acknowledge the additional members of my thesis committee: Second Reader Nick Ponvert, for guiding me through a number of technical difficulties during my time in the lab, for assisting with edits for a fellowship proposal as well as my thesis, and for being a friend; and Clark Honors College Representative Professor Helen Southworth, for helping me refine my research and writing skills, and for being brave enough to accept a position on my committee despite being a professor of literature.

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Introduction

Decision-making is a fundamental component of human life. From choosing what time to wake up in the morning to what to eat for dinner in the evening, we make decisions of various magnitudes practically every moment of our lives. Many of these decisions appear to be made without hesitation, but in reality an assortment of factors underlie the decision-making process. One factor that can play a crucial role in decisions is incentive (Farrell, Goh, & White, 2014). An incentive is broadly defined as a thing that motivates or encourages one to do something. Incentives can be established with rewards. For example, money is a common reward used to motivate people to do something like show up to work on time. Not only do rewards provide incentive, they also have the potential to alter decisions.

Imagine a parent who is trying to feed their child broccoli, which the child does not want to eat. With no success, the parent offers the child a reward of dessert if the broccoli is eaten. Despite no changes in the food or the child's food preferences, the child now chooses to eat the broccoli because of the reward. While it is clear that reward influences choices, the mechanisms by which the brain takes reward size into account when making decisions are not well understood.

Although at times decision-making may seem spontaneous, with each decision there is an applicable cost-benefit analysis regarding potential outcomes. Our brain gathers information from sensory stimuli in our surroundings and compares it with variables like past experience, context, and reward to weigh possible conclusions and ultimately make a decision that results in an action.

For example, imagine it's 10pm and you are outside on your porch when you hear a loud boom. How you react to that boom depends on the circumstances. If it were July 4th, you'd likely attribute the sound to a nearby firework and not think twice about it. On the other hand, if you hear the same boom on an evening in August, you might decide to return inside your house, or even investigate the sound due to concern for your safety.

In another scenario, picture a mouse that sits in a nook at the edge of an alley eyeing a piece of cheese in the alley. They mouse is prepared to retrieve the cheese when it hears a cat's meow off in the distance. The mouse must determine whether the reward of the cheese is worth the risk of being caught by a cat. Here the mouse uses external stimuli (the sight of the cheese and the meow) as well as information about reward size (size of the cheese) to help make a decision. For humans and mice alike, the brain's capacity to rapidly integrate these factors and formulate a decision allows us to take the most appropriate action in a given situation.

As researchers, we want to better understand the neural basis of decision-making by analyzing the activity of neurons. An animal model provides the most expedient opportunity to record the activity of individual neurons in the brain. By conducting experiments while monitoring the behavior of single neurons in animals, we can learn more about the mechanisms of the decision-making pathway. Neurons are cells within an animal's nervous system that are specialized to transmit information. A neuron fires an electrical impulse, known as an action potential, or a spike, which triggers either an electrical or chemical signal that is sent to another cell, forming connections throughout

the body. The number of action potentials per unit time is known as a neuron's firing rate, which represents the activity of a neuron.

Sound provides a simple external stimulus for scientists to manipulate when analyzing neural mechanisms of animals. By associating sounds with meaning, animals can be tasked with making choices depending on the sounds they hear. To further characterize the neural pathways implicated in decision-making, we recorded the activity of individual neurons in the brains of mice when the animals were making choices dependent upon sounds that had different meanings.

The auditory striatum and auditory cortex likely facilitate reward dependent decision- making in auditory decisions

The neural pathways by which the brain uses sound information to guide action are well characterized, but the integration of information about reward size in these auditory pathways when decisions are reward-dependent is not well understood. Sound travels from an exterior source to the animal's ear, where it is converted from vibrations to electrical signals that ultimately arrive at the brain region called the auditory cortex (AC). After reaching the AC, auditory information is relayed to a number of brain regions that help trigger a motor response to the sound. Previous research has shown that a brain region informally known as the auditory striatum (AStr)(Figure 1), uses information received from the AC to help drive behavioral choices of mice during auditory-discrimination tasks (Znamenskiy & Zador, 2013).

Other studies have reported that the striatum mediates specific action–outcome associations and the selection of actions on the basis of expected reward value (Balleine, Delgado, & Hikosaka, 2007). Furthermore, experiments in monkeys have

found that neurons in the AC have varied responses to different reward feedback that is used to motivate monkeys to perform auditory-categorization tasks (Brosch, Selezneva, & Scheich, 2011). Together these findings suggest the AC and AStr play a role in integrating sound and reward information that is used to facilitate proper motor outputs. Further research is necessary to better understand how reward size is incorporated into the auditory pathway during decision-making.

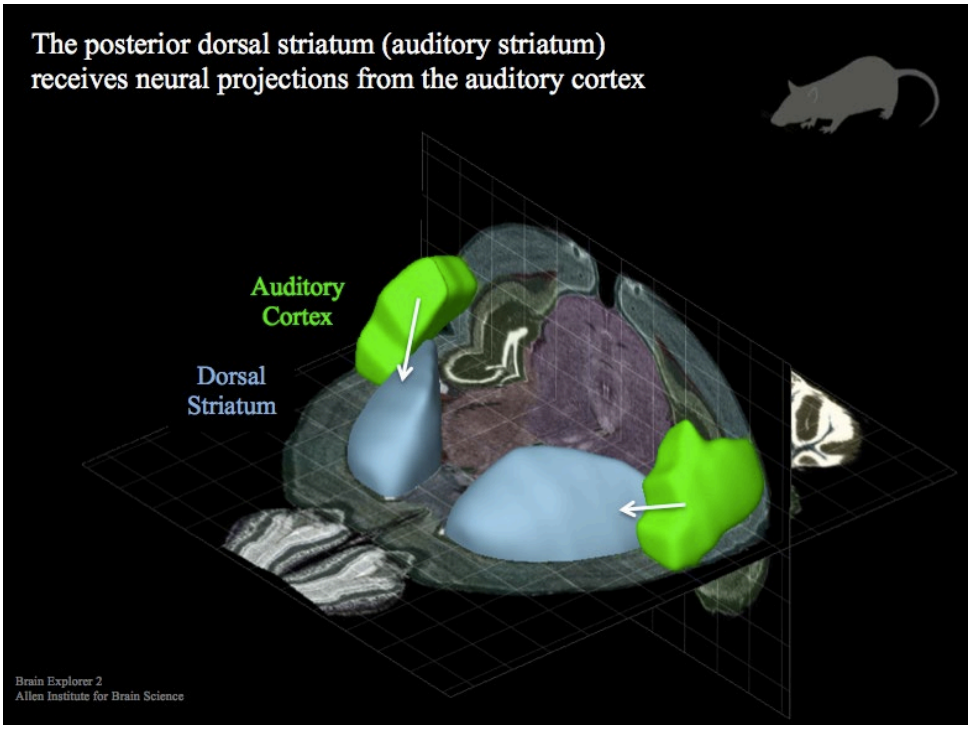
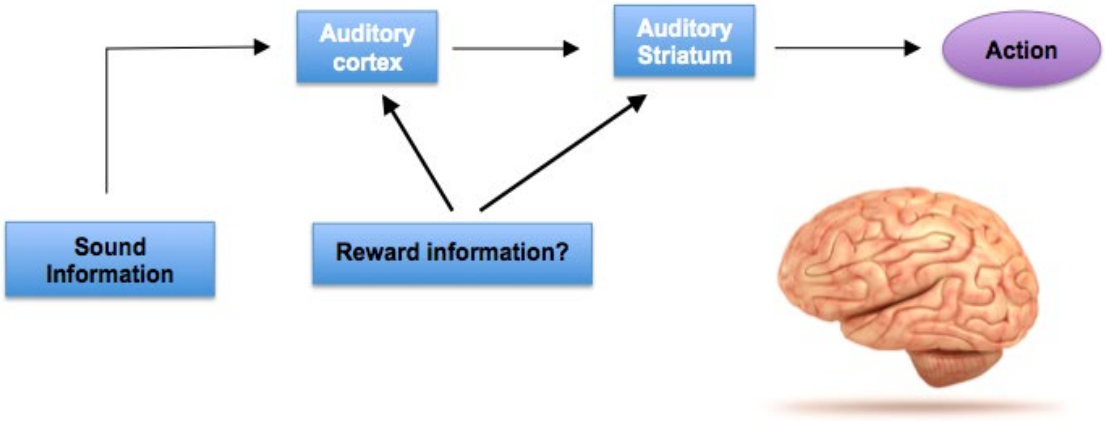


Figure 1: The auditory cortex projects to the posterior tail of the dorsal striatum in the auditory pathway

This model circuit depicts how information about sound travels through the brain. Sound information from the outside world travels through the ears where it is transduced into electrical signals, which are eventually transmitted from the auditory cortex (green) to the posterior tail of the dorsal striatum (blue), also known as the auditory striatum, which facilitates motor output.

Recent work in our lab indicates that some neurons in the AStr fire differently in response to a sound when the sound is paired with a large reward versus a small reward (Guo et. al., Manuscript submitted for publication). These data suggest that the AStr



may be integrating information about sound and reward size in a way that could support sound-action association learning. However, it is important to determine whether the observed alterations in neuronal activity due to reward changes arises in the AStr, or if it is already present in the inputs to this region arriving from the AC (Figure 2).

Figure 2: The auditory cortex and auditory striatum may incorporate information about rewards during decision-making

This model illustrates the proposed pathway of sound information and reward information when decisions about sound lead to action. Sound information from the outside world travels through the ears where it is transduced into electrical signals, which are eventually transmitted from the auditory cortex to the auditory striatum, which facilitates motor output. When making decisions about reward that is prompted by sound, reward information may be integrated with sound information at the auditory cortex, or auditory striatum, to ultimately influence the chosen motor output.

It is well known that the activity of some AC neurons changes in response to sounds that are associated with a reward. However, it is unknown if the activity of AC neurons changes when the same sound is associated with different size rewards. The

mechanisms by which the AC may integrate sound and reward information to discriminate between different reward associations with the same sound can be investigated by analyzing changes in AC neuronal activity as the brain processes knowledge about sound and reward. This research used mice as a model organism. We explored the possibility that the AC mediates the integration of sound and reward information by using surgically implanted electrodes to record the activity of individual neurons in the AC as mice performed a reward-change task with auditory stimuli. We compared the firing rates of AC neurons between two conditions: one in which the animals received a large reward in response to a sound, and one in which the animals received a small reward in response to the same sound. We hypothesized that some AC neurons would be modulated by a change in reward, meaning they show a significant change in firing activity, depending on the reward associated with the sound with which the mouse was presented.

Project Development and Research

This project was conducted under the direction of Professor Santiago Jaramillo in the Jaramillo lab at the University of Oregon's Institute of Neuroscience. I began training mice to do behavior tasks for various lab experiments in September of 2015. I was introduced to this project in January 2017 by Stacy Levichev, a graduate student who started the project during her rotation in the Jaramillo Lab. Over the course of the winter I became familiar with data collection for the experiment as Stacy and I recorded neural activity from two mice. I took over the project in March of 2017 and proceeded to record data from four additional mice through the spring and summer. I controlled parameters for the tasks run by the mice, recorded neural activity and documented

observed neural information, conducted histology of the mouse brains, and assisted with data analysis. I collaborated with Lan Guo, PhD on most of this project. Lan did the implant surgeries on all of the mice, as well as final data analysis, and assisted with numerous technical difficulties throughout the project.

Methods

Experimental Approach

The goal of this project was to determine the extent to which neurons in the AC change their activity during sound presentation when a different reward is associated with the same sound and the same action. We recorded electrical signals, extracellularly, from individual neurons in the AC of mice while they performed a sound-characterization task. In the task, each sound was associated with one action, but the meaning of the sound changed periodically throughout the task to represent different amounts of reward. We compared the firing rates of AC neurons both during and after sound presentation when the sound-reward association was different. We hypothesized that when mice were presented with sounds during a reward-change task, the firing rate of some neurons in the AC would differ depending on the meaning of the sound at that time. We found some neurons that changed firing rate in regards to change in sound, some neurons that changed firing rate in regards to reward, and some neurons that changed firing rate in regards to action. However, the majority of sound responsive neurons we recorded did not show a significant change in activity during the task when the same sound was paired with different reward.

Mouse Model

To best explore how animals make decisions based on sound and reward information, it was necessary to find a model organism. In this study we used six adult male wild-type (C57BL/6J) mice. Because of the ease of maintaining and breeding mice in the laboratory, their physiological similarity to humans, and the availability of many

genetically identical strains, mice have long served as models of human biology and disease (Perlman, 2016). Studies have also highlighted many genetic homologies between mice and humans (Chinwalla et al., 2002). These, together with the ability to create transgenic, knockout, and knockin mice, have provided robust incentives to use mice as model organisms for neural analysis.

The use of male mice controlled for fluctuations in female behavior as a result of reproductive cycles. The animals had continual access to food, but water was restricted, only being administered during experiments. Free water was provided on days with no experimental sessions. After each experiment, it was ensured that the mice obtained a healthy amount of water. All animal procedures were overseen by the University of Oregon Animal Care and Use Committee.

Behavioral Paradigm

We first trained water-restricted mice to perform a frequency-discrimination behavioral task, where they learned to associate sound with reward (Figure 3).

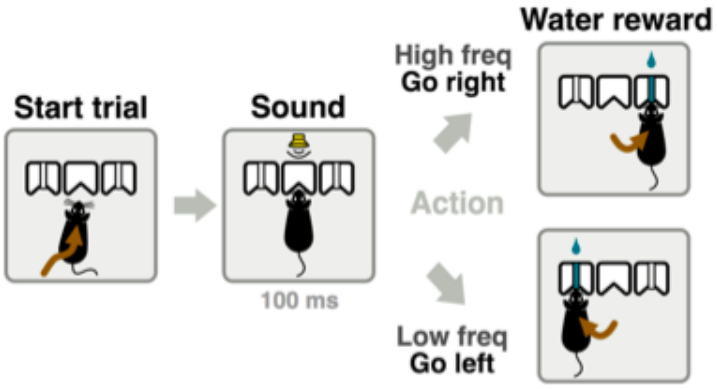


Figure 3: Mice performed an auditory frequency-discrimination task

Male C57BL/6J mice were trained to do an auditory frequency-discrimination task in which they initiated a trial by poking in the center port to trigger a sound. The mice then poked in a reward port to the right or left of the center port, depending on whether they believed the sound to be of high or low frequency, respectively. Mice received a water reward if they went to the correct port, according to the sound that had been played.

In this task, mice were placed in a box with three adjacent ports. A mouse initiated a trial by poking its nose in the center port and waiting for between 150-200 milliseconds until a sound was presented. Each sound was played for 100 milliseconds. In the most simple behavior task, the mice heard either a high frequency (19.2 kHz) or low frequency sound (6.2 kHz). A high frequency sound meant the mice must go to the right port to receive a water reward of 2.25 microliters, while a low frequency sound meant the mice must go to the left port to obtain the same amount of reward. No reward was issued if the mice traveled to the incorrect port. Following a choice, or if no choice was made within 4 seconds, a new trial could be initiated. Each session was one hour in length.

During training and testing periods, food was readily available, while mouse water intake was restricted. The animals were examined and weighed daily to ensure

they remained adequately hydrated, and their health was monitored daily by the veterinary staff of the UO Animal Care Facility.

After mice learned to perform the frequency-discrimination task, we modified the task such that the amount of reward available at the left or the right reward ports changed either every 150 or 200 trials. Each of these sets of trials constituted one block. For example, in some experiments with 200 trials per block, for the first block the mice received 4.9 microliters of water on trials where they traveled correctly to the right compared to 1.25 microliters of water on trials they traveled correctly to the left. Then for the second block of 200 trials, the amount of reward was switched and the mice received more water on trials where they traveled to the left compared to the right. The third block would return to the same reward setup as the first block. Under such conditions, within two adjacent blocks the mice experienced trials where they heard the same sound and traveled correctly in the same direction, but received different amounts of reward (Figure 4).

After mice learned to perform the reward-change task, we modified the parameters of the task to include eight different frequencies, ranging from 6.2 kHz to 19.2 kHz. Doing so confirmed the behavioral context of changing the amount of reward mice received during the task. After mice could perform the reward-change task with an accuracy of 60% correct and complete at least 600 attempted trials per session, they received an implant with eight tetrodes to the right AC to explore these neural implications.

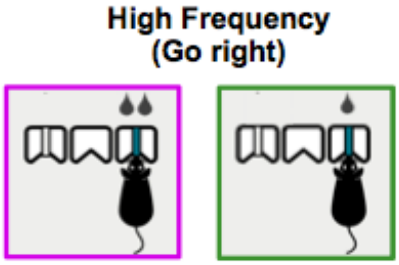


Figure 4: Mice performed an auditory reward-change task

After learning a frequency-discrimination task, the male C57BL/6J mice were trained to do an auditory reward-change task where the amount of reward varied during the task. Mice initiated a trial by poking in the center port to trigger a sound. The mice then poked in a reward port to the right or left of the center port, depending on whether they believed the sound to be of high or low frequency, respectively. Mice received a water reward if they went to the correct port, according to the sound that had been played. For a set block of trials determined by the experimenter, mice received more reward on one side port compared to the other. In the proceeding block of trials, the mice received more reward at the opposite port.

Mouse Training Timeline

Over the course of this study, we recorded data from six mice. All of these mice underwent initial frequency-discrimination training and reward-change training prior to surgical implantation of tetrodes to record neural activity. The average mouse required a minimum of three weeks of training before meeting our criteria for surgical implantation. The average age of mice at the start of data collection was 232.67 days (n=6). Data was collected from mice for an average of 39.17 days with a range from 28-57 days (n=6).

Surgical Procedure

Lan Guo, PhD conducted all tetrode implantation surgeries for this study. Determining the location for implantation of the tetrodes into the brain was accomplished using a coordinate system based landmarks on the skull of the mouse.

During surgery, the anesthetized mice were placed in a stereotax device for stability. For each animal, the skull was exposed and the mouse's head angled in the stereotax so that the lambda and bregma landmarks were level in a horizontal plane. The location for the craniotomy above the right AC was calculated using the following measurements: 2.8mm from bregma in the anterior-posterior plane, 4.5mm right in the medial-lateral plane, and +0.5mm in the dorsal-ventral plane (Figure 5).

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

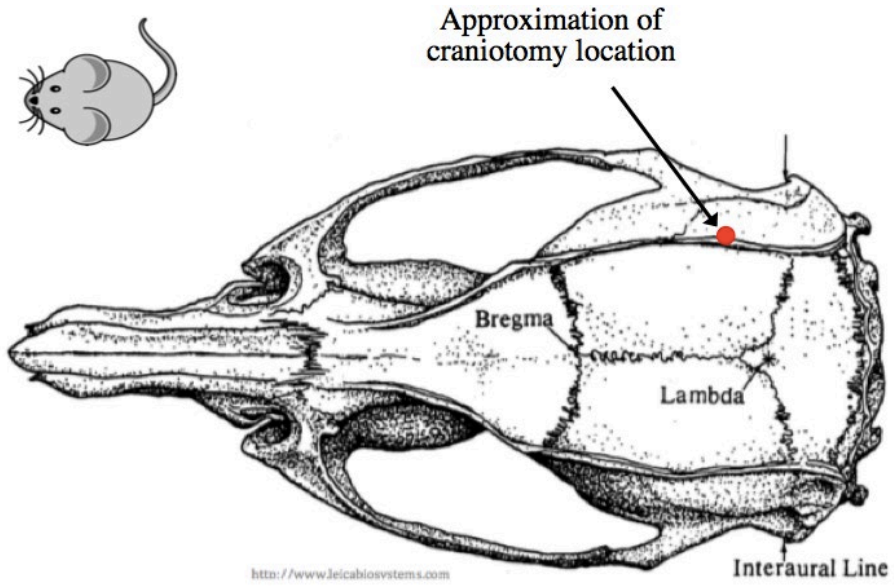


Figure 5: The bregma and lambda skull landmarks were used to determine craniotomy location for implanting tetrodes into the right AC of male C57BL/6J mice

The figure above shows the approximate location of the craniotomy in regards to the lambda and bregma skull landmarks when implanting electrodes into the right AC of mice. Previously determined coordinates based on the positions of lambda and bregma were used to locate the region of the skull covering the auditory cortex. The craniotomies of all six mice used the same set of coordinates. Each mouse had a distinct skull size, so the location of the craniotomy was subject to slight variation between animals.

Following screw placement, a small craniotomy for the ground wire was made as far back as possible in the right side of the skull, and the ground wire was inserted and secured with tissue adhesive. The same adhesive was also applied around the skull, except where the primary craniotomy was to be made. The tips of the tetrodes in the implant were covered with DiI, a fluorescent dye used for staining brain tissue to later aid with histological analysis. The craniotomy was then made in the marked location, all of the skull fragments were cleared, and the dura layers were scraped away to allow smooth entrance of the tetrodes. The implant was aligned with the tetrodes positioned

over the craniotomy, and the tetrodes were lowered 0.5 millimeters into the brain. Outer parts of the implant were cemented to the skull and skull screws with dental acrylic. After the acrylic dried and the mouse was removed from the stereotax, a plastic cover was taped over the outer part of the implant in order to protect the device.

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. Each tetrode varied slightly in length, and was composed of four electrodes. The device to move the tetrodes was suspended on stainless steel hypodermic tubing, and contained a screw that controlled vertical movement of the tetrodes. 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 were reintroduced first to the frequency-discrimination task until they could perform with an accuracy of 60% correct and complete at least 600 attempted trials per session. Upon reaching this benchmark, the mice transitioned to the reward-change task. The first 2-3 sessions of reward-change tasks featured no data collection as the mice were getting re-accustomed to the task. Prior to each behavior 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 (Figure 6), and the mice were placed into the soundproof behavior box with the reward

ports covered to prevent access. The doors to the behavior box were closed and the electrophysiology program was started to initiate a spike trace, or real time graphical measurement of recorded electrical activity, for each tetrode (Figure 7).

The electrode with the least recorded neuronal activity and electrical noise was set as a reference for comparison of the voltage changes picked up by the other tetrodes. Once the reference was established, the sound responsiveness of neurons was tested by recording neural activity during the presentation of 100 millisecond bursts of white noise. These noisebursts were played for 100 seconds. Then the frequency selectiveness of neurons was tested by playing sounds that occurred at 1-second intervals and featured 16 different chords that ranged from 2 kHz to 40 kHz. These sessions lasted 320 seconds. If neurons had responses to sound and were frequency selective, electrophysiology was recorded while the mice performed the behavioral task.

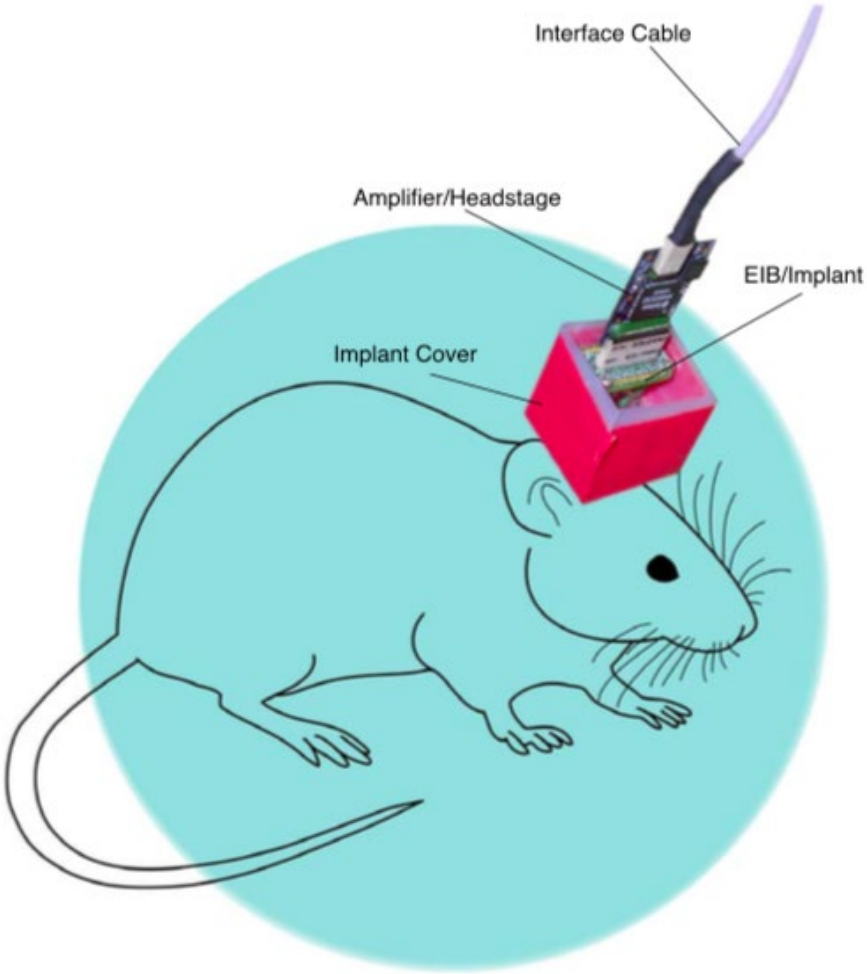


Figure 6: Experimental setup with implanted mouse

This schematic illustrates the experimental setup of an implanted mouse. The EIB/implant and implant cover were surgically attached to the mouse. During experimentation, the top of the protective implant cover was removed and the headstage was plugged into the EIB on the implant, allowing electrophysiology to commence.

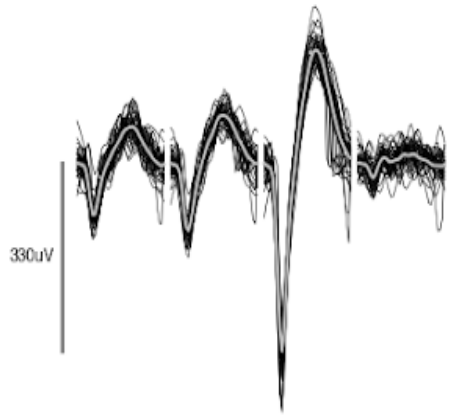


Figure 7: An overlay of spikes recorded from an individual neuron via electrophysiology

This figure illustrates action potentials, or spikes, from an individual neuron. Each single black line represents the electric potential difference recorded by an electrode, creating a spike trace. The white trace shows the average across several recorded spikes. The third electrode from the left shows the most dramatic change in electric potential, as well a classic neuron spike shape, signifying that this electrode is positioned closest to the neuron. The remaining three electrodes show noise from same neuron being recorded, as well as other nearby neurons and additional electrical activity in the brain.

After confirming neurons were sound responsive and frequency tuned, a new electrophysiology recording window was established, the behavioral paradigm was started, the reward ports were uncovered, the acoustic chamber door was closed, and mice engaged in the task for a one hour session. The animals were monitored via video camera and neural activity was projected on a monitor for observation.

After a successful recording in which a mouse completed a minimum of 2.75 times the number of trials per block during a session, the tetrodes were advanced approximately 40 micrometers deeper into the brain to access different AC neurons. Tetrodes were moved at the end of a behavioral session, approximately 24 hours in advance of the next recording, which allowed the tissue to settle in place. If a mouse did

not have a successful recording session, the tetrodes were not advanced, and an additional behavior session was recorded at the same depth the following day. Tetrodes were advanced in 40 micrometer increments and electrophysiology was recorded until neurons were no longer sound responsive, indicating the tetrodes had vacated the AC.

Lesions for Analyzing Location of Recordings

At the completion of recording for each animal, histological analysis was conducted to determine the path taken by the tetrodes through the brain. Animals were anesthetized with Isoflurane and electrolytic lesions were created at the end of the shortest and longest electrodes, scarring the surrounding tissue to mark their relative locations in the brain. To create lesions, wires were plugged into a 12V DC source and the power wire was placed in the animal's mouth, while the ground wire was touched to the gold pins on the EIB corresponding to the longest and shortest tetrodes.

After lesioning, mice were injected with Euthasol, perfused with a paraformaldehyde/ saline solution, and the brains were extracted. The brains were soaked in paraformaldehyde overnight, allowing the tissue to stabilize. The following day, brains were sliced in a coronal fashion via vibratome at a thickness 100 micrometers. Later, images of the brain slices were captured using a Zeiss Axio Imaging Microscope at magnifications 1.25x and 2.5x with aid of ZEN software. These images were analyzed to ensure the tetrodes traveled through the AC. Florescent images allowed the injected DiI to expose the pathway traveled by the tetrodes (Figure 8).

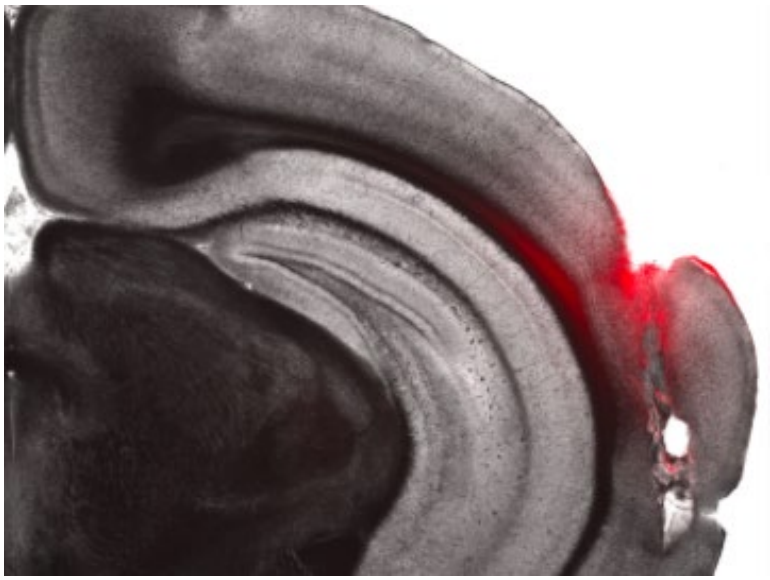


Figure 8: A coronal brain slice illustrating the tetrode track through the right AC

After mice were perfused and their brains were removed, the brains were sliced coronally to allow for histological analysis. A fluorescent microscope was used to capture images of the brain slices. DiI that was coated on the tips of the tetrodes prior to implantation is visible in red, helping illuminate the track of the tetrodes in the right AC as they were advanced downward over the course of the experiment.

Data Collection and Analysis

Software

Electrophysiological recordings were conducted using the Open Ephys GUI ("OpenEphys GUI,") and a custom behavioral paradigm developed within the lab using the python programming language. Within Open Ephys, a reference channel was selected and thresholds for action potential recording were set for each electrode based on the visual representation of the spikes provided in the Open Ephys interface. Thresholds were set to record the majority of neuron action potentials while excluding as much noise as possible. A bandpass filter from 300-6000Hz was applied during

recording, and the sampling rate was 30.0 kilosamples/second to filter out high/ low frequencies.

Valid Sessions

Data was deemed usable only when mice had sound responsive neurons that were tuned at and between frequencies of 6.2 kHz and 19.2 kHz, and when the mice completed a valid behavioral session. Mice were required to complete a total of 2.75 times the number of trials in one block. If the mice did not complete enough trials, if they became unplugged from the head stage during the session, or if any technological malfunctions occurred, the session was unusable for data analysis. Under these circumstances, the tetrodes were not advanced, and thus the same neurons were recorded from the following day.

Clustering

The program Klustakwik was used to sort spike data. For each spike, the peak and valley were calculated, and Klustakwik sorted the spikes into 12 clusters. 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.

Behavior Data

A Jaramillo Lab algorithm was used to analyze the electrophysiology behavior data and find cells while eliminating background noise. The algorithm automatically cells with selected criteria, such as good wave form and consistent firing. For these cells, the algorithm used knowledge of spike time and sound presentation to generate

graphs known as raster plots that plot individual neuron action potentials during each trial over a period of time during which sound is presented. The algorithm also separated trials based on where more or less reward was being administered. A graphical representation of each raster plot, or peristimulus time histogram (PSTH) was also created by the algorithm.

Neuron Sound Responsiveness

Sound responsiveness of individual neurons was determined by a Wilcoxon rank sum test using a threshold for significance of 0.05.

Neuron Modulation

The Wilcoxon rank-sum test was used to determine whether or not cells were modulated by reward at specified times, such as during sound presentation or during movement, using a threshold for significance of 0.05.

Results

Although the AC and AStr are well implicated in auditory-discrimination learning (Znamenskiy & Zador, 2013) and it has been shown that neurons in the AC may have varied responses to reward feedback in primates (Brosch, Selezneva, & Scheich, 2011), it is unknown if the activity of AC neurons changes when the same sound and same action are associated with different rewards. This study aimed to characterize the activity of neurons in the AC of mice during an auditory reward-change task. We recorded activity from 487 AC neurons during an auditory reward-change task and analyzed the patterns of activity when the same sound stimulus represented different amounts of reward. Of 160 sound responsive neurons analyzed during conditions when a different reward was associated with the same sound and the same action, we found 12 neurons that had a significant difference in firing rate during sound presentation. These neurons were modulated by reward during sound presentation. Additionally, we found 133 AC neurons that responded after sound presentation had concluded and the mouse was moving to receive reward. Of these neurons, 29 had a significant difference in firing rate when the mouse was moving the same direction, but receiving different amounts of reward. These neurons were modulated by reward after sound presentation, during movement.

We successfully trained mice to complete an auditory reward-change task and maintain their performance following electrode implantation. We recorded activity from individual neurons in the AC that were both sound responsive and frequency tuned at frequencies that ranged from 6.2 kHz to 19.2 kHz. We found that most AC neurons did not significantly change their firing rate when the same sound stimulus was associated

with different amounts of reward. However, a small percentage of AC neurons had a significantly different response when the same sound was associated with different amounts of reward. Additionally, we found a small percentage of AC neurons that had a significant difference in activity when the animals were traveling different directions (e.g. moving to the left versus moving to the right).

Mice show behavioral bias during auditory reward change frequency-discrimination task

During auditory reward change frequency-discrimination tasks prior to implant surgery, mice favored the direction that offered more reward. A psychometric curve plotting the behavior during the task showed that at middle frequencies where the mice were making a 50/50 guess as to the correct rewarded direction, in blocks of trials where the mice received more reward on the right, their decisions were biased to the right. Likewise, in blocks of trials where the mice received more reward on the left, their decisions were biased to the left (Figure 9).

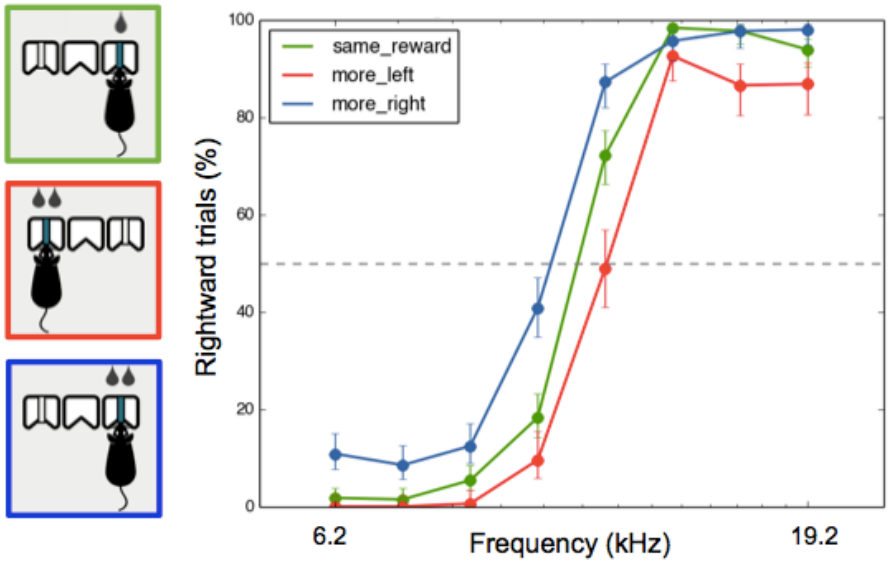


Figure 9: Mice show behavioral bias during auditory reward change frequency-discrimination task

This graph shows three psychometric curves plotting mouse behavior during a reward change frequency-discrimination task, using 8 frequencies that ranged from 6.2 kHz to 19.2 kHz. Under all reward conditions, at low frequencies (signaling go left to receive reward) the mouse traveled to the left during most trials. Likewise, under all reward conditions, at high frequencies (signaling go right to receive reward) the mouse traveled to the right during most trials. During trials where an intermediate frequency was played, the mouse traveled left or right approximately 50% of the time because there was no definitive high or low frequency sound to prompt an informed decision of traveling right or left, respectively. The green curve signifies trials of equal reward, while the red curve represents trials with greater reward on the left and the blue curve represents trials with greater reward on the right. The shift of the blue curve upward (more rightward) during trials where more reward is received on the right compared to trials with equal reward indicates bias toward choosing to travel to the right. The shift of the red curve downward (less rightward/ more leftward) during trials where more reward is received on the left compared to trials with equal reward represents bias toward choosing to travel to the left. Both shifts indicate bias toward the direction with more reward. Similar results were seen in all six mice.

These results indicated the mice were taking reward quantity into account when making decisions in this task where they used auditory information to determine which direction to travel to receive a reward.

Mice are able to perform auditory-discrimination tasks with equal ability before and after AC Implantation

Following surgical implantation of electrodes in the right AC of mice, animals were able to perform auditory frequency-discrimination tasks with the same accuracy and endurance compared to sessions prior to surgery (Figure 10).

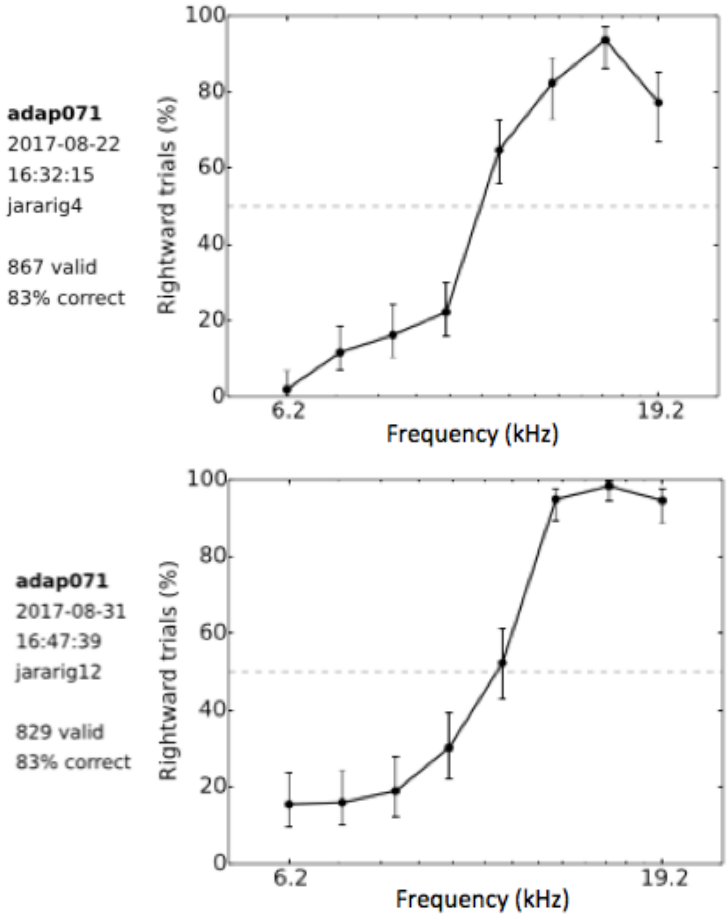


Figure 10: Mice are able to perform auditory-discrimination tasks with equal ability before and after AC Implantation

These psychometric curves of an animal pre and post tetrode implantation in the right AC show animals can still discriminate between sound frequencies to obtain a reward after implantation. During low frequency trials (signaling go left to receive reward) the mouse most often traveled left. During high frequency trials (signaling go right to receive reward) the mouse most often traveled right. At intermediate frequencies, the mouse traveled left and right approximately 50% of the time. In sessions both before and after tetrode implantation, similar numbers of valid trials were completed with the same level of accuracy. Similar results were seen in all six mice.

Neurons in the AC are sound responsive and frequency selective

Electrophysiological recordings from 487 neurons in the AC of mice showed that 160 AC neurons were sound responsive. Many neurons in the AC responded to

sounds with an increase in activity at sound onset (Figure 11). Other neurons had a decrease in activity at sound onset (Figure 12). In both cases, neurons responded to sounds of a selective range of frequencies. Some neurons were neither sound responsive nor frequency tuned, and thus had no change in activity when sound was played.

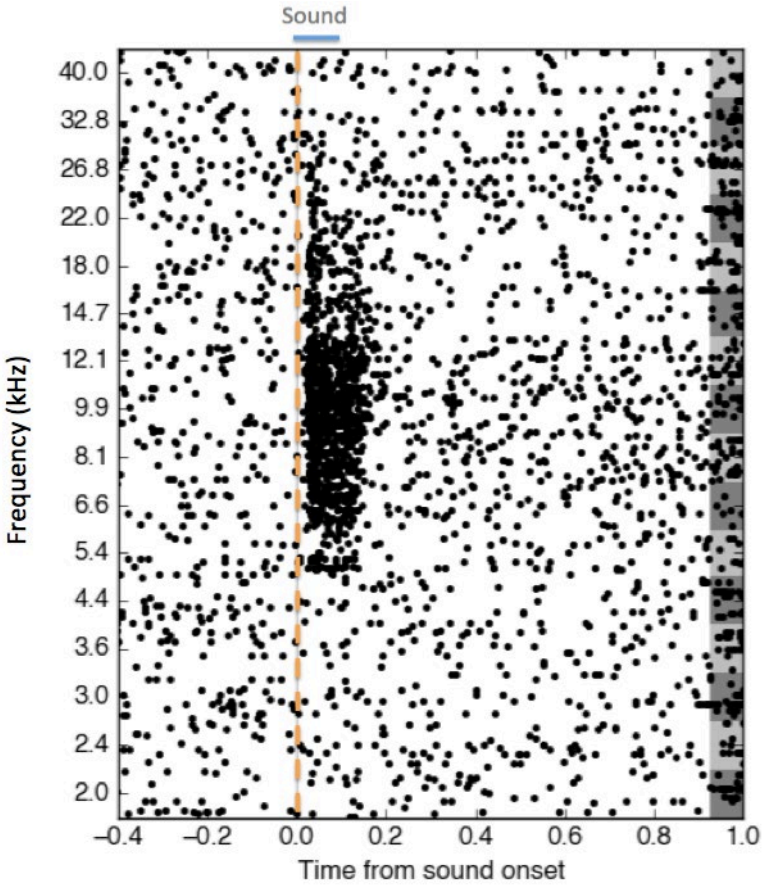


Figure 11: Some neurons in the AC increase their firing rate in response to specific frequencies of sound during sound presentation

This raster plot shows neuron activity before, during, and after sound presentation, where sounds of different frequencies are played from t=0 to t=0.1. Neuron spikes are represented by black dots. During sound presentation, the neuron increased its firing rate when the sound was between roughly 5 kHz and 32 kHz. The neuron was sound responsive and frequency tuned.

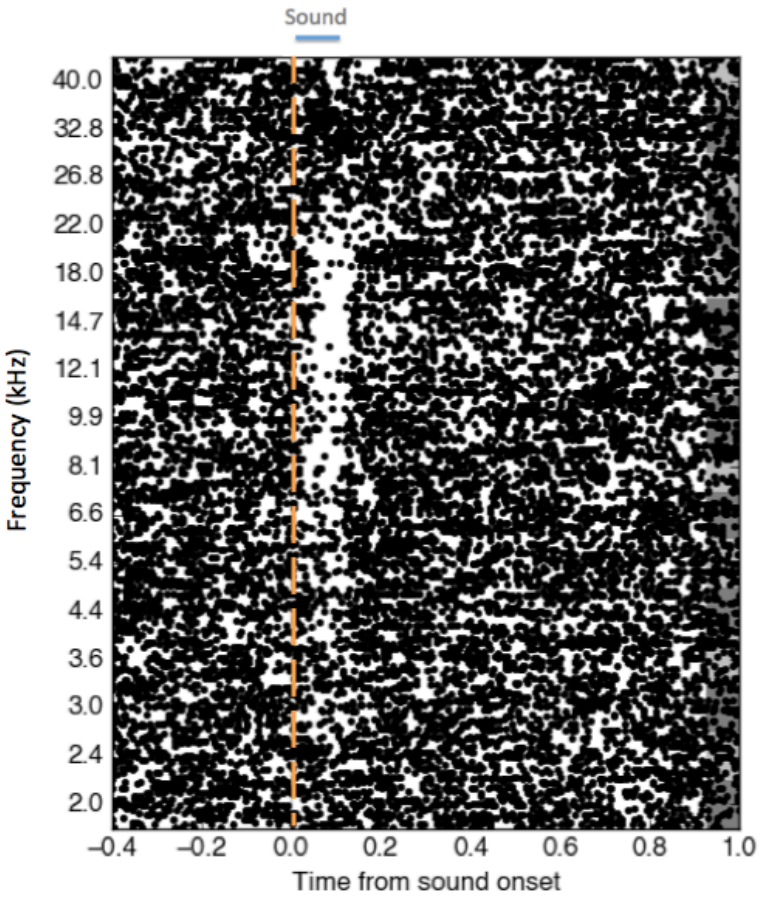


Figure 12: Some neurons in the AC decrease their firing rate in response to specific frequencies of sound during sound presentation

This raster plot shows neuron activity before, during, and after sound presentation, where sounds of different frequencies are played from t=0 to t=0.1. Neuron spikes are represented by black dots. During sound presentation, the neuron decreased its firing rate when the sound was between roughly 2.5 kHz and 24 kHz. The neuron was sound responsive and frequency tuned.

Some sound responsive AC neurons respond differently to the same sound during sound presentation depending on reward implications

Some neurons fired more frequently when the sound was associated with more reward (Figure 13). Other neurons fired less frequently when the sound was associated with more reward (Figure 14). Most neurons did not change their activity when the same sound was associated with different amounts of reward (Figure 15).

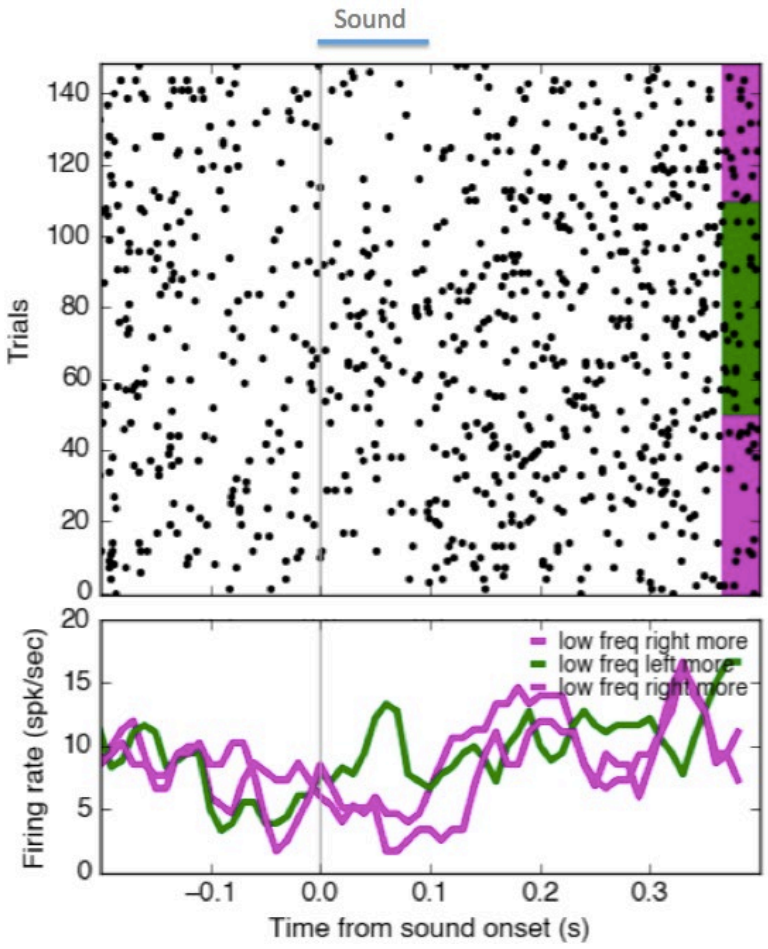


Figure 13: Some AC neurons respond differently during sound presentation depending on the amount of reward to be received

During sound presentation (t=0 to t=0.1), the firing rate of this neuron for trials in which a mouse heard a 6.2 kHz sound and traveled left to receive reward was significantly greater when the mouse received more reward at the left port (green) compared to when it received less reward at the left port (purple).

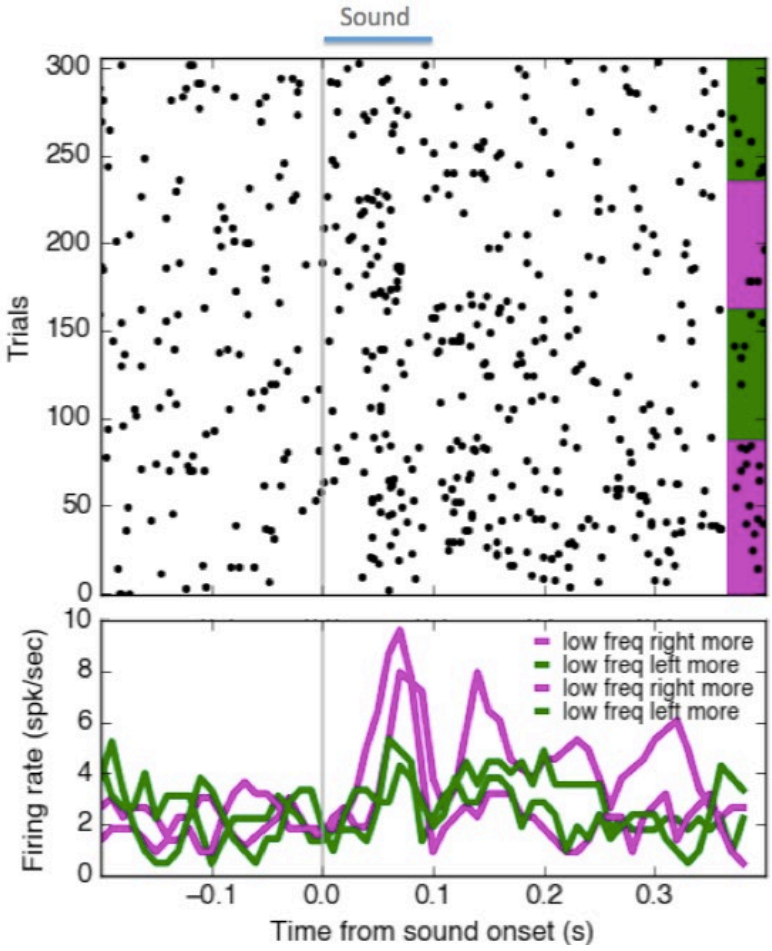


Figure 14: Some AC neurons respond differently during sound presentation depending on the amount of reward to be received

During sound presentation (t=0 to t=0.1), the firing rate of this neuron for trials in which a mouse heard a 6.2 kHz sound and traveled left to receive reward was significantly greater when the mouse received less reward at the left port (purple) compared to when it received more reward at the left port (green).

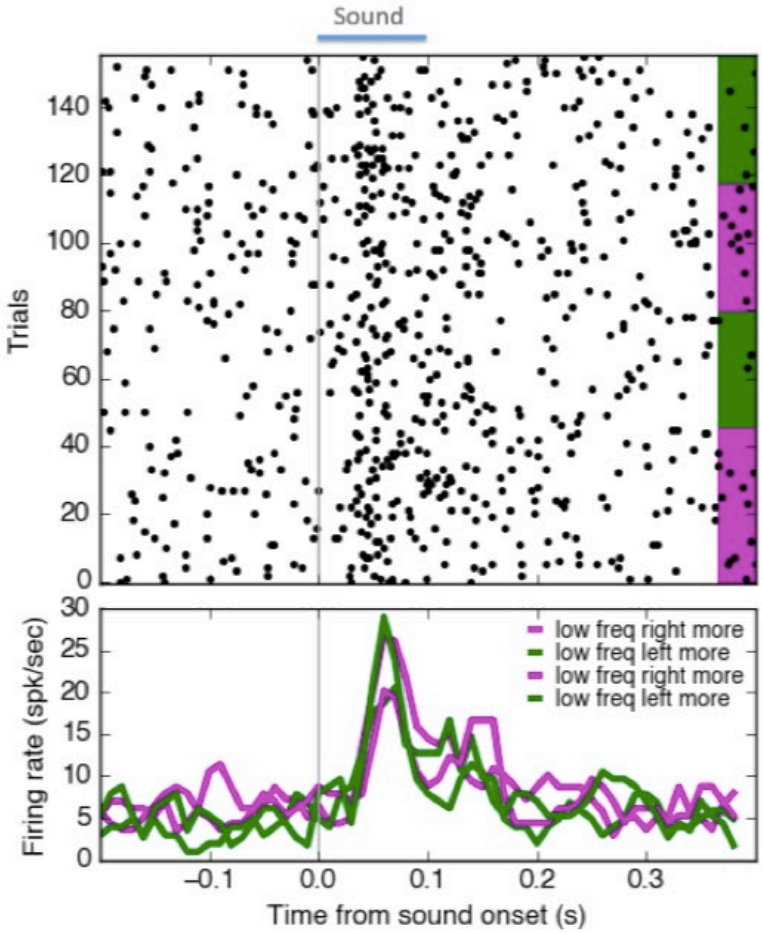


Figure 15: Many sound responsive AC neurons do not change their activity during sound presentation depending on the amount of reward to be received

During sound presentation (t=0 to t=0.1), the firing rate of this neuron for trials in which a mouse heard a 6.2 kHz sound and traveled left to receive reward was unchanged when the mouse received different amounts of reward at that port (green and purple).

Electrophysiological recordings from 160 sound responsive neurons in the AC of mice showed that 12 (7.5%) significantly changed their activity during sound presentation when the same sound was associated with different amounts of reward (Figure 16).

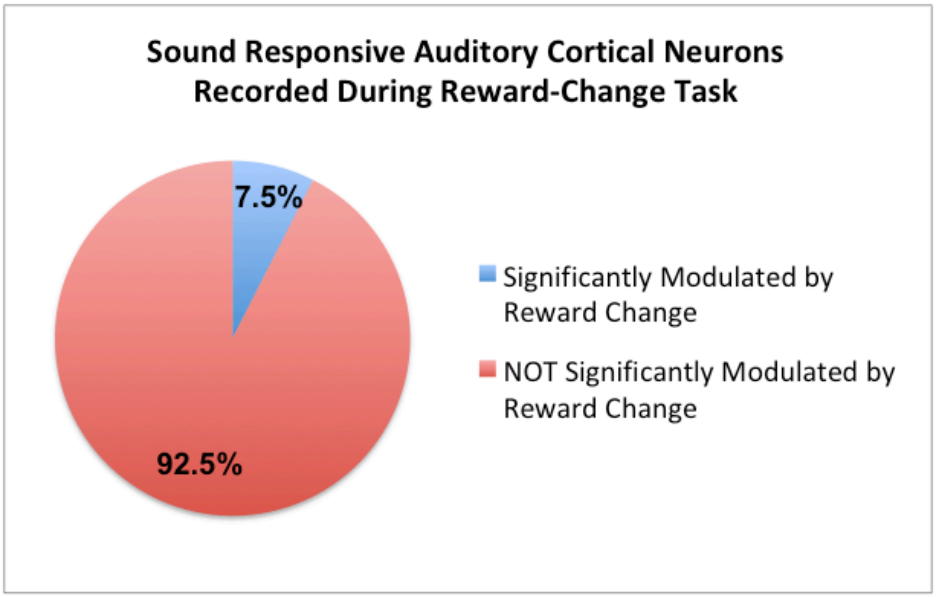


Figure 16: 7.5% of AC neurons have activity modulated by differences in reward during sound presentation

In this chart, neurons significantly modulated by reward during the 100 millisecond period of sound presentation are represented by blue, and neurons without significant modulation are represented by red.

Some movement responsive AC neurons respond differently to the same sound after sound presentation, during movement, depending on reward implications

Some neurons fired more frequently when the direction of movement was associated with more reward (Figure 16). Other neurons fired less frequently when the direction movement was associated with more reward (Figure 17). Most neurons did not

change their activity when the same movement was associated with different amounts of reward (Figure 18).

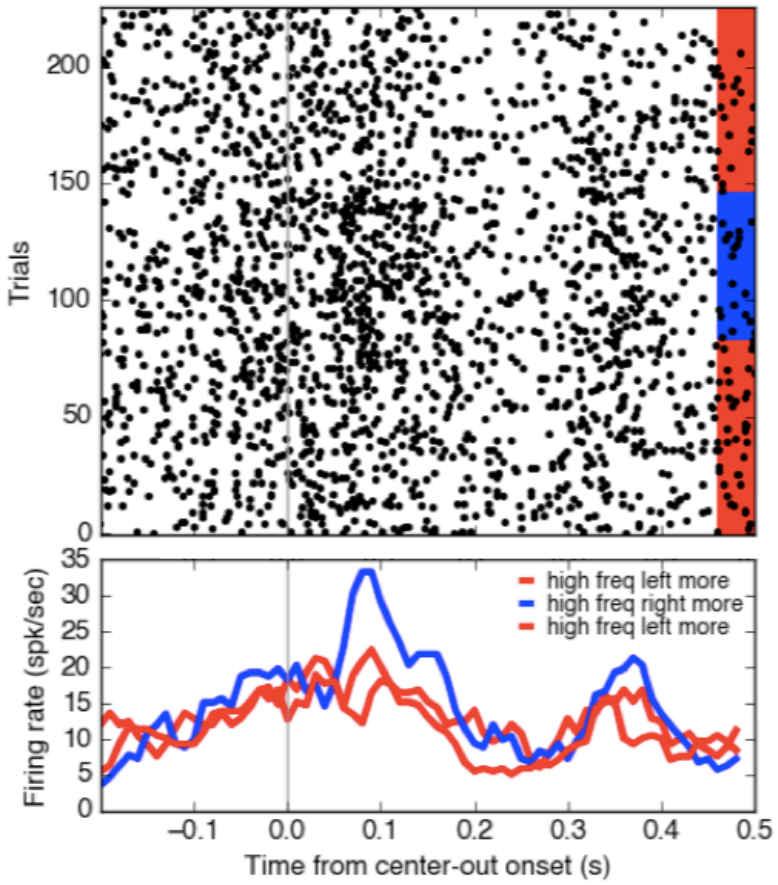


Figure 17: Some AC neurons respond differently after sound presentation, during movement, depending on the amount of reward to be received

After the mouse departed the center port (t=0) and was traveling to the right to receive reward, the firing rate of this neuron for trials in which a mouse heard a 19.2 kHz sound and traveled right to receive reward was significantly greater when the mouse received more reward at the right port (blue) compared to when it received less reward at the right port (red).

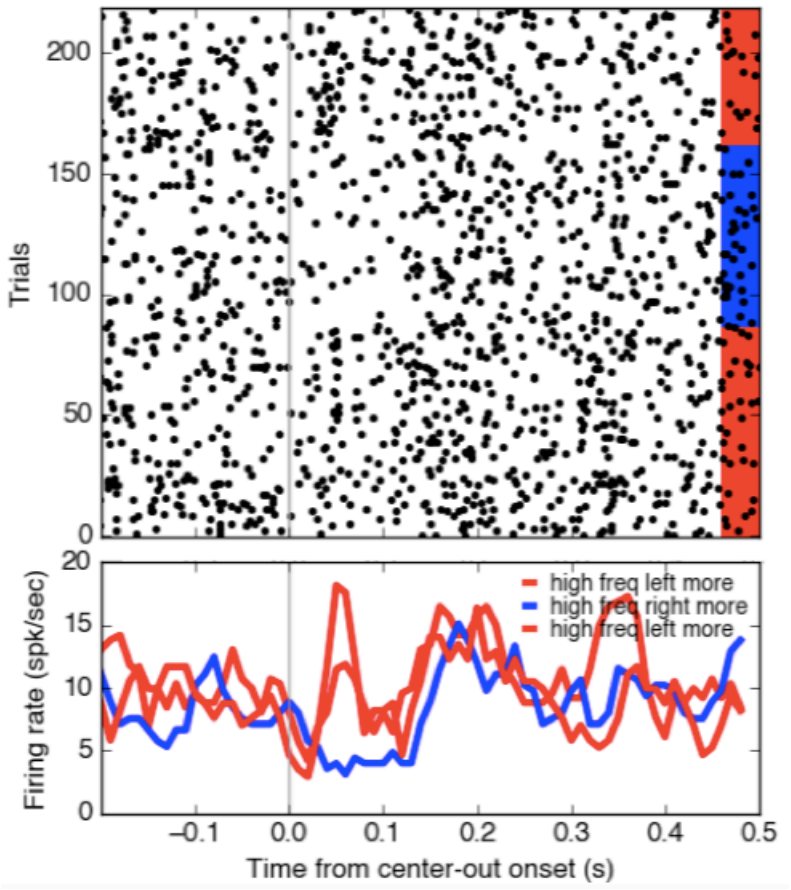


Figure 18: Some AC neurons respond differently after sound presentation, during movement, depending on the amount of reward to be received

After the mouse departed the center port (t=0) and was traveling to the right to receive reward, the firing rate of this neuron for trials in which a mouse heard a 19.2 kHz sound and traveled right to receive reward was significantly greater when the mouse received less reward at the right port (red) compared to when it received more reward at the right port (blue).

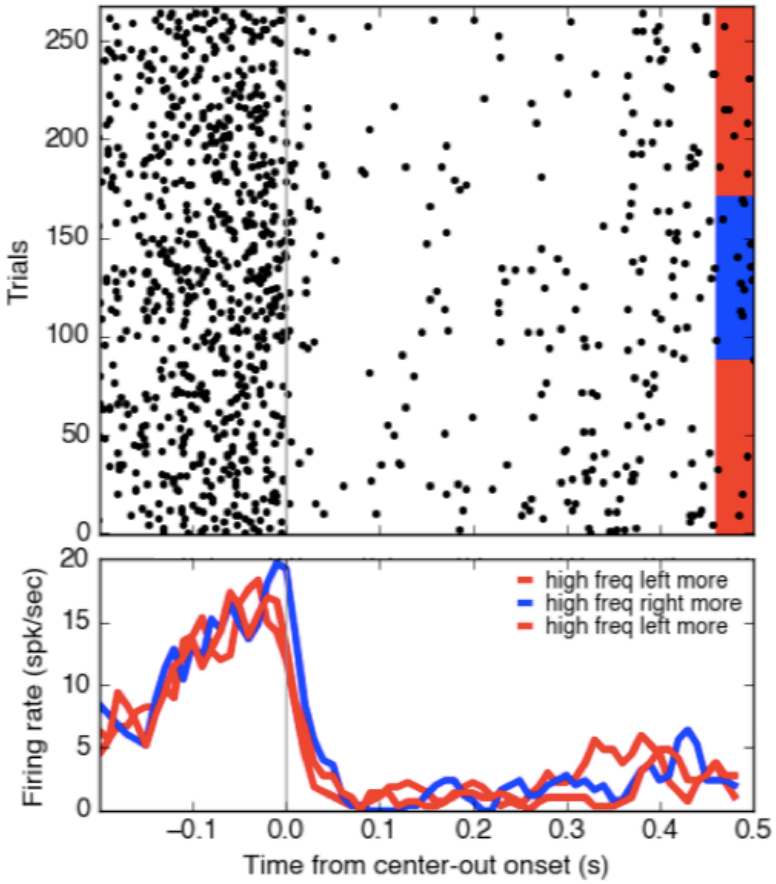


Figure 19: Many movement responsive AC neurons do not change their activity after sound presentation, during movement, depending on the amount of reward to be received

After the mouse departed the center port (t=0) and was traveling to the right to receive reward, the firing rate of this neuron for trials in which a mouse heard a 19.2 kHz sound and traveled right to receive reward was unchanged when the mouse received different amounts of reward at that port (red and blue).

Electrophysiological recordings from 133 movement responsive neurons in the AC of mice showed that 29 (21.8%) significantly changed their activity after sound presentation, during movement, when the same movement was associated with different amounts of reward (Figure 20).

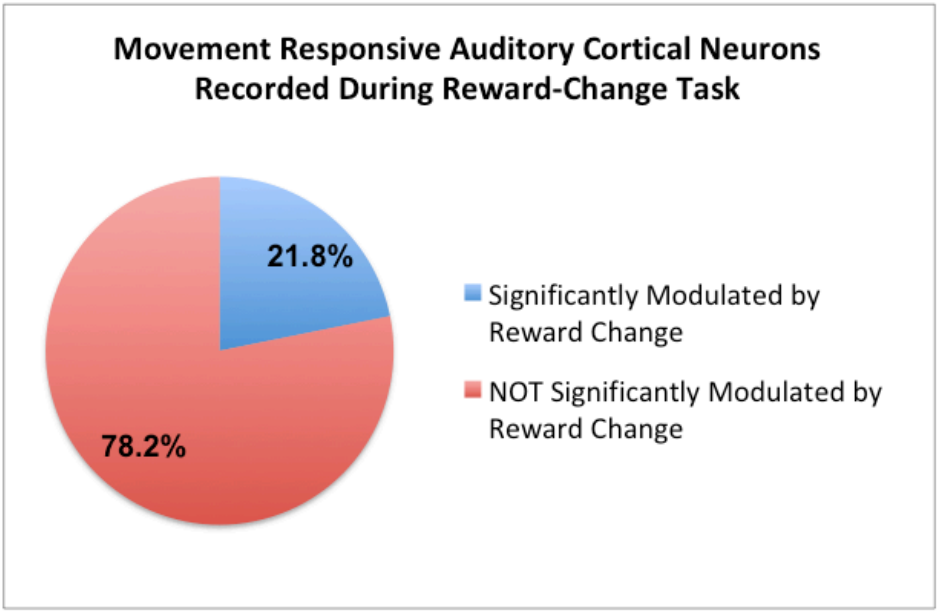


Figure 20: 21.8% of AC neurons have activity modulated by differences in reward during movement

In this chart, neurons significantly modulated by reward during movement, or the 100 milliseconds after the mice left the center port, are represented by blue, and neurons without significant modulation are represented by red.

Discussion

The key aim of this research was to understand whether the auditory cortex (AC) is involved in processing information about changes in reward when animals make decisions prompted by sounds. To investigate the role of the AC in mediating rewarded decisions, we examined the activity of AC neurons in male C57BL/6J mice, via chronically implanted electrodes, while they performed an auditory reward-change task in which mice heard the same sound and moved the same direction to obtain a water reward, but received a different amount of reward. We compared the firing rates of neurons in the AC under different contextual conditions, and found that, both during sound presentation and during movement, few neurons in this population have activity that is modulated by difference in reward.

Our recordings show that some neurons in the AC are sound responsive, as well as frequency selective, providing opportunities for researchers to explore decision-making in AC neurons in the future. Given the complexity of the brain and the location of the AC in the middle of the auditory circuit, it is possible that other brain regions play a more prominent role in making decisions based on reward.

7.5% of sound responsive neurons recorded in the AC showed modulation by changes in reward (Figure 16). This suggests that the activity of some neurons in the AC represent not only the auditory features of a stimulus, but also its meaning. However, since these neurons comprise only a small portion of the AC population, it is possible they collaborate with additional neurons in the AC, or elsewhere in the brain, to procure decisions based on the meanings of sound.

Many of the neurons we recorded from had activity changes correlated with movement, rather than with sound presentation. 21.8% of these movement responsive neurons had activity modulated by reward with respect to choice direction (Figure 20). One possible explanation for the presence of movement responsive neurons in the AC is a phenomenon known as motor efference copy. Motor efference copy is thought to be when motor neurons send information back to brain regions that process stimulus information, such as the AC (Niziolek, Nagarajan, & Houde, 2013). This would occur, for example, when the brain predicts auditory feedback it will receive as a result of speaking. In the future, characteristics of the neurons recorded in these experiments can be examined to determine how exactly they differ from AC neurons modulated during sound presentation, and whether or not they receive projections from additional brain regions, which could potentially explain why these neurons were modulated during movement rather than during sound.

The primary limitation of this research in regards to its relevance to human decision-making is the fact that these data were collected from mice as opposed to humans. Despite the earlier cited similarities between mice and humans, we can only suggest that the decision-making mechanisms explored in this project might relate to those in humans. It is not until we analyze the activity of neurons in the brains of humans (a challenge of its own) that we can better understand these pathways in people and their connections to mice. An additional challenge to this project was that we were unable to confirm the area of the brain we recorded neuronal signals from until after we conducted histology on the brain. In one implanted mouse, we discovered during histological analysis that the tetrodes had advanced superficially into the AC, but never

advanced downward through the entirety of the region. This meant the majority of our experiments recorded neural activity from the same cells rather than cells throughout the AC. Other complications to the project were fluctuations in mouse behavior. Some mice completed an adequate number of trials on one day, followed by an insufficient number of trials the next, meaning the tetrodes were not advanced that day, and thus it is possible the brain region continued to settle around the tetrodes and the opportunity to record from some neurons could have been missed. Technological difficulties also resulted in various equipment changes, which could be an additional source of error in results.

Many people are interested in learning more about decision-making for a number of reasons. Most animal behavior is influenced by some form of decision-making. By better understanding how and why animals make decisions, we can better understand their behavior. Within the scope of this project, we worked with mice to establish how the information regarding decision-making based upon reward travels through the auditory pathways of the brain, which offers us an opportunity for insight into animal behavior. Given shared physiology (Perlman, 2016) and genetics (Chinwalla et al., 2002) between mice and humans, the findings in mice can hopefully be applied to humans, with the acknowledgment that discrepancies exist between these mechanisms. If we can use this information to better understand how and why humans make certain decisions, we can potentially improve our decision-making capability, and reduce so called “human error”.

This research is also significant beyond animals (including humans). Computers are devices that make decisions based on available information. As technology

improves, computers are increasingly making decisions for us. Though there is clear distinction between the decision pathways in animals and computers, we can apply knowledge of the animal pathway to aid the decision-making process in computers so they can make more accurate decisions.

From a medical standpoint, understanding the decision-making system could allow specified areas of the brain to be isolated in patients with decision-making complications, such as those with Alzheimer's disease, Parkinson's disease and Huntington's disease (Gleichgerrcht et al., 2010). Additionally, avenues are opened for further decision-making research, such as additional decision-making pathways that may play a role in disease in humans, as well as how the pathways of research animals relate to those in humans.

Continued improvement of the understanding of the neural mechanisms behind reward discrimination in decision-making can be achieved in the future by investigating the potential role of other brain regions in reward based decision-making. A likely target in the auditory pathway, aside from the AC, would be the thalamus, which sends neural projections to the AC. It may also make sense to further investigate the striatum, which plays a significant role in the auditory pathway during decision-making. A follow up experiment within the pathway explored during this research could analyze the activity of AC neurons in response to punishment rather than reward and compare how these neurons fire under such conditions. Together, these subsequent research avenues could supplement what we already know about decision-making.

Bibliography

Balleine, B., Delgado, M., & Hikosaka, O. (2007). The Role of the Dorsal Striatum in Reward and Decision-Making. *Journal of Neuroscience*, *27*(31), 8161–8165.

Brosch, M., Selezneva, E., & Scheich, H. (2011). Representation of Reward Feedback in Primate Auditory Cortex. *Frontiers in Systems Neuroscience*, *5*, 5.

Chinwalla, A. et al., (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature*, *420*(6915), 520–562.

Farrell, A., Goh, J., & White, B. (2014). The Effect of Performance-Based Incentive Contracts on System 1 and System 2 Processing in Affective Decision Contexts: fMRI and Behavioral Evidence. *The Accounting Review*, *89*(6), 1979–2010.

Gleichgerrcht, E. et al., (2010). Decision-making cognition in neurodegenerative diseases. *Nature Reviews Neurology*, *6*(11), 611–623.

Guo, L. et al., (2017). Stable representation of sounds in the posterior striatum during flexible auditory decisions. Manuscript submitted for publication.

Niziolek C., Nagarajan S., & Houde J., (2013). What Does Motor Efference Copy Represent? Evidence from Speech Production. *Journal of Neuroscience, 33*(41), 16110–16116.

Perlman, R. (2016). Mouse models of human disease: An evolutionary perspective. *Evolution, Medicine, and Public Health*, *2016*(1), 170–6.

Znamenskiy, P., & Zador, A. (2013). Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. *Nature*, *497*(7450), 482–485.