CHARACTERIZATION OF MEDIUM SPINY NEURONS IN POSTERIOR STRIATUM

by

MATTHEW BLAKE NARDOCI

A THESIS

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Approved: *Santiago Jaramillo, PhD*
Primary Thesis Advisor

This research project focused on two types of **neurons**[1] in the **striatum** (a brain area dealing with decision-making) and how they respond to sound: neurons on the direct pathway and neurons on the indirect pathway. These two subsets of neurons were characterized using **electrophysiology** recordings from head-fixed mice that are presented with a variety of **auditory stimuli** to identify possible differences in how the two populations respond to sound. Neurons on the direct pathway express dopamine receptor D1, which is not found on indirect pathway neurons. Using **transgenic** mice, **channelrhodopsin-2** (ChR2), a light sensitive protein, was expressed at these receptors allowing the identification of direct pathway neurons using blue laser light. No significant differences in response were found between direct and non-direct pathway neurons in naïve animals.

[1] Words in bold are further defined in the glossary at the end of this document

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1. Introduction

Every day, animals of all shapes and sizes perceive various stimuli with every inherent sense. It can be a bird feeling the wind ruffle its feathers, a bear watching fish leap out of a river as they push upstream for spawning season, or a human turning to face the direction of an approaching ambulance's blaring sirens. Consciously or not, these inputs influence the actions that we take. To improve our understanding of how our decisions are affected by sensory inputs, we can dive into one sense in particular, the auditory system, as there is much still unknown about how it works. In the grand scheme of the auditory decision-making **pathway**[2], previous research has found that an **auditory stimulus** enters the ear, and becomes **transduced** into an electrical signal. This signal is then carried to the **auditory cortex** (AC) from where some **projections** are then made to the **striatum**. Previous research has also investigated the motor cortex and how its **neurons** project to muscle which results in an action, such as muscle contraction. The path connecting the AC and motor systems is still ambiguous. A possible region connecting these two areas is the striatum, as it is known that the striatum both receives sensory input from auditory cortex and plays a role in motor initiation (Guo et al., 2018).

Research centered around the striatum is important as previous studies indicate that the dorsal region, or upper region in four-legged animals, of the striatum is a part of both the reward and decision-making pathway (Balleine et al., 2007). Many of the neurons in the striatum that project to other regions contain dopamine **receptors** and the neurons' responses can be **mediated** by the presence of dopamine (Tritsch and Sabatini,

[2] Words in bold are further defined in the glossary at the end of this document

2012). Decision-making is driven in part by reward and reward is associated with the release of specific **neurotransmitters**. Dopamine is a key neurotransmitter for feelings of reward and pleasure in response to actions an organism makes (Carlsson, 1959). Dopamine can be released from various regions of the brain and then bind to specific receptors on a cell, similar to a key into a lock. This causes the cell to have a stronger response or different response entirely to any given input in a form of modulation (Surmeier et al., 2007). Certain cells in the posterior, or rear end, of the striatum (PStr) can express dopamine receptors which means the PStr has the potential to represent reward information about auditory signals (Surmeier et al., 2007; Tritsch and Sabatini, 2012). The striatum is also a good region for research and exploring decision-making in relation to auditory systems specifically because it receives signals from the AC as well as other possible auditory regions (McGeorge and Faull, 1989; Hunnicutt et al., 2016). These signals are integrated in the PStr. This plus other features make the PStr an ideal region to study to learn more about auditory-related decision-making.

There are two pathways that make up the striatum: the direct and indirect pathways. The direct pathway involves neurons in the striatum that project to brain areas that ultimately promotes movement (Balleine et al., 2007; Kravitz et al., 2010; Freeze et al., 2013). In contrast, indirect pathway neurons project to a different brain region and send a signal ultimately leading to an inhibition of movement (Kravitz et al., 2010; Freeze et al., 2013). Two relevant dopamine receptors for this study are dopamine receptor D1 (DRD1) and dopamine receptor D2 (DRD2) (Hallett et al., 2006; Surmeier et al., 2007). DRD1-expressing cells are on the direct pathway and DRD2-expressing

cells are on the indirect pathway. These receptors will help in identification of cells on these pathways while recording inside a mouse.

Living models can be much more useful for identifying interactions than theoretical models in a computer. Mice serve as excellent animal models because there is evidence that neural mechanisms in mice can be related to how the human brain neurobiology functions as well (Leung and Jia, 2016). The mouse genome is also well understood and documented meaning that it is significantly easier to make genetic modifications and measure effects from those modifications with high accuracy compared to other animal models. Mice also have a long history of being used as animal models and because of this have been bred to several specialized **strains** that are useful in research. These strains also have many physiological and genomic details recorded about them, even down to the exact gestation period and chromosomal crossings, allowing for as many variables to be as controlled for as possible and limiting variation from animal to animal.

A recent development in **optogenetics** as well as **transgenics** has greatly advanced the field in its ability to identify cells within organisms. For common lab animals, like mice, the complete **genome** has been mapped out even for specific mouse strains (Gong et al., 2003; Madisen et al., 2012). By identifying the gene responsible for DRD1, it is possible to genetically modify the gene itself or the area around it. One way optogenetics can be combined with this knowledge is by inserting a gene that encodes for the protein **channelrhodopsin-2** (ChR2). This protein is special in that **rhodopsins** can be activated by specific wavelengths of light depending on the type of rhodopsin. When the correct wavelength is used, ChR2 becomes activated and will activate the cell

to which it is a part of. Control over the light allows the researcher to controllably activate a ChR2-tagged cell, giving confirmation on what cell type is likely being recorded from (Lima et al., 2009). For this experiment, cells containing DRD1a are targeted for ChR2 expression. This allows the application of blue light to activate DRD1-containing cells to verify that the sound responses being recorded are likely from the correct cell in the correct location. This is done by looking at the shape of the waveform which is characteristic to a specific neuron when activated. If the waveform from light-activation and sound-activation are the same or very similar, it can be concluded that the same cell is being recorded from in both cases allowing us to link the identity of the cell to the sound response of the cell. Before this type of optogenetic technique existed, it would have been very difficult to identify exactly which cells are expressing DRD1 compared to other cells in the region that are also sound-responsive.

One way cells can be characterized in auditory systems is by how they respond to sounds. By presenting an auditory stimulus and measuring the responses of cells in the PStr there may be a measurable difference in how direct versus indirect pathway cells respond to the same sound stimulus. Varying responses alone would not lead to a conclusion that the cells are different as there would be no way of linking a sound response to a cell just through a recording because the neurons of interest are intermingled throughout the PStr.

This paper explores the possible connection between the PStr and eventual action in the form of movement by studying a point where the decision-making path splits with direct pathway DRD1- and indirect pathway DRD2-expressing cells. We predict cells on the indirect pathway react to sound differently from cells on the direct

pathway because indirect pathway neurons lead to an inhibition of movement while direct pathway neurons promote movement. Determining if the cells respond to sound differently could help with understanding how the direct and indirect pathway are activated respectively. Understanding under what conditions these pathways are activated could lead to the creation of models that allow the prediction of what sounds lead to what types of actions and how those actions are formed when a subject is exposed to a similar situation in the future. This is important as a mechanism cannot be fully understood until the results can be predicted and the mechanism can be replicated *in silico*. A better understanding of how these systems work is important from a medical perspective as deficiencies in a person's reaction to an auditory stimulus could be indicative of an issue along the pathway that is being studied.

2. Methods

2.1 Generalized methods

Each mouse underwent a surgery where two holes were made in the skull, one over the left auditory striatum and one over the right auditory striatum. A headbar was then attached to each mouse that is used to hold their head in place when a probe is inserted into the brain to record electrical signals. Certain cells in the brain have been genetically modified to be activated by light due to the protein called channelrhodopsin-2 (ChR2). Only cells containing the target receptor, DRD1, contain ChR2, which allows us to identify the type of cell we are possibly recording from while working inside the brain.

Once the final recording from the mouse is complete, they are euthanized and their brain is extracted. This brain is then sliced using a vibratome and imaged under a powerful microscope with different types of light to highlight the dyes used on the probes. These images are then aligned to a brain atlas of the average mouse brain (http://mouse.brain-map.org/experiment/thumbnails/100048576?image_type=atlas) and the point of penetration is identified. From this information, it becomes possible to estimate where in the brain each recording took place with reasonable accuracy.

Software that was developed in the Jaramillo lab in Python (www.python.org) is used to sort the recorded electrical signals from the brain into possible cells using multiple parameters to classify which cells are considered “good” cells. These cells’ data are then processed to identify more specific properties of the cells.

2.2 Electrophysiology from ChR2-tagged neurons

In order to perform **head-fixed** awake electrophysiological recordings in the subjects, a headbar was fixed to the skull of the animals while they were anesthetized during surgery. The animals were anesthetized with 2.5% **isoflurane** and were placed on to a **stereotaxic surgical apparatus** to fix their head during surgery. The scalp is cut partially away to make room for the headbar (**AP**: +2.0 mm from bregma, skull screws at ML: ±1.8 mm from midline) and **craniotomies** over auditory striatum (AP: -1 mm to -2 mm from **bregma**, ML: ±2.9 mm to 4.1 mm from midline). To reduce pain felt by the mouse during recording, the **dura mater** was removed. Plastic wells were attached around each craniotomy and filled with a silicone elastomer (Sylgard 170, Dow-Corning) to protect the surface of the brain and retain moisture. The animals recovered for a minimum of three days while being monitored before beginning electrophysiology experiments.

For recording, animals were head-fixed while allowed to run on a wheel inside a single-walled sound-isolation box (IAC-Acoustics). The silicone elastomer was removed and the electrodes were inserted through the craniotomy into the brain. All recordings were performed with 4-shank, 32-channel silicon probes with electrodes arrayed in tetrodes (A4x2-tet-5mm-150-200-121, NeuroNexus). The shanks of the probes were marked with a fluorescent dye (DiI: Cat #V22885, or DiD: Cat #V22887, Thermo-Fisher Scientific) before penetration of the brain to assist in identifying shank location post-mortem. The probes were fitted with a 50 μm diameter **optical fiber** (Polymicro Technologies) placed approximately in the center of the probes and

extended to approximately 100 μm from the top tetrode. The optical fiber delivered a 445 nm laser light (2.5 mW at the fiber tip).

To find DRD1-expressing neurons, we presented 100 ms bursts of white noise at 60 dB SPL (sound pressure level) followed by 100 ms **pulses** of laser light to identify ChR2-expressing neurons (**Figure 1B**). Recording only continued if both a light-evoked and sound-evoked response was found. Next, **trains** of 10 ms light pulses at 5 Hz were presented in order to distinguish between neurons directly expressing ChR2 and those indirectly activated via synaptic input from ChR2-positive neurons (Lima et al., 2009). Finally, we presented the ensemble of auditory stimuli to determine the frequency tuning. After the recording was complete, the silicone probe was removed and the well was re-filled with a silicone elastomer. Multiple recordings were performed for each animal. After the final recording session, the animal was sacrificed and its brain was extracted.

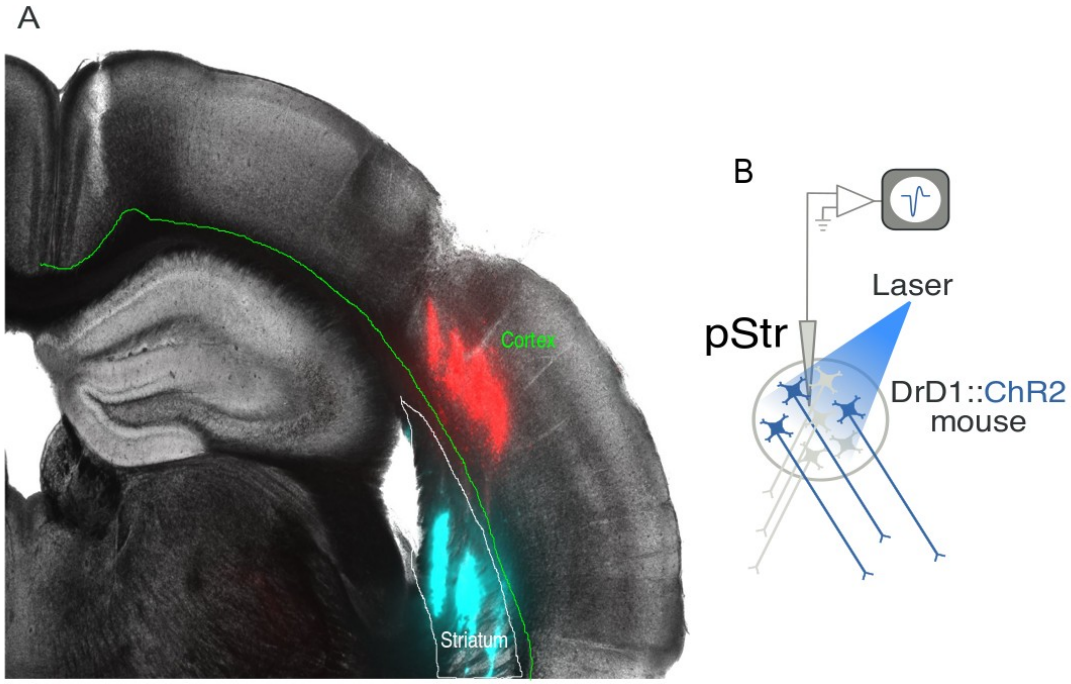


Figure 1. Histology information of animal subjects.

(A) Fluorescent images of DiD (false-colored blue) and DiI (false-colored red) in a 100 µm section of a mouse brain. The white-outlined area is the dorsal striatum and the area superior to the green line is cortex. DiD fluorescence is from the deepest point of the penetration indicating the electrodes were in striatum while recording. DiI fluorescence is in cortex but is not from the deepest point of that penetration. (B) Cell types in the striatum are intermingled. DRD1 cells express ChR2 allowing them to be activated by 445 nm laser light (blue). Cells in grey can either be a part of the indirect pathway and project somewhere else or not be medium spiny neurons at all.

2.3 Auditory stimuli

Auditory stimuli were presented in open-field configuration from a speaker (MF1, Tucker-Davis Technologies) **contralateral** to the side of recording. Speakers were calibrated using an ultrasonic microphone (ANL-940-1, Med Associates, Inc.) to obtain the desired **sound intensity** level for **frequencies** between 1 kHz and 40 kHz. Stimuli were created through the *taskontrol* platform (www.github.com/sjara/taskontrol) developed in our lab using the Python programming

language (www.python.org). The ensemble of auditory stimuli was composed of **white noise** bursts (100 ms duration, 60 dB SPL), pure tone pips at 16 frequencies logarithmically spaced between 2 kHz and 40 kHz (100 ms duration, 15-70 dB SPL in 5 dB steps). All stimuli had a 2 ms ramp up and ramp down. Interstimulus intervals were randomized to prevent the mouse from being able to predict the onset of the stimulus.

2.4 Histology

Animals were deeply anesthetized with **euthasol** and then perfused through the heart with 4% **paraformaldehyde**. Brains were extracted and left in 4% paraformaldehyde for at least 24 hours before slicing. Brains were sliced under phosphate-buffered saline on a **vibratome** (Leica VT1000 S). Slice thickness for electrode tracts after electrophysiology experiments was either 50 μm or 100 μm depending on probe orientation in the brain during recording. Brain slices were imaged using a fluorescent microscope (Axio Imager 2, Carl Zeiss) with a 1.25x and 2.5x objective (NA 0.16). Fluorescence was imaged from the dyes with DiI being false colored red and DiD being false colored blue (**Figure 1A**) and superimposed on top of a brightfield image of a brain slice, which is shown in greyscale. These superimposed images were used to identify where the electrophysiology recordings occurred. We set the deepest part of the fluorescence equal to the max depth the probe went and the brain surface as zero and calculated where the various depths each of our recordings took place would physically be in the brain.

2.5 Statistical analysis

All statistical comparisons were done using two-sided non-parametric **rank-sum test** with no assumption of normality.

2.5.1 Spike sorting

Spiking activity was detected by applying a low threshold (40-55 μV) to band-pass (300-6000 Hz) filtered continuous data. Spiking activity of individual neurons was isolated offline using the automated expectation clustering algorithm KlustaKwik (Kadir et al., 2014). Isolated clusters were only included in the analysis if less than 2% of inter-spike intervals were shorter than 2 ms because real cells cannot have responses more often than approximately every 2 ms due to a refractory period. We calculated a spike quality index, defined as the ratio between the peak amplitude of the waveform and the average variance, using the channel with the largest amplitude. We included cells with spike quality indices greater than 2.

2.5.2 Automatic selection of ChR2-tagged neurons

We used an automatic method to select neurons that were directly tagged with ChR2. We required neurons to have statistically significant responses above baseline (Mann-Whitney U test, $p<0.05$) within the first 10 ms of a 100 ms pulse of 445 nm laser light.

2.5.3 Estimation of frequency tuning

We used an automated method to determine the **characteristic frequency** (CF), **threshold**, and frequency tuning bandwidth of each cell. We first determined the frequency response area (FRA) defined as the set of frequency-intensity pairs for which

the cell's response was greater than a response threshold, defined as the **baseline firing rate**, the amount of action potentials a cell has when no stimulus is presented, plus 20% of the difference between baseline and the cell's maximum firing rate under any condition (Sutter and Schreiner, 1991; Schumacher et al., 2011). The neuron's CF was defined as the frequency with the lowest sound intensity inside the FRA where 85% of the intensities above were also within the FRA.

For neurons with sound intensity thresholds at 60 dB SPL or below, we fit a Gaussian curve to the individual trial stimulus-evoked spike counts frequencies at 10 dB above the sound intensity threshold. We then found the upper intersection (f_{upper}) and the lower intersection (f_{lower}) of this curve with the same response threshold value that we used to define the FRA. We defined the bandwidth at 10 dB above the neuron's sound intensity threshold (BW10) to be: (f_{upper} - f_{lower})/CF. Our comparisons of tuning width included only neurons for which the Gaussian function fit the data with an R-squared above 0.04 (to remove cells with very noisy responses) and had a midpoint below 32 kHz (to prevent estimation of upper tuning flanks outside the range of the frequencies tested).

2.5.4 Comparison of onset versus sustained response to sound

We calculated the firing rate in response to presentations of each cell's characteristic frequency during two time bins after sound onset. Only trials in which the CF was presented at 50-70 dB SPL were used in this analysis. The onset period was defined as the time range from the response latency of the cell to latency + 50 ms. The sustained range was defined as the latency + 50 ms to latency + 100 ms. Defining time ranges relative to the latency of each individual neuron was necessary to prevent the

underestimation of the onset component for neurons with longer latencies. We then calculated an index for the relationship between the onset and sustained portions of the response, defined as: (onset - sustained)/(onset + sustained).

2.5.5 Estimation of monotonicity of response with respect to sound level

We estimated the **monotonicity** of each neuron's response to presentations of its characteristic frequency at different intensities. We first calculated the firing rate during the sound. We then calculated an index of monotonicity used in previous studies (de la Rocha et al., 2008; Watkins and Barbour, 2011), defined as the ratio between the firing rate at the maximum intensity and the maximum firing rate at any intensity.

3. Results

3.1 Sound frequency tuning within direct and indirect pathway neurons

To determine whether direct and indirect pathway neurons respond to different features about sound frequency, we recorded responses of identified direct pathway neurons and unidentified non-direct pathway neurons to pure tones at frequencies between 2-40 kHz and intensities between 15-70 dB SPL. Figure 2 shows example frequency-intensity tuning curves for a ChR2-tagged direct pathway neuron (**Figure 2B**) and an untagged neuron (**Figure 2A**). We first analyzed the latency of the response to the sound and found that there was no statistically significant difference between direct and non-direct pathway populations ($p=0.183$, Mann-Whitney U test, **Figure 2E**).

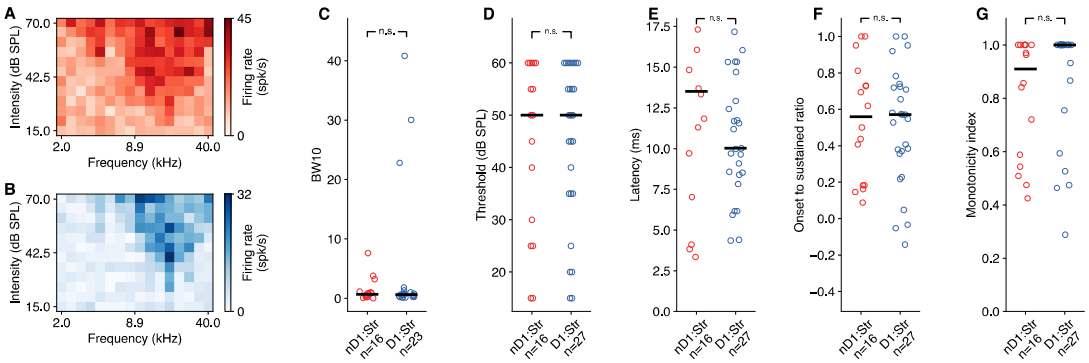


Figure 2. Sound response properties in direct and indirect neurons

(A) Example frequency-intensity tuning curve from a non-direct pathway neuron. Tuning curves were generated by recording neural responses to 100 ms pure tone pips at 16 frequencies (2-40 kHz) and 12 intensities (15-70 dB SPL in 5 dB steps). (B) Example frequency-intensity tuning curve from a direct pathway medium spiny neuron. (C) No statistically significant difference in tuning bandwidth 10 dB above threshold (BW10) was observed between direct pathway (n = 23) and non-direct pathway (n = 16) neurons. (D) No statistically significant difference in intensity threshold for response to pure tones was observed between direct pathway (n = 27) and non-direct pathway (n = 16) neurons. (E) No statistically significant difference in response latencies to pure tones was observed between direct and indirect pathway neurons. (F) No statistically significant difference was observed in the ratio of onset spike rate to sustained spike rate was observed between direct and indirect pathway neurons. (G) No statistically significant difference in monotonic response level in relation to sound level was observed between direct and non-direct neurons. Stars represent $p<0.05$. Black bars indicate the median value of each group.

We then determined the characteristic frequency (CF) for each neuron and the bandwidth of the tuning curve 10 dB above that threshold (BW10). We found that there was no statistical difference between thresholds of direct and non-direct pathway neurons ($p=0.454$, Mann-Whitney U test, **Figure 2D**). Calculation of BW10 was only possible for neurons that were tuned for frequency and had intensity thresholds below 60 dB. We found 16 non-direct pathway neurons and 23 direct pathway neurons that fit

these criteria. Both populations of neurons displayed similar BW10 values (p=0.483, Mann-Whitney U test, **Figure 2C**).

Additionally, we estimated how **monotonic** each neuron was in its response to presentations of the characteristic frequency at different intensities. We found both direct and non-direct pathway neurons displayed monotonic responses with respect to sound level (p=0.209, Mann-Whitney U test, **Figure 2G**). Lastly, we evaluated whether temporal response dynamics differed between direct and non-direct neurons. We calculated an index that compared the strength of the onset response to sound (the first 50 ms of a neuron's response) with the sustained component of the response (from 50 ms to 100 ms after the beginning of the response). We observed no difference in this index between the two neuronal populations (p=0.744, Mann-Whitney U test, **Figure 2F**).

Overall, both direct and non-direct pathway neurons are capable of responding to sound frequency information.

3.2 Histology analysis

Silicon probes that were inserted into the brain were coated in a fluorescent dye to track where cells were recorded. Fluorescence shows penetrations reached striatum (**Figure 1A**).

4. Discussion

Between direct and non-direct pathway cells, there appears to be no significant differences in how they respond to pure tone auditory stimuli. This could be due to both the direct and indirect pathways being mechanisms for a behavioral response that affects movement, such as running from a frightening sound. In the naïve animals used for this study there is no mechanical response conditioned with the presented sounds. It is possible that by training the mice to associate high frequency pure tones with movement to the left and low frequency and mid-range frequency pure tones with no movement that there may start to be changes in the cell response patterns due to plasticity. A mouse trained under these conditions may get high cell response rates in the form of neuronal activity from direct pathway cells when a high frequency sound is being presented as the expected response would be movement in a direction. In this situation, the direct pathway neurons may become very sharply tuned to specific high frequencies while ignoring all others that do not deal with a movement response. Indirect pathway neurons may become tuned to the wide range of frequencies that are associated with no movement.

In addition to pure tone pips, there were amplitude modulated (AM) sounds presented to the mouse with a range of modulation rates. It is possible that while the cells recorded from do not respond to pure tones, they would respond to modulated sounds. This data has not been included as no analysis for significance could be completed in time for presentation. A preliminary look through the data shows no sign of difference in the activity between the two pathways, but without rigorously testing

the data no conclusions can be drawn from it. A positive result from the AM sounds could provide a second avenue of future research to pursue.

Even with no definitive results found, the lack of a result is a result in and of itself. Many problems relating to technique and hardware/software were revealed by this process of trying to record from striatum. Because of this process, the way surgical implants are attached was massively improved to accommodate the requirements of recording so deeply and the silicon probes used for electrophysiology had several issues resolved also related to depth. Future experiments this lab conducts in the region of the posterior striatum will now be much more accurate. From the histology images, we now have a record to refer to for what depths corresponded to what approximate regions of the brain. This allows avoidance of the cortex, which can also express ChR2 as DRD1-expressing cortical cells will be modified by the transgene creating false positive cells that respond to both light and sound.

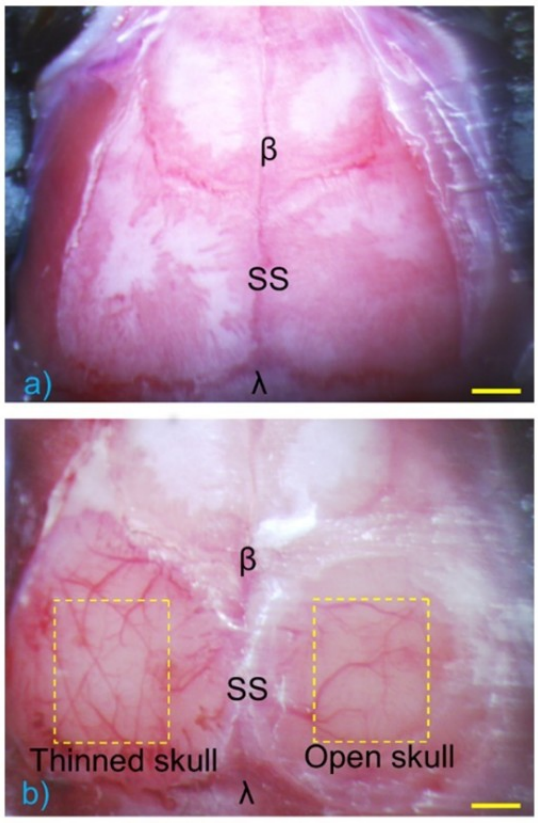
This research and the future research that comes from this project will continue to advance our knowledge of how organisms hear the world around them and react to it. Advancements made in this field can lead to better localization of problems in patients with hearing disabilities and robotic systems that can better process vocalized commands, such as Siri or Alexa. As we learn about auditory-based decision making in the striatum, it may be possible to apply some of these mechanisms to other sensory systems that pass through the striatum to better understand how visual cues or what an organism physically feels affects the choices they make in different situations.

5. Glossary

AP: Anterior-posterior. Anterior means towards the front/nose of the animal while posterior refers to towards the tail or rear of the animal. In the case of surgery coordinates, positive numbers mean in front or **bregma** towards the snout while negative coordinates refers to behind bregma towards the tail.

Auditory cortex: A region on the external, or superficial, part of the brain which is collectively called the cortex. This region is responsible for interpreting basic sounds.

Auditory stimulus: A sound wave that is within the hearing range of a subject that causes a possible response, whether it be physically or on a neuronal scale.

Bregma-lambda: 

The distance between Bregma (Greek letter Beta (β) in the image) and lambda (λ). These are defined

by where cranial sutures, or the lines in the skull, come together on the anterior part of the skull (β) and the posterior part of the skull (λ). (image found at https://openi.nlm.nih.gov/imgs/512/227/4245213/PMC4245213_pone.0113658.g001.png)

Channelrhodopsin-2 (ChR2): A particular type of **rhodopsin** that responds to laser light of 485 nm (blue light). When exposed to blue light, the cell ChR2 is attached to will become activated.

Characteristic frequency (CF): The frequency which has the lowest threshold **intensity** for activation.

Contralateral: Opposite sides. Example: The left brain processes stimuli from the right ear.

Craniotomy: A hole in the skull of an animal.

Dopamine: A neurotransmitter associated with reward, happiness and voluntary movement initiation. There are many types of **receptors** for dopamine throughout the brain that it can bind to, some receptors cause feelings of happiness and others use dopamine to activate other functions.

Dura mater: A layer of tissue surrounding the brain that is especially tough. It is normally removed during surgery because it can block the silicon probes from penetrating the brain, but also contains nerve endings. By removing the dura mater while the animal is anesthetized it minimizes/removes completely the pain a subject may feel from a probe entering the brain while the subject is awake.

Electrophysiology: Measuring the electrical activity of living cells and tissues. For this paper, it involves measuring the electrical signals that **neurons** give when they

activate. These electrical signals can be linked to a **stimulus** to identify if a particular cell activates from a specific type of stimulus, such as a specific sound or tone.

Euthasol: A chemical that acts as a heavy sedative and when administered in a non-small dose depresses the nervous system enough to cause death. For this paper, it is a drug that is injected into a subject at a lethal level after the subject is unconscious from **isoflurane** so it does not feel the needle piercing.

Gene: A specific sequence of DNA that codes for a certain protein, such as a pigmented protein that causes a person's hair to be a certain color.

Genome: A map of all of the genes that make up an organism.

Globus pallidus external: A region of the brain that projects inhibitory signals to other regions of the brain, including parts of the direct **pathway**. Part of the indirect pathway.

Globus pallidus internal: Normally inhibits the thalamus, but signals from the striatum can prevent this. Part of the direct **pathway**.

Head-fixed: Using a device to hold the animals head so it cannot move. This is done so the subject cannot move their head and break off the probe while the probe is inside the subject's brain.

Intensity: See **sound intensity**.

Isoflurane: A drug used as a general anesthetic. It is generally administered as a gas the subject inhales until it falls unconscious. Non-lethal in most doses.

Mediate: Directly controlling the response of a cell by binding or releasing from a receptor. Example: When dopamine binds to a receptor on Cell X, we become happy when doing an activity because Cell X creates a lot of action potentials. When

dopamine unbinds from Cell X receptor, we no longer have positive associations with an activity as Cell X no longer responds at all.

Neuron: Cells that make up the nervous system including the brain and spinal cord that communicate through electrical signals or chemical signals in the form of neurotransmitters.

Neurotransmitters: Small chemicals of various types found throughout the brain for signaling purposes. These signals are normally sent directly from one individual cell to one or more cells nearby.

Optical fiber: A coated cylinder of glass used to conduct light.

Optogenetics: The field of using light to control cells or tissues that have been genetically modified to be sensitive to light in some capacity.

Paraformaldehyde: A chemical used as a tissue/body preservative. When pumped through an animal's circulatory system it hardens the tissues and prevents decay.

Pathway: A series of steps a **stimulus** or other signal takes from a starting point to an ending point. In a simple auditory model, it is the steps a stimulus takes from entering the ear all the way through the brain. So first it enters the ear, then goes through the brain stem, then thalamus etc. There can be points where a signal branches off to multiple different locations. The points where these branches occur is where separate pathways begins.

(protein)-Positive/expressing neuron: A **neuron** that has a (chemical) attached to it, either through a virus injecting it into the cell or the animal naturally expressing a specific protein on cells. An example would be ChR2-expressing neurons. This refers to

a neuron that has the protein **channelrhodopsin-2** and therefore will activate when blue light is shined on the cell.

Project(ing): Sending a signal to another region of the brain by making a **synaptic** connection in the target region.

Pulses (of stimulus): A long, continuous presentation of a stimulus. An example would be a 100 ms pulse of light, which means for 100 milliseconds light is presented and then shuts off.

Rank-sum test: A statistical test on two individual, non-normally distributed populations that relies on ranking the values of each population by size and taking the sum of the ranks assigned in each population to calculate a value. This test tests the assumption that one randomly selected value from one population will be equally likely to be bigger or smaller than one randomly selected value from the other population, meaning they can average out to be the same. The calculated value is then linked to a p-value for significance based on the parameters of the populations, with a p-value falling between 0 and 1. For example, $p = 0.05$ means there is a 5% chance that if the two groups are not different you would observe distributions like this. $p = 0.95$ means that if these two populations are not different, there is a 95% chance they would form distributions like what we observed. A $p < 0.05$ is generally considered the minimum for biological results to be considered significant and not just due to populations like the ones we observed forming from luck or chance.

Receptor: A protein that is made to allow a very particular chemical to bind to it. A more generalized way of viewing a chemical and a receptor is a lock and key, where

the lock is a receptor and the key is the specific chemical that can bind to the receptor and cause it to activate.

Rhodopsin: A family of light-sensitive proteins. These proteins are structured in such a way that when a very specific color of light, determined by the wavelength of the light, is presented the protein will activate and cause some effect in whatever system the rhodopsin is a part of. This can involve activating or deactivating a cell outside of normal circumstances amongst other possibilities.

Single units: Individual cells that have been separated out based on the parameters of our algorithm.

Sound intensity: The power or volume of a sound.

Stereotaxic surgical apparatus: A device used to hold the subject's head still while surgery is occuring. It allows the surgeon to very precisely measure the distance between surgical coordinates so that an exact amount of the skull is removed for a **craniotomy**.

Strain: A specific lineage of mouse, similar to breeds of dogs.

Striatal: Something pertaining to the **striatum.**

Striatum: A brain region that integrates signals from many regions of the brain and plays a role in decision-making.

Substantia nigra reticulata: An area of the brain dealing with inhibition.

Synapse: The point where part of one neuron connects to a different neuron and signals or information can be exchanged.

Trains (of a stimulus): Presenting many short stimuli to a neuron in quick succession. An example would be a 10 ms train of light at 5 Hz. This means that light is

shown for 10 millisecond intervals at a frequency of 5 times per second, so there would be five 10 ms presentations of light every second.

Transduction: The process of changing from one form to another. In the case of this paper, it is the process by which an **auditory stimulus**, which is a soundwave, is converted to an electrical signal that can be conducted throughout the brain.

Transgenic (Possibly too much overlap with strain?): Mice that naturally express some protein that is not produced in wild mice. The alternative to cause expression is injecting a virus to produce the protein at the desired location.

Vibratome: A machine that cuts something to a very specific thickness using a quickly vibrating blade.

White noise: Sound that includes all ranges of frequencies. So rather than sounding like a specific musical note or pure tone, white noise sounds like “shhhhh” or like static from an old TV that lost its signal.

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