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Neuroscience Underlying Temporal Cue Discrimination in the Auditory Cortex

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Abstract

Animals, including rats and humans, use the auditory cortex to discriminate auditory cues for communication and survival. It has been shown that individual neurons in the A1, ventral auditory field (VAF), and suprarhinal auditory field (SRAF) of the rat auditory cortex respond to different noise burst lengths to help the rat comprehend an auditory stimulus. In this experiment, we demonstrate the behavioral ability of male Long Evans rats to discriminate between noise bursts of different temporal lengths: 12 ms and 66 ms, in a Two-Alternative Forced Choice task. The study of temporal cue discrimination in the auditory cortex can be used clinically to help patients with timing cue deficiencies and to improve cochlear implants.

Introduction

Animals use auditory discrimination for communication and survival. For example, natural sounds, like running water, are used as indicators that the source of the sound, the water, is nearby. When that sound is reproduced artificially by matching the temporal cue statistics of natural water sounds, even humans will still identify that sound as 'water' (Geffen, Gervain, Werker, & Magnasco, 2011). This indicates that the human brain analyzes temporal and other cues in sound to perceptually categorize sounds based on combinations of basic acoustic features. Given that survival is often dependent on sound processing, recognition, and association, deficiencies in auditory processing may be fatal.

In humans, auditory processing deficiencies in young infants can be an early indicator of later language development issues (Chonchaiya et al., 2012). Specifically, temporal processing conducted by the auditory cortex begins important maturation within an infant's first year that later affects both language development and reading abilities. Deficiencies in auditory brainstem response (ABR), a non-invasive electrophysiological diagnostic signal, can indicate developmental disorders like dyslexia and autism (Mai et al., 2015).

In an ABR, waves are generated due to evoked action potentials and can be measured for diagnostic purposes. Animal model studies find that Wave I of the ABR is the result of the mechanical deflection of a hair cell in response to a sound wave, which elicits an excitatory response from the cochlear nerve or cranial nerve (CN) VIII. Wave II results from the conduction between CN VIII and the cochlear nuclei. The activation of the superior olivary complex forms Wave III. Wave IV begins at the arrival of the signal at the lateral lemniscus. Finally, Wave V is generated by signaling at the inferior colliculus (Salamy, 1984). The human auditory cortex, itself, is found within Heschl's gyrus of the temporal lobe. Thus, experimental studies indicate that each synapse in the ascending auditory pathway contributes a unique and sequential sound-evoked wave within the ABR. Though the scalp ABR provides an excellent non-invasive metric of how neurons synchronize and respond to temporal cues in sound, it is not a suitable approach for determining the source location (topographic positions) for neuronal response sensitivities to acoustic features including tonal frequency, timbre, rhythm, and location of sound.

Electrophysiologic mapping and metabolic imaging studies reveal similar topographic organization of neurons according to sensitivities to tones and temporal cues. Like the rat, humans have multiple cortical fields with neuronal or metabolic responses that are organized topographically to respond to tone frequency, in a manner like the tonotopic organization of the cochlear surface (Formisano et al., 2003; Higgins et al., 2010; Hackett, 2015).

The rat tonotopic auditory cortex consists of a primary (A1) auditory field, as well as two other non-primary cortical fields called the ventral (VAF) auditory field and suprarhinal (SRAF) auditory field (Lee, Osman, Volgushev, Escabí, & Read, 2016). As observed for the human tonotopic cortex, all tonotopic cortical fields in the rat respond to its audible frequency range – each field with a unique anatomic direction of tonotopy (Polley et al., 2007; Higgins et al., 2010; Formisano et al., 2003). Thus, the rat is a good animal model for examining sound processing abilities within tonotopic auditory cortex.

Recent studies indicate that A1, VAF and SRAF cortices have unique sensitivities to temporal cues in sound. Based on the individual neuron responses, Lee and colleagues (2016) found that the ventral auditory field (VAF) and suprarhinal auditory field (SRAF) follow the shape of the sound better at longer sound durations. In contrast, A1 has a brief duration response to sound burst onset. That is, A1 spikes at the beginning of the sound and does not follow the shape of the sound as the VAF and SRAF do (Lee, Osman, Volgushev, Escabí, & Read, 2016). Based on this information, we propose that the VAF and SRAF work in conjunction with A1 to discriminate complementary temporal cues during sound bursts.

Hypothesis

In this experiment, we seek to use a behavioral task to define the roles of A1, the VAF, and SRAF in discrimination of different auditory temporal cues. It has been shown that the auditory cortex is important for temporal cue discrimination in rats in tasks like gap discrimination (Threlkeld, Penley, Rosen, & Fitch, 2008). However, it was not known which specific locations within the auditory cortex are responsible for decoding these cues. Here, we test temporal discrimination using sound bursts, rather than gaps, using sounds that resemble those used in our lab's prior study of the rat auditory cortex sensitivities to temporal cues (Lee et al., 2016).

We hypothesize that Long Evans rats can discriminate between 23 Hz (12 ms) and 4 Hz (66 ms) noise bursts. In this experiment, we set out to demonstrate these temporal cue discriminations through rat behavior. This requires that the animals perform with minimal bias – when selecting a side randomly, it is chosen with equal probability – and requires that the animals perform this discrimination at greater than 75% accuracy for the most different sound burst lengths.

Materials and Methods

This study used fifteen male Long-Evans rats obtained from Envigo, strain Hsd Blu:LE. The rats were housed in the vivarium of the Weston A. Bousfield Psychology Building at the University of Connecticut and were kept following IACUC protocol A15054. Rats 3, 5, 9, 17, 19, 10, and 20 (Group A), as well as Rats 4, 6, 7, and 11 (Group B), were received at 28 days old. Finally, Rats 25, 26, 27, and 28 (Group C) were received at 83 days old.

Weights

Following protocol, rats undergoing training – Groups A and C – were maintained at an 85% body weight restriction for the first two weeks of training. They were then kept at 80% body weight restriction for the third week, as protocol allows. The rats were kept on food restriction to increase motivation while performing the task. Group B was allowed *ad libitum* access to the Harlan brand chow as weight controls and was not trained to perform the paradigm. The rats' body weights were maintained following a modified trend line provided by Envigo. The difference between the rats' starting weights were compared to the graph, and were shifted up or down depending on the weight difference (**Fig. 1**).

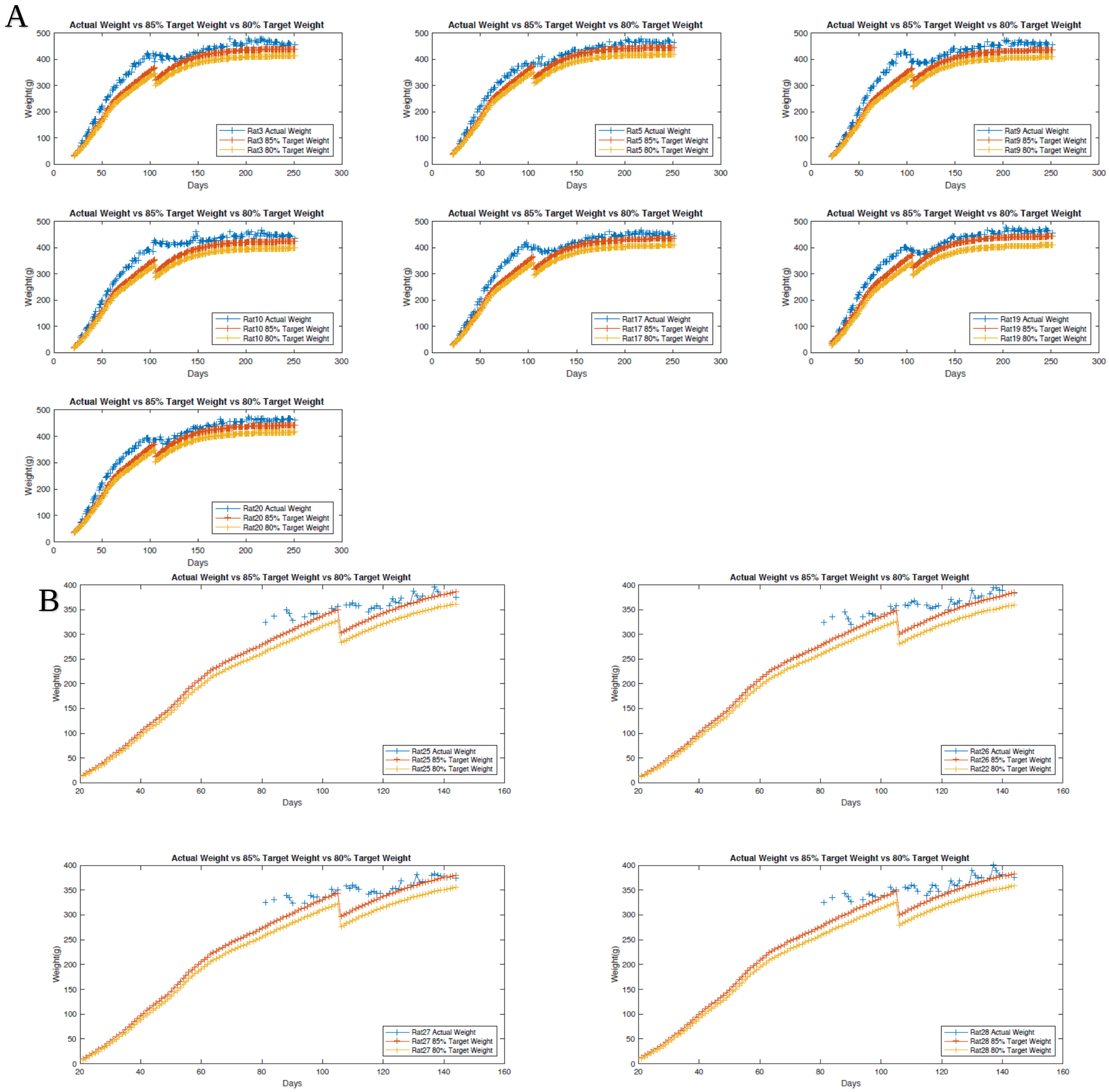

Figure 1: Rat weights were maintained at 85% Envigo desired body weight for the first two weeks of training, then maintained at 80% Envigo desired body weight for a week. (A) Group A test weights based on the 85% and 80% Envigo target weights. (B) Group C test weights based on the 85% and 80% Envigo target weights.

Chow was given according to the test weights of the rats relative to their target weights.

The general equation for calculating grams of food given was

$$\text{Food (g)} = ((\text{Test Weight}/100)*x) - 5*(.2)$$

where the subtracted expression represents additional BioServ protein pellets given to the rats as a treat, and *x* is the variable dependent on the rats' "Test minus Target" value. The following *x* values were plugged into the food equation, on the following given conditions:

Test - Target	*X* value
≥ 20g	3.5
15-20g	4
10-15g	4.5
≤ 10g	5

Table 1: *X* value used in food equation for determining amount of food rats from Groups A, B, and D received after training. These values were dependent on the difference between the test weights of the rats and their target weight determined by Fig. 1. If the rat's Test minus Target fell under 10g, then it was further supplemented (10 – Test – Target) in grams of chow.

Experimental Setup

The rats were trained in a soundproof training booth consisting of an outer box and an inner box. The outer box is lined internally with cone-shaped foam to absorb sounds after they have been played from the speakers and to prevent any sounds from outside the box from being heard by the rats. The outer box is made of medium-density fiberboard to further soundproof and prevent vibration in the training booth, and is hinged to swing open like a door with latches to seal the box and prevent outside sounds from penetrating the box. The inner box is acrylic, with small holes for sound and air to perforate.

Inside the inner box, there are three ports for the rats to poke during training. The ports are located on opposite sides – right and left – and in the wall between the two sides of the inner box. Within each port is a diode beam that is broken when a rat pokes and that sends a signal to the Bpod that the rat has poked. Two Ultrasonic Vifa speakers from Avisoft Bioacoustics, one behind each port, are used to play the sounds used in this paradigm. When the rat pokes its nose into the port, a diode beam within the port breaks, and sends a signal to the Bpod. The Bpod, which is controlled by a MATLAB program, then sends two other signals to the Tucker-Davis

Technologies (TDT) program running sounds played by the high-fidelity sound-producing RZ6 processors, and either to the syringe pumps dispensing the Ensure reward or to initiate a timeout via Pulse Pal. The timeout consists of a bright light and a time interval, during which the rats cannot hear the next sound. If the timeout is due to the rat not holding the center poke for enough time, 0.15 seconds, then the timeout lasts for 3 seconds; if the timeout is due to an incorrect port choice, then it lasts for 50 seconds. The pumps use 10mL syringes to pump 0.075mL of 33% diluted Ensure per correct choice (**Fig. 2**).

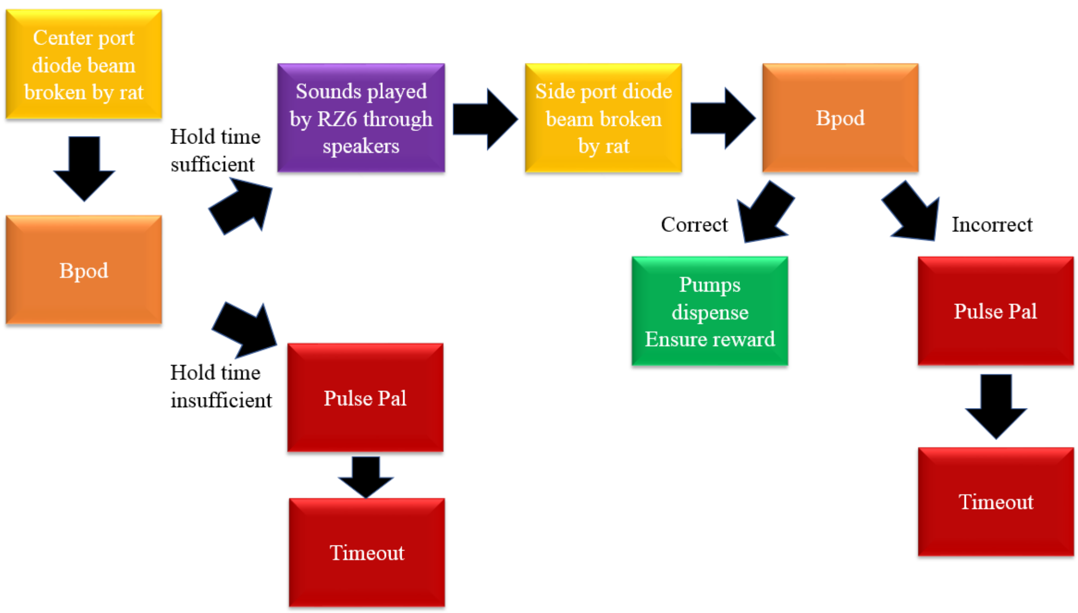

Figure 2: Logic flow of the programs controlling the paradigm. After the rat either earns the reward or enters timeout, it must begin the paradigm again by poking at the center port.

The reward Ensure solution is made from strawberry-flavored Ensure. It is mixed in the Read Lab with purified water to produce its 33% concentration.

To monitor the training activity, a camera and sound amplifier system were installed. The camera hangs directly above the inner box so that the entire inner box, especially the ports, are visible on a monitor in another room. A sound amplifier and BatBox are used to be able to listen to the sounds being played within the box via headphones in the other room.

Training

Training for the paradigm used in this experiment began at 196 days old for Group A and 89 days old for Group C. The rats were kept on a 12-h light/dark cycle (lights off at 0830 hours) to encourage activity in the morning and afternoon, when they are normally dormant. They were trained following a Two-Alternative Forced Choice (2AFC) operant task to discriminate between long and short noise burst sequences. Once the rat is placed in the booth, it can initiate either repeated 23 Hz (12 ms) noise bursts or repeated 4 Hz (66 ms) noise bursts through the speakers by poking the center port for at least 0.15 seconds. The rat receives a light cue from the center port when the port is available to be poked. Once the rat has poked the center port, the cue light turns off. Next, the rat must make a decision based on the noise it hears. If the noise burst is 23 Hz, then the rat should pick and poke the left port; if the noise burst is 4 Hz, then the rat should pick the right port. If the rat makes the correct choice, then pumps will dispense 0.075mL of 33% Ensure solution from the correct port. If the rat makes the incorrect choice, then no reward is dispensed. Instead, a timeout is initiated, where a bright light above the inner box turns on and no sound can be initiated for 50 seconds. Once the rat has either finished a timeout or received its reward, the center port cue light turns on again to indicate that the rat make initiate the next sound.

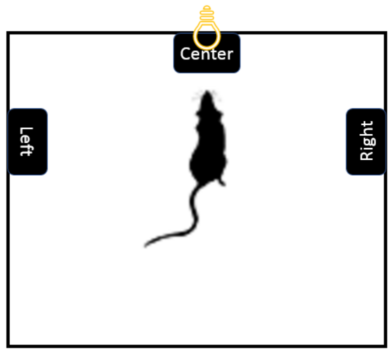

Figure 3: Representation of the training booth layout. Behind the right and left ports are the speakers producing the sound. Within the right and left ports are the tubes containing Ensure that are connected to pumps that push the reward through after a correct choice. Above the training booth is the light that is activated during a timeout. In the center port is the cue light.

Training for each rat consisted of one 50-minute session per day, five days per week. While they were training, the rats were not given access to food but were allowed access to water. Chow and protein pellets were given only after all the rats had completed their training.

Sounds

The sounds used in this lab are synthetically produced using a MATLAB program that mirrors a rat vocalization, especially temporally. The purpose of using synthetic sound is to be able to easily adjust the frequency of the signal to test our hypothesis of frequency discrimination. We use randomized jitter – the temporal spacing between noise bursts – but still maintain, on average, about 4 bursts/sec (**Fig. 4**).

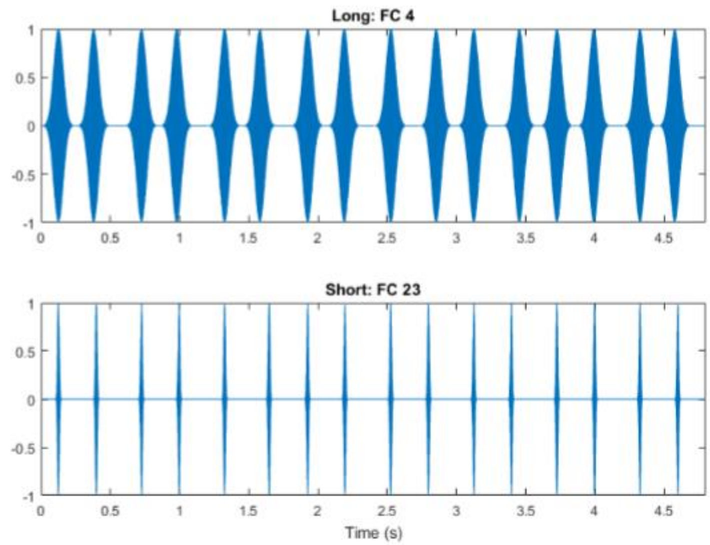

Figure 4: Sample 4 Hz and 23 Hz noise bursts. Note the randomized jitter used to prevent pattern recognition.

Results

Figure 5 shows an example of the data from single, 50-minute session. The program is designed such that future sounds that will be played are displayed as solid, blue circles. For the first twenty-five trials, these circles are distributed equally on either the right- or left-side rows, representing the equal probability of either sound being played by the speakers, but with the same sound playing no more than three times in a row.

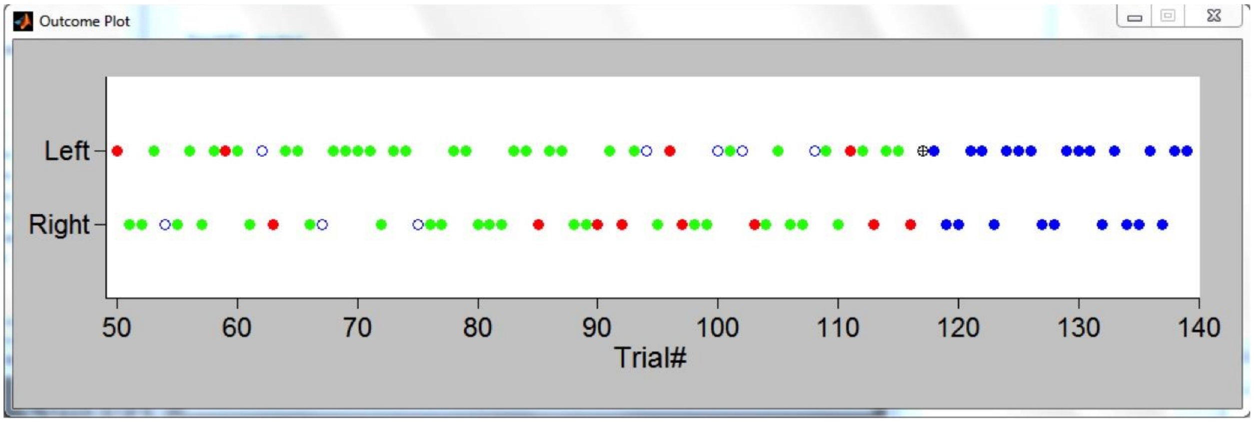

Figure 5: Session data from Rat 17. Rat 17 performed for 50 minutes, though not all trials are shown. Because there is no significant bias, the program did not require Rat 17 to poke on one side more than the other after 25 trials.

However, after the first twenty-five trials, the sounds may play with more frequency on one side than the other. This is to try to counter a left- or right-hand bias from the rats. The side that the rat is choosing to ignore more will have the sound played more frequently than the other sound, to encourage the rat to also choose that port. Once the rat learns the task, then the number of times either sound plays is more likely to equalize.

When the rat initiates a poke, it can either hold it poke for long enough (≥0.15 seconds) or for an insufficient amount of time (>0.15 seconds). If the rat does not hold its nose in the center port for enough time, the display will represent the poke as an empty blue circle. If the rat completes a successful center port hold, then the color of the circle reflects the rat's following decision based on the sound. If the correct port is chosen, then the circle displays as green. If the wrong port is chosen, then the circle becomes red.

Training for this experiment began on April 17, 2017. Figure 6 shows rat performance in discriminating long sounds (4 Hz; 12 ms), while Figure 7 shows rat performance in discriminating short sounds (23 Hz; 66 ms). Figure 8 shows biases towards choosing either the right- or left-side ports when randomly selecting a port in response to the noise burst playing.

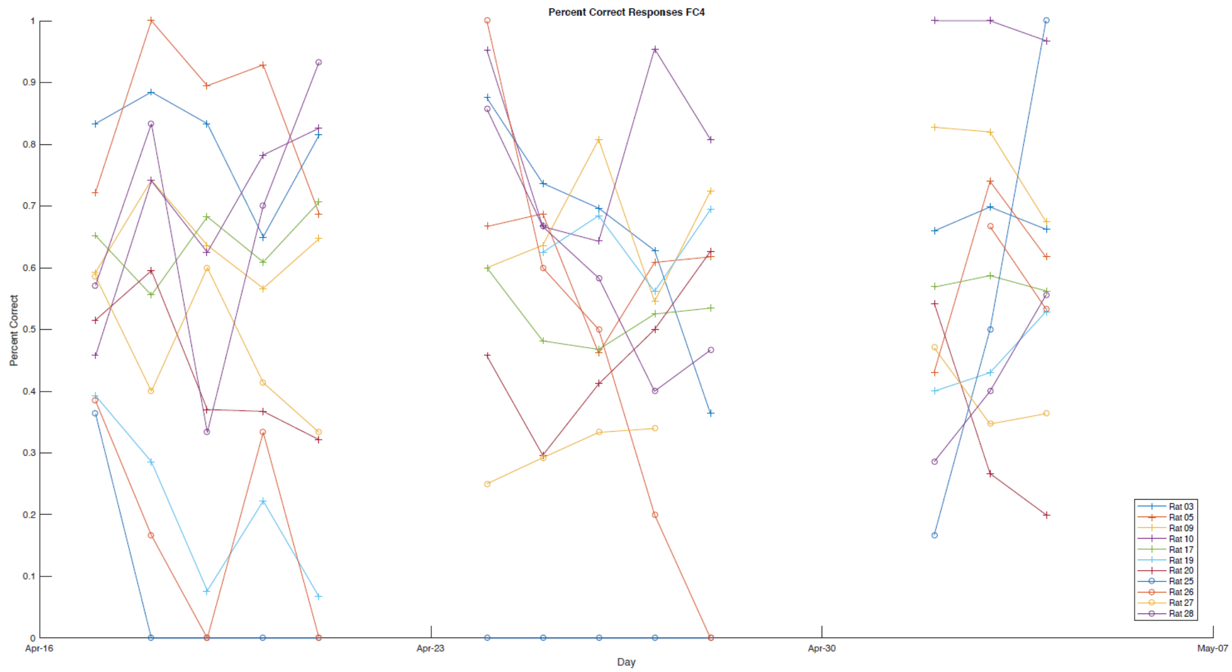

Figure 6: Percent correct responses for the 4 Hz sound. The 4 Hz sound came from the right speaker.

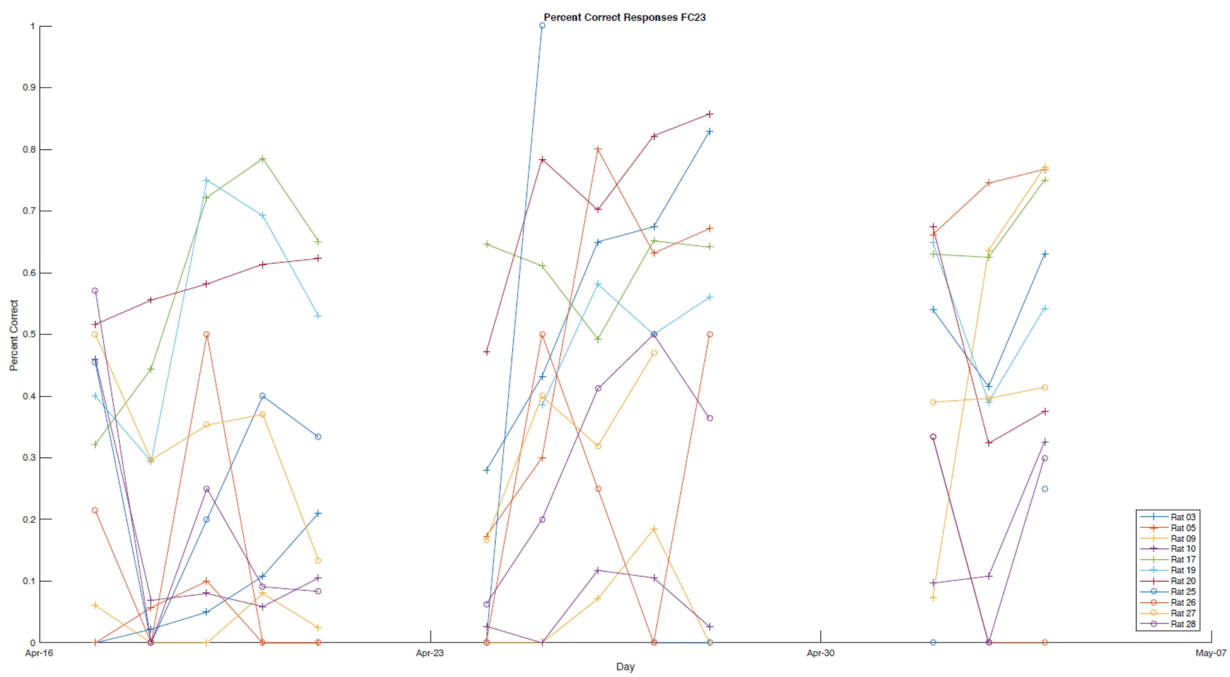

Figure 7: Percent correct responses for the 23 Hz sound. The 23 Hz sound came from the left speaker.

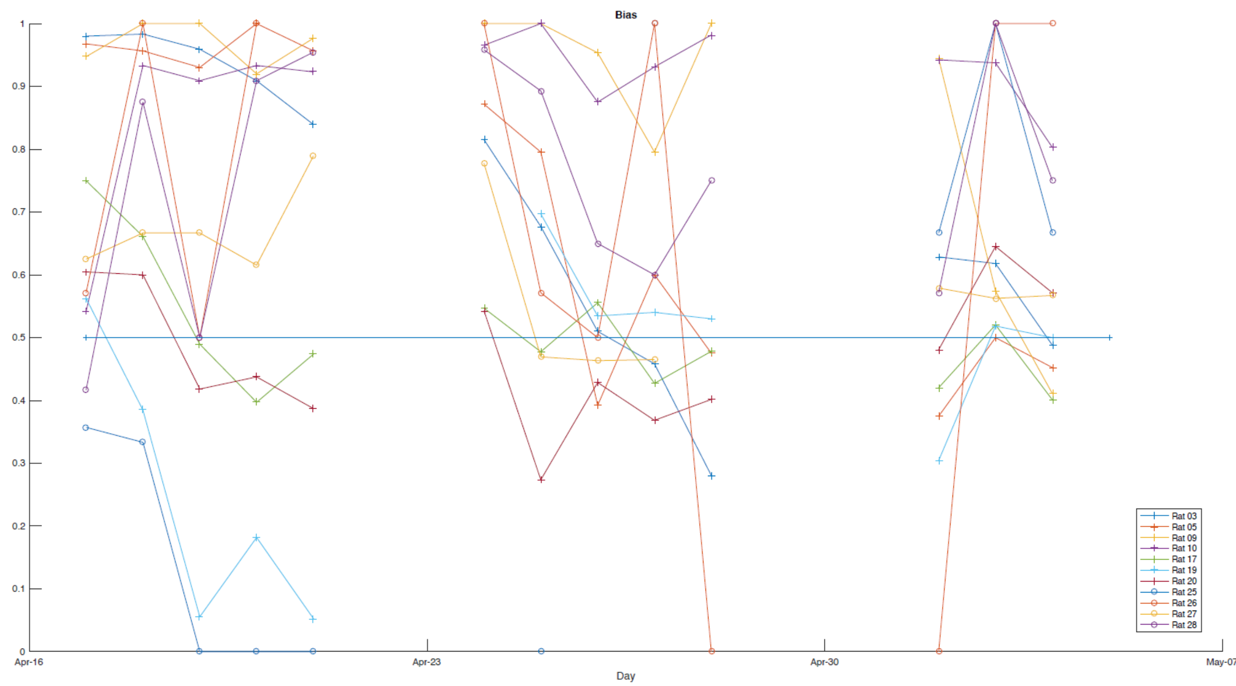

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Figure 8: Right-handed rat biases. A score of 0.5 on the bias graph indicates having no bias towards a particular side. A score of 1 on the bias graph indicates having a bias for the right side, and a score of 0 indicates a bias for the left side.

During the first week, Rats 17 and 27 were randomly choosing either the left or right side, with little bias and about 50% accuracy on both sides. Rats 3, 5, 9, 10, and 28 were all biased towards the right side during week one. During week one, Rats 19, 25, and 26 were performing poorly on the long sounds; however, while Rat 19 had was randomly getting the short sound correct, Rats 25 and 26 were performing poorly on the short sound. Both Rats 19 and 25 had left-side biases, and Rat 26 had a right-side bias. Finally, for the first week, Rat 27 was performing with random accuracy on the long sounds but was performing poorly on the short sounds, and had a slight right bias (**Fig. 6**, **7**, **8**).

By the second week, Rats 3, 5, and 28 all improved by becoming less biased, though half of their responses were incorrect on each side. Rats 9, 10, and 25 all performed the same as they did during Week 1; however, Rat 25 did go to the left side more often than in Week 1. Rats 17 and 19 had equal probabilities of choosing either side incorrectly but still had little bias. Rat 20 has slightly more correct on choosing the left side than the right, which caused a slight left shift on the bias graph. Rat 26 improved on its left side pokes, which resulted in a bias shifted to the left compared to the first week. Finally, Rat 27's right-side performance decreased, while its left side performance increased; this caused its slight right bias to decrease throughout the week (**Fig. 6**, **7**, **8**).

In the third week, Rats 3, 5, 17, 19, and 20 were all performing well on the short sound and randomly on the long sound, except Rat 3, who was performing better than the other rats. These rats also had minimal bias. Rat 9 improved from Week 2 by performing better on the short sound and decreasing bias. Rats 10 and 27 did not improve from Week 2. Rats 25 and 28 improved on discriminating long sounds throughout the week but still performed poorly on the short sound, indicating right-side biases. Finally, Rat 26 was only getting half of the long sounds correct and most of the short sounds wrong, showing a right-side bias week (**Fig. 6**, **7**, **8**).

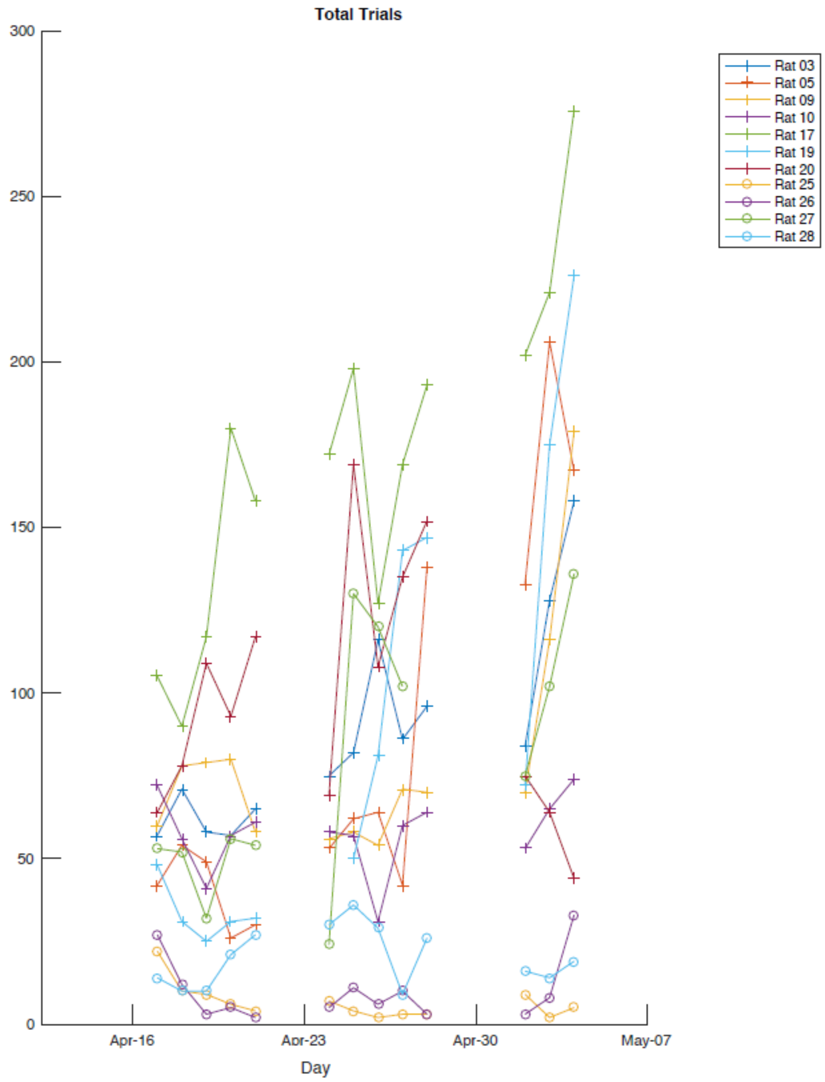

Figure 9: Total number of trials performed by rats from Groups A and C. Each session in which the trials were performed lasted 50 minutes total.

During Weeks 1 through 3, rat trial initiation generally increased in Rats 3, 5, 9, 17, 19, and 27. There was no real change in Rat 10, 25, 26, and 28, though Rat 10 tended to initiate more trials than 25, 26, and 28. Rat 20's trial number decreased throughout the weeks of the experiment (**Fig. 9**).

Discussion

According to this study, we have shown that rats can discriminate between 12 ms (23 Hz) and 66 ms (4 Hz) sound bursts with randomized inter-burst intervals of 4 Hz on average, in a Two-Alternative Forced Choice task – verifying our hypothesis.

As the rats continue to undergo behavioral training, we see a general improvement in bias, percent correct on both 4 Hz and 23 Hz sounds, and number of trials (**Fig. 6**, **7**, **8**, **9**). Trial number increased in Week 3 likely because the rats were given food per an 80% body weight restriction, as allowed by protocol. Despite this, the number of trials for some of the rats – particularly Rats 25, 26, and 28 – remained low, making the data from Figures 4, 5, and 6 very dynamic.

Bias

Biases in rats have been explained in previous studies to be due to a right or left paw preference (Guven, Elalmis, Binokay, &Tan, 2003). In attempt to counter these biases, we used the 25 trial, anti-biasing program, where the side the rat is biased against has its sound played more frequently to encourage the rat to poke on that side. In addition, we added a center poke to orient the sound to the rat's ego, rather than have the sounds play in a spatially oriented way. However, as seen in Figure 8, biases still are present in the rat behavior when the rats are still randomly choosing a side port in response to a noise burst cue. As the rats continue this training and understand the task, we hope to see a decrease in bias.

Future Studies

As we continue to train the rats in the paradigm, eventually all the rats should be performing with at least 80% correct on both the long and short sounds before moving on to the next phase – though, preferably, we expect the rats to perform at around 90% correct. The next phase will be to remove the speakers behind the right and left ports and replace them with a single speaker that will play both sounds behind the central port. This is to ensure that the rats are not using spatial location to make their choice. Once that phase has completed, we will begin narrowing the temporal difference in the noise bursts. We will have the rats discriminate between 23 Hz (12 ms) and the following: 6 Hz (46 ms), 8 Hz (32 ms), 11 Hz (24 ms), and 16 Hz (16 ms). With this information, we will determine a threshold for auditory temporal discrimination.

The next experiment we will perform to study the importance of A1 in temporal cue discrimination with sound bursts of differing temporal lengths is an optogenetic experiment that targets the A1 in the rat brain to silence it. We will then have the rats perform the same behavioral task as in the previous experiment to determine if the same auditory threshold is reached or if it requires a larger threshold.

This research can be clinically applied. Cochlear implant technology can be improved to help patients understand speech by improving the technology to have better temporal cue recognition. Furthermore, with aging and cognitive development disorders, like autism, patients experience timing cue deficiencies – particularly in differentiating between conversation and background noise (Marsh & Campbell, 2016). Thus, by furthering our understanding of auditory temporal cue discrimination in rats, we can apply the knowledge to human temporal discrimination in clinical settings.

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