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Rat Behavioral Discrimination of Temporal Cues in Species-Specific Vocalization

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Biological Sciences Senior Honors Thesis

University of Connecticut

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Abstract

Current behavioral and neurophysiologic studies propose that many animals can detect and discriminate the invariant statistics found in natural vocalization (Geffen et. al., 2011; Rodriguez et al., 2010). However, according to current research the neuronal mechanisms underlying the sound discrimination process is still unclear. While numerous auditory statistics have been manipulated, none has varied the temporal and tonal frequency cues independently in their synthetic call sequences, thus it is still uncertain whether rats rely on temporal cues in the sound envelope for communication.

The aim of this research is to determine whether or not rats rely on temporal cues in the sound envelope for call recognition and communication, as humans do similarly during speech perception. Studying the behavior and neural recordings related to sound and speech recognition can aid in the generating a biologically accurate computational model to further medical research of cortical response and pathways of sound and speech recognition.

Background

Humans are known to utilize spectral and temporal cues to recognize speech to such an extent as degrading temporal cues can dramatically alter speech recognition (Drullman et al., 1994; Souza et al., 2015; Shannon et al., Science 1995). Rats are an ideal animal model to explore the neurobiological mechanisms for discrimination of these varying temporal cues in vocalizations. They are social creatures that discriminate vocalization sequences in order to communicate like other mammals, indicating their ability to perceive and respond to sounds of varying periodicity, shape and spectral cues (Fig.1, compare A and B) (Wohr et al., 2007; Burgdorf et. al., 2008).

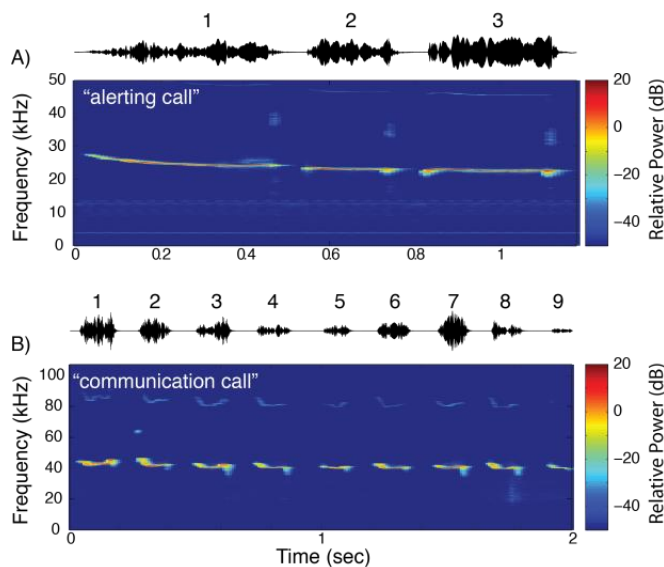


Figure 1. Temporal cue differences for two types of rat call sequences. Sound pressure level (SPL) waveforms and frequency spectrograms are shown on top and bottom, respectively. Numbers indicate individual calls. (A) An “alerting” call sequence with 3 call repeats creating ~2.5 Hz frequency modulations. (B) An “approach” call sequence with 9 call repeats creating ~ 4 Hz modulation frequencies. (Original recordings provided by our collaborator Markus Wöhr).

Wohr and colleagues discovered that rats are more likely to approach audio speakers playing back rat pro-social communication calls (Figure 1B), but avoided speakers playing back rat alerting calls (Figure 1A) (Wohr and Schwarting, 2007, 2009). Rats similarly to humans are able to detect and discriminate temporal variations in natural non-vocalized sounds (Geffen et al., 2011; Long and Clark, 1984; Kelley et al., 2006). Kelly et al. examined the behavioral capacity of rats to respond to sinusoidal amplitude modulated sounds and sounds of different

duration, and found that performance was best for larger modulation depths, lower modulation frequencies, and shorter sound durations (Kelly et al., 2006). Auditory cortical neurons spike in time with temporal cues in the sound envelope and computer simulations can accurately discriminate sound sequences based on these spiking patterns alone (Osman et al., SFN 2015). These results provide basic information about the neurobiological and perceptual limits of temporal cue perception in rodents that could play a role in perception of vocalization sequences.

Though Drullman and Shannon clearly demonstrate that temporal sound cues independent of tonal frequency cues are critical for humans to discriminate speech vocalizations, few studies have directly examined whether rodents have similar perceptual limits. Rodents have been shown to be able to discriminate a variety of species-specific vocalizations, such as when male and female rats approach a speaker upon the play of an unfamiliar pro-social call sequence (Wöhr and Schwarting, 2007), and female rats that have had pups will approach a speaker playing a rat pup vocalization sequence (Wöhr and Schwarting, 2008). In addition, Wöhr and Schwarting discovered that rats would approach synthetic call sequences that preserved tonal frequency as well as many of the basic temporal cues. This is significant as it indicates that there may be a subset of critical temporal cues used for recognition of communication sequences. As Wöhr did not vary the temporal and tonal frequency cues independently in their synthetic call sequences, it is still uncertain whether rats rely on temporal cues in the sound envelope for communication.

Hypothesis

Previous pilot studies conducted on humans (Figure 2) and brown rat (Figure 3) have concluded that performance on a modulation frequency discrimination task decreases as the difference between the modulation frequency decreases. Following the pilot study paradigm we

hypothesize that Long-Evans rats will discriminate natural rat vocalizations from the synthetic vocalization sequences in which the temporal cues have been “smoothed” by low-pass filtering the sound envelope using a basis spline (B-spline) filter, creating shaped modified vocalizations.

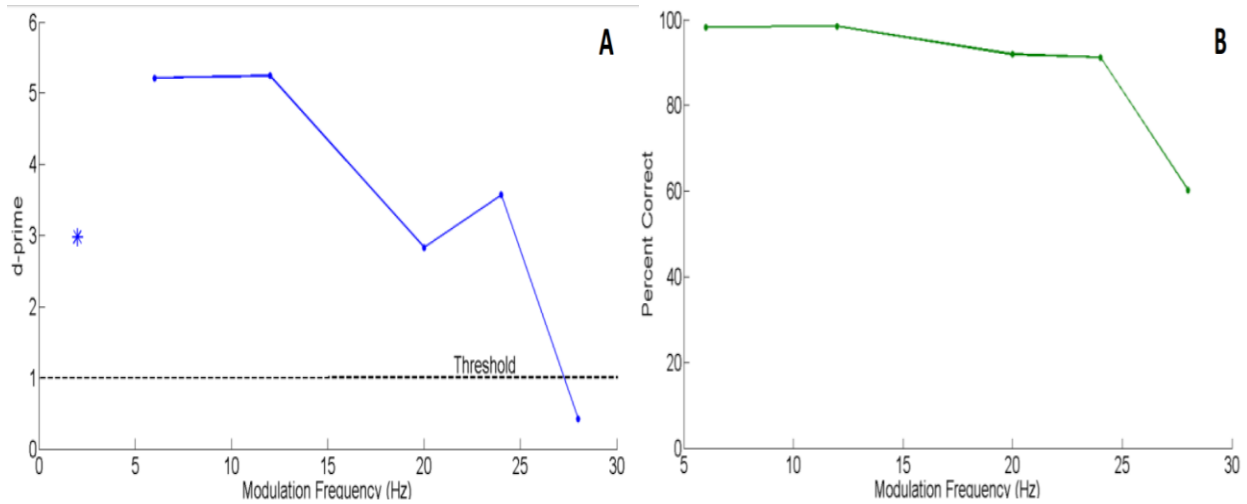


Figure 2: Threshold for Discrimination of Modulation Frequency in Humans (N=3). (A) Psychometric function for discriminating 5 No-Go sound modulation frequencies from a reference Go-Sound in Human. Humans can discriminate 23 Hz (No-Go) from a 32 Hz (Go) sound with a ~70% probability (d' -prime~1), indicating above chance discrimination. Threshold of d' -prime=1 indicates level at which discrimination is at chance (50%). Blue star indicates a d' -prime of 3 from rat pilot data (N=4) at 2 Hz. (B) Percent correct detection of modulation frequency in humans. The response curve indicates humans can discriminate 28 Hz (No-Go) from 32 Hz (Go) sounds at above 50% chance levels.

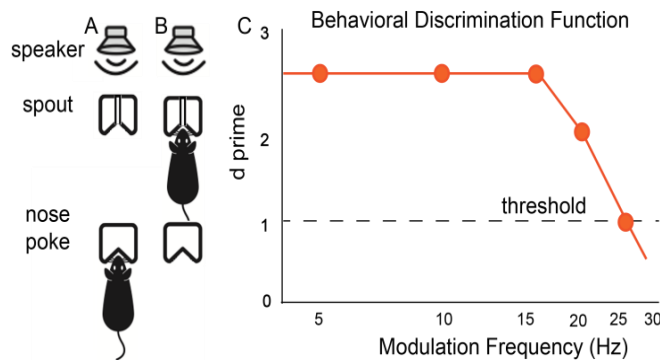


Figure 3. Sound discrimination go/no-go task and behavioral discrimination of MF. A) Animals are trained to nose-poke a cup to initiate a trial. B) Then to approach and lick the spout when they hear the “go” sound for reward. C) Pilot data from one animal with a go-sound noise envelope MF= 30Hz. Threshold is d' -prime=1. Von Trapp, Read, Sanes

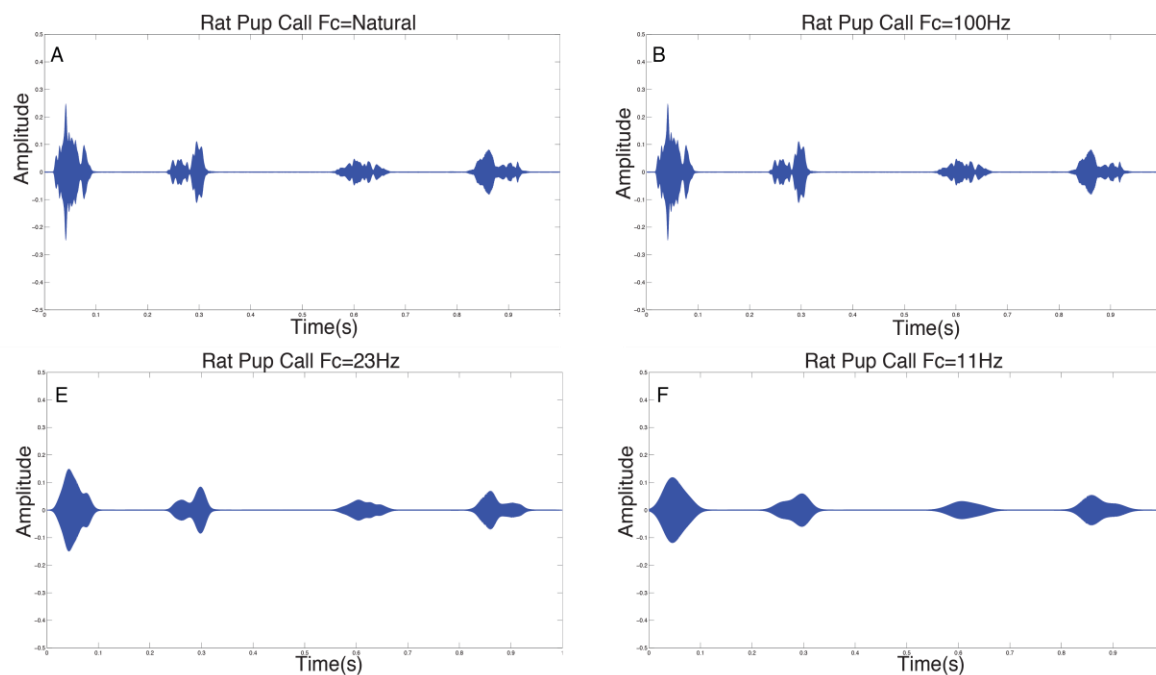
Specifically, we hypothesize that rats discriminate differences a natural rat vocalization “go” sound from a set of synthetic vocalization sequences “no-go” sounds that have been low-

pass filtered to remove fast modulations in the sound envelope. The methods used to create the sound filtering and discrimination test are similar to those used to demonstrate the dependence of speech discrimination on temporal cues (Drullman et al., 1994).

Experimental Design

Twelve Long-Evan rats obtained from Charles River and housed in Bousfield vivarium at the University of Connecticut were maintained in accordance to IACUC protocol A15-054. Ten male young adult rats were incrementally trained to discriminate natural vocalization sequences (go sounds) and five variations of the B-spline smoothed vocalization sequence (no-go sounds) while two additional rats will remain as the weight controls. The rats were food restricted and their weights monitored daily to maintain a healthy goal weight of at least 85% of control animal body weight according to IACUC protocol A15-054.

The rats were food restricted during the weekday and put on *ad libitum* food (protein pellets) during the weekend. However, in order to ensure that the rats began the experimental weekday with enthusiasm, twenty-four hours prior to training the rats were placed back on food



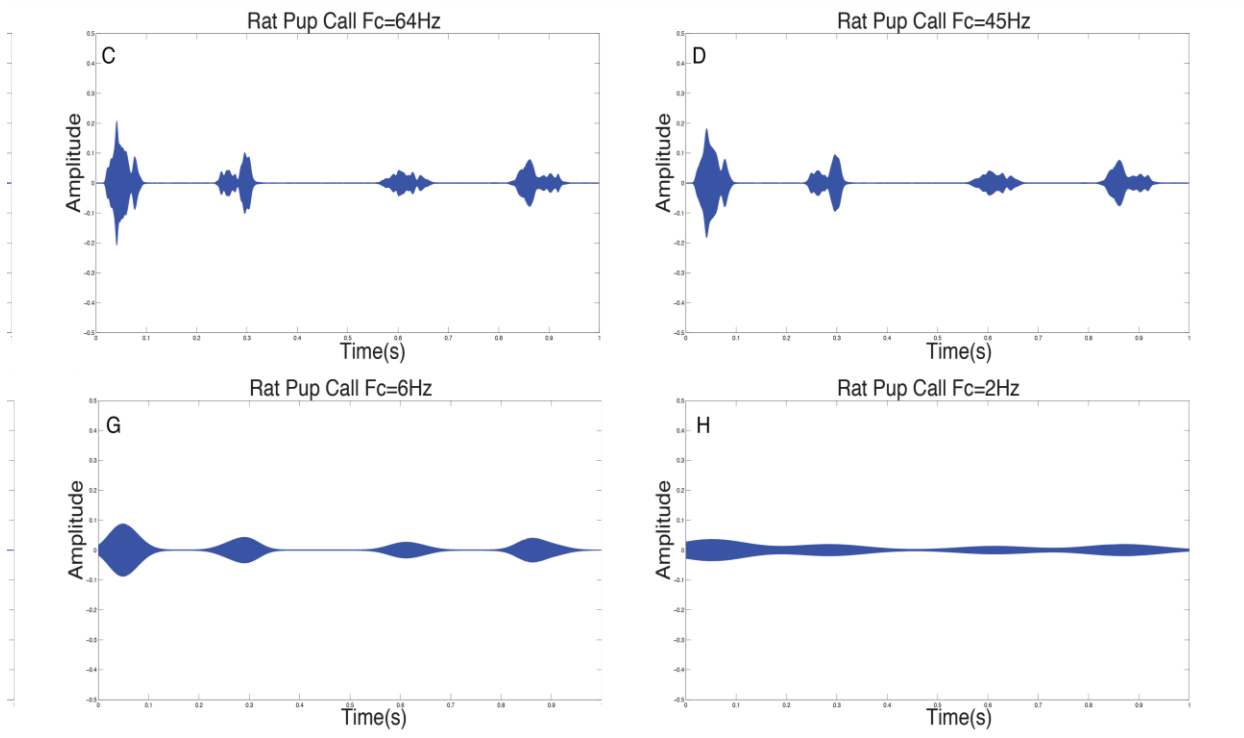


Figure 3. Natural and B-spline filtered vocalization sequences. (A) Natural rat pup vocalization. (B-H) Filtered vocalization sequences generated using a B-spline filter cutoffs of 100Hz, 64Hz, 45 Hz, 23 Hz, 11 Hz, 6 Hz and 2 Hz of the natural vocalization.

restriction. The rats were at no point placed on any water restriction. The minimum daily food intake was set at 5% body weight. The food was accounted for by weighed protein pellets (rodent meal) and strawberry protein liquid, Ensure Plus, (assuming a density of 1g/ml). Rats were weighed at the end of the day's training and if the weight of a rat was found to be below 85% of the control weight or the rat showed signs of poor health, such as porphyrin staining or abnormal skin turgor they were taken out of training and put on ad libitum food until the problem was resolved.

The ten food restricted rats were trained and tested on their ability to discriminate natural rat pup vocalization sequences (Figure 3A) and pro-social calls, from B-spline filtered vocalization sequences. Synthetic variations of the no-go sounds will be generated using a B-

spline filter cutoffs of 45 Hz, 23 Hz, 11 Hz, 6 Hz and 2 Hz (Figure 3B, C, D, E, F, G, H) on the original go sound. This range of temporal filters should yield the best results as previous data from pilot studies in the Read-lab and on a report by Drullman demonstrates that speech perception declines with low-pass filters of 32 Hz or greater.

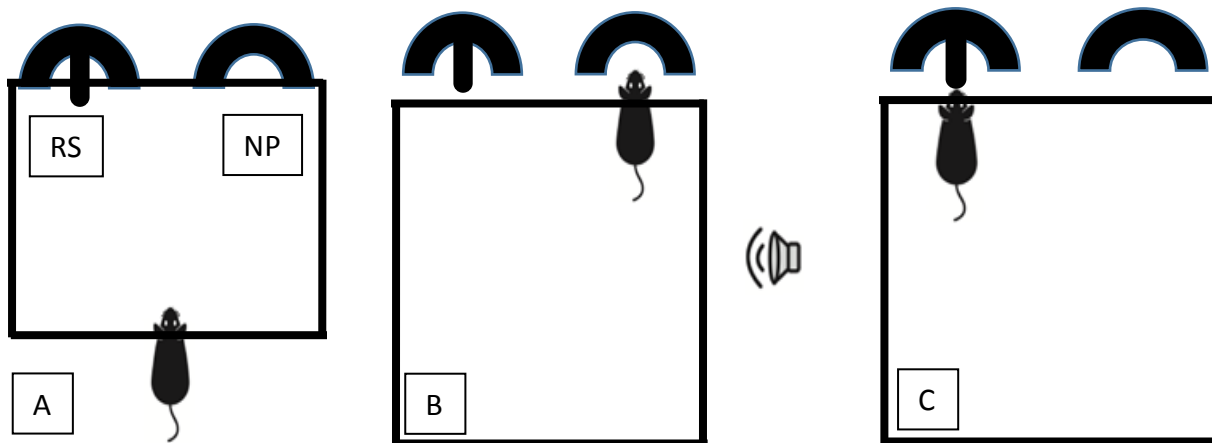


Figure 4: Behavior paradigm. (A) The rat is placed within a 2x2 foot cage that contains a reward spout and a nose poke. (B) Triggering the nose poke (NP) plays a randomly selected vocalization to the rat. (C) Upon correctly identifying the go sound and approaching the spout, 0.2 ml reward is dispensed from the reward spout (RS). If the rat approaches the RS during a no-go sound instead of a reward, a bright light is displaced as an aversion.

In all behavioral studies here, animals were trained to discriminate natural and filtered communication sequences in an operant “go/no-go” behavioral task (Figure 4). Sounds were delivered through a speaker mounted above the rats within the Read-lab frequency calibrated sound isolated chamber. During the initial training, “go sounds” were played each time a rat serendipitously poked his nose to interrupt a photo-diode beam located in the “nose poke” and a strawberry protein liquid was delivered automatically as a reward. Rats were trained incrementally to initiate operant trials themselves by making a nose poke that signaled our computer to play one of the sound sequences and deliver reward under appropriate conditions.

In initial study, subjects were trained and tested to discriminate the modulation frequency of a synthetic noise burst sound sequence. To monitor performance and learning and to determine perceptual thresholds, we plot the “percent correct” performance curves for each stage of training and testing. Figure 6 (A, green line), indicates that rats learned with 80% correct performance on average to navigate to the spout upon hearing the “Go-Sound” to obtain liquid strawberry protein as a reward. In the second training phase, rats were trained to “turn the speaker on” by making a trial initiation nose-poke. This training took about 10 days to reach almost 80% correct performance as shown (Fig. 6B, blue line). In the third training phase, rats were trained and tested for ability to discriminate the “Go-Sound” from a newly introduced “No-Go” sound. In phase three, whenever they approach the food dispenser after a go sound, the response is considered a “hit” and whenever they approach the dispenser after hearing a no-go sound, it is considered a “miss”. A bright light is flashed and a 6 second time-out is introduced after a “miss” trial to deter the rats from making the same mistake. In phase three, about 40% of the sounds played after animals made a trial initiation nose-poke were “no-go” sounds and 60% were “Go-Sounds”. As illustrated (Fig. 6C, 33 Hz, red line), animals continued to showed a high percent correct performance (near 80%) for detecting the “Go-Sound” under these conditions. It took about 40 days for animals to learn to correctly withhold the response to the “No-Go” sounds such that the “No-Go” percent correct performance curve on day 65 was near 0% and by day 40 was above 50% correct performance (Fig. 6D, 2Hz, orange line). .

To monitor performance and learning and to determine perceptual thresholds, we plot the “percent correct” and a discrimination index (d'). Percent correct is the number of hits divided by the total sounds presented (Go or no-Go) multiplied by 100. The percent current

detection of the “go-sound” during the learning phase is illustrated in Fig. 6A. The d-prime is based on hit rate (HR) and false alarm rate (FAR) and calculated as shown below (Green, 1966):

$$HR = \frac{\text{number of hits}}{\text{(number of hits+ number of misses)}}$$

$$FAR = \frac{\text{number of FAs}}{\text{(number of FAs + number of hits)}}$$

$d' = Z(HR) - Z(FAR)$, where $Z()$ is the inverse of the cumulative normal functions.

Results

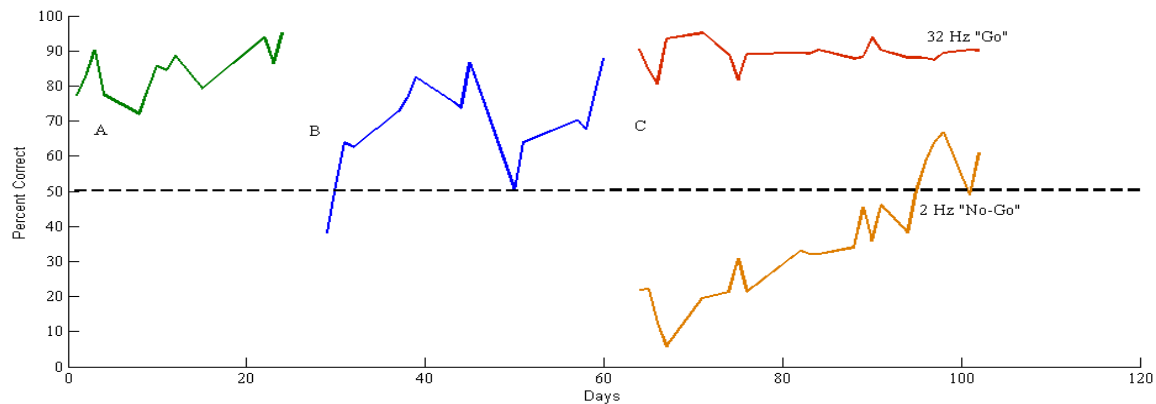


Figure 6. Percent Correct Response in Long Evans Rats to Sound Modulation Frequency. (A) Introduction of reward spout and 32 Hz “Go” sound. (B) Introduction of nose-poke trial initiation. (C) Introduction of 2 Hz modulation frequency “No-Go” sound (Orange). The dotted line indicates the threshold for better than chance performance.

In this initial study using synthetic noise burst sequences instead of vocalization sequences we discovered that as predicted in the hypothesis, the Long-Evans rats were able to successfully discriminate the modulation frequency. As shown in Figure 6, rats discriminate between the 32 Hz “go sound” and the 2 Hz “no-go sound” with increasing accuracy over time. As we predicted, there is a decrease in the percent correct detection of a “no-go sound” as the modulation frequency approaches that of the “go sound”, with frequencies higher than 16 Hz being below the 50% correct rejection rate.

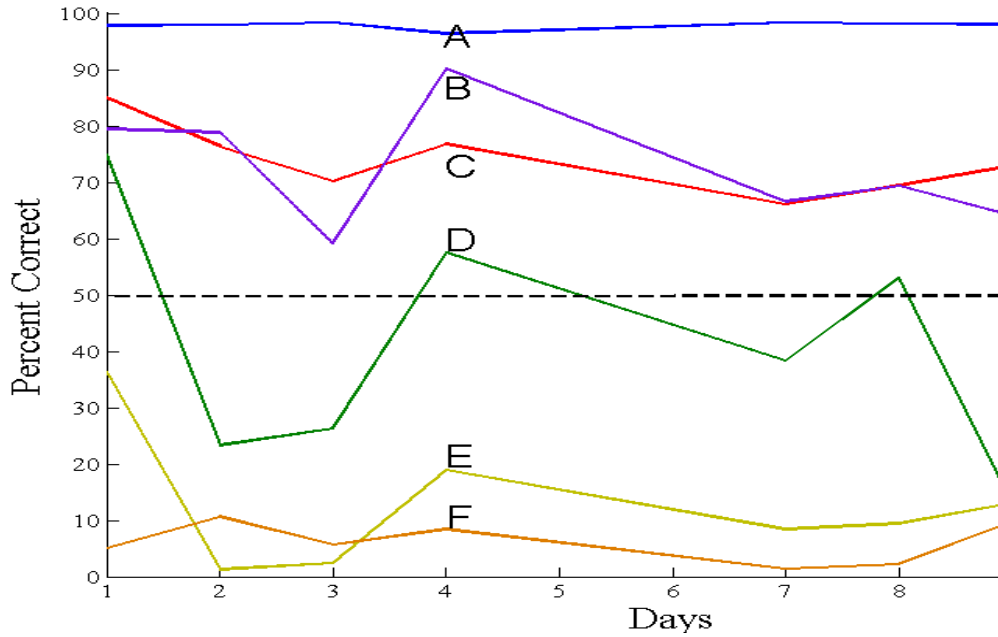


Figure 7. Percent Correct Response in Long Evans Rats to Sound Modulation Frequency. (A) 32 Hz “Go” (B) 10 Hz (C) 2 Hz (D) 16 Hz (E) 23 Hz (F) 28 Hz.

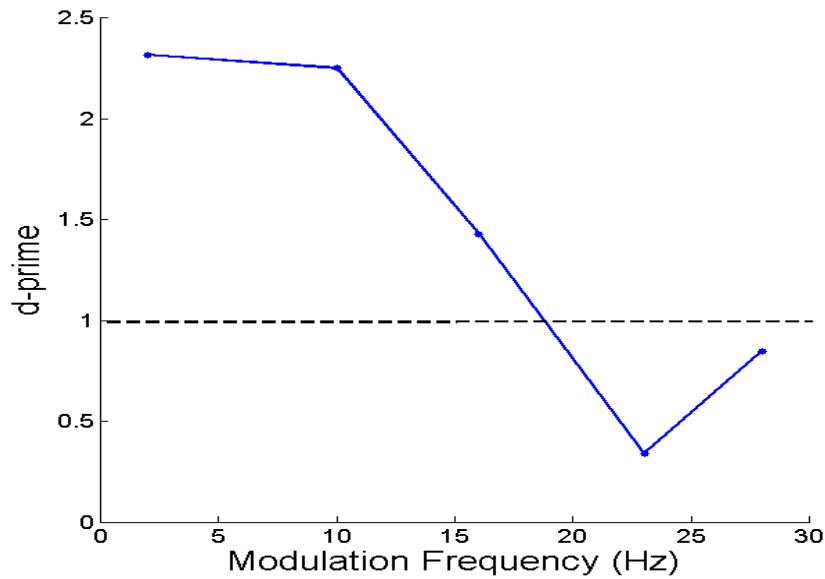


Figure 8. Threshold for Discrimination. d-prime of ~1. (i.e. ~70% probability)

Though percent correct response functions are useful for monitoring learning rates they do not determine the perceptual limits for discriminating sound cues. Hence, we computed a d-prime index for all pairwise comparisons between a 32 Hz “Go” sound versus each of 5 “no-go”

sounds that varied in modulation frequency. In Figure 8, the perceptual threshold is indicated with a dotted line where discrimination is a better than chance. The 16 Hz modulation frequency was the threshold for behavioral discrimination. Note that discrimination is maximal (>2) when the go-sound is compared with the 5 and 10 Hz no-go sounds. Rats failed to discriminate 23 and 29 Hz modulation frequencies from the 32 Hz “Go” sound. The d-prime response function is steep and indicates that modulation frequency must be about half as fast as the go sound in order for animals to detect a difference. This initial study indicates the time-course for discrimination learning with our paradigm and the perceptual threshold for this discrimination.

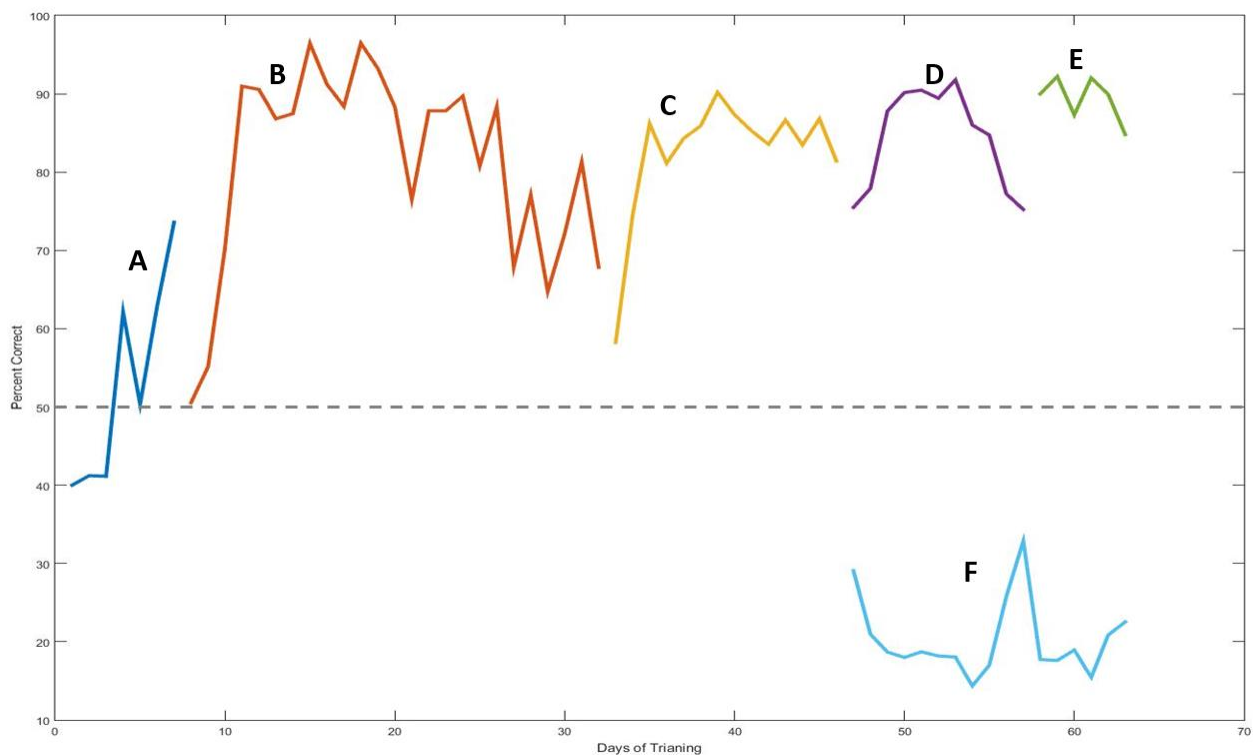


Figure 9. Percent Correct Response in Long Evans Rats to Vocalization Modulation Frequency. (A) Introduction of reward spout and 100 Hz “Go” sound (fundamental frequency only). (B) Introduction of nose-poke trial initiation. (C) Continuation of “B” after the introduction of the first harmonic. (D) Continuation of “C” after change of amplitude from 70 to 73 dB. (E) Introduction of red filtered light in the training booth. (F) Introduction of 2 Hz modulation frequency “No-Go” sound. The dashed line indicates the threshold for better than chance performance.

In the next study we used the same behavioral paradigm to train and test rats ability to learn to discriminate more complex natural vocalization sequences. As in the prior study, rats quickly learned to approach the spout for strawberry ensure reward (Figure 9A, blue line). In phase two, it took about a week for rats to learn to play the speaker and initiate a trial with a nose poke and to correctly navigate back to the spout for reward (Figure 9B, orange-brown line). It took about a week for animals to reach > 80% correct performance in phase two. In this phase, while waiting for a consistent performance metric we noticed numerous instants of a sudden drops indicating a fault in the behavioral training methodologies.

For example, as evident in Figure 9B, there is a continuing decrease in the performance metric from higher than 95% to below 70% accuracy. As it can be seen in Figure 10, the spectrogram of the ultrasonic natural rat vocalization has a harmonic at 80k Hz in addition to the fundamental frequency at 40k Hz. In efforts to improve the behavioral detection, we added the natural harmonic to all the synthetic variations we created in order to better fit to the natural vocalization and we noticed a sudden increase in the percent correct detection over about 10 days (Figure 9C, yellow line). As the days of training continued there was another decrease in the performance metric, (Figure 9D, purple line), which we attributed to the decreased hearing of the rats due to their age and changed the amplitude from 70 to 73 dB resulting in restoration in the accuracy of discrimination (Fig. 9E, green line). We are currently in the final phase of testing discrimination of natural and temporally smoothed vocalization sequences. Fig. 9E (cyan colored line) indicates that rats do not yet correctly withhold a response for the “No-Go” vocalization sequence. Hence, the percent correct detection of the “No-Go” sound is less than 50% or lower than chance. Indeed the overall performance dropped. We took measures to enhance the light flash sensory by installing a red filter over the background light for the training

booth. As a result, the red filtered light increases the perceived brightness of the “miss” trial light evoking a more aversive response from the rats. Based on our first study, we anticipate this group of animals will learn to discriminate natural versus synthetically smoothed vocalization sequences within the next month (Figure. 6).

Discussion

Temporal timing cues are important for all animals to communicate with each other and the environment surrounding them. The aim of the project is to determine whether or not rats rely on temporal cues in the sound envelope for communication.

We were able to determine the ability of the rats to behaviorally differentiate between different sound modulations. We hypothesized that the ability of the rats to discriminate the “go” from the “no-go” sounds would decrease in accuracy as the modulation frequency increased. This was confirmed as our data shows that there is decrease in discriminating between sound modulation frequencies as the “no-go” sound became increasingly similar to the “go” sound.

The d-prime and average percent correct performance metrics displayed a sudden drop in performance at 16 Hz sound modulation frequency, with discrimination dipping below 50% correct for modulation frequencies above 16 Hz (Figure 7) and d-prime falling below 1 (Figure 8). This indicates that the behavioral limit for discriminating sound rhythm in rats is close to 16 Hz.

Our next behavior study was to examine the ability of the rats to discriminate another temporal cue, shape. In this study we were successfully able to train the rats to discriminate between a natural vocalization (go sound) and the most shape modified vocalization (nogo sound $F_c=2\text{Hz}$). Further training needs to be completed to generate a full discrimination curve as we did with the modulation frequency timing cue.

At the completion of the training, we will examine the neural and cortical mechanisms underlying this behavioral limit for discriminating shape and periodicity timing cues in vocalization sequences.

As shown in previous studies, midbrain and cortical neurons spike responses separately encode sound envelope shape and periodicity information using onset spike and sustained spike activity, respectively (Zheng & Escabi, 2008). Spike timings also have been shown to increase in precision with increasing envelope slope and modulation frequency of sinusoid amplitude modulated sound (Zheng & Escabi, 2013). This indicates that auditory pathway neurons encodes shape and periodicity cues through the use of spike timing precision in their spiking patterns.

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