Spring 5-1-2022

# Case Study: Effects of Ultrasonic Vocalizations on Rat Behavior and Place Cell Remapping in the Hippocampus

Qingli Hu
qingli@uconn.edu

*Case Study: Effects of Ultrasonic Vocalizations on Rat Behavior and Place Cell Remapping in the Hippocampus*

Qingli Hu

qingli@uconn.edu

Advised by: Etan Markus, PhD

## Abstract

Spatial information is known to be encoded in the hippocampus, and small changes in the environment can alter the way that it is represented by our hippocampal place cells in a process called remapping. Hearing is an important sense that can be used to orient ourselves and react to the environment accordingly. In this case study, a rat model is used to test the effects of emotional auditory stimuli, behaviorally significant ultrasonic vocalizations (USVs) (50 kHz, emitted during play; 22 kHz, emitted during danger), on rat behavior on a linear track and place cell remapping in the hippocampus. Behaviorally, it was found that the rat slowed down significantly going in one direction on the track when exposed to these novel USVs. On a neuronal level, the firing rates of place cells trended to increase as the rat was exposed to the 50 kHz sound but not the 22 kHz sound. Some place cells remapped, and those changes either persisted or reverted after the auditory stimuli were turned off, while others did not.


## Introduction

The hippocampus is the brain's memory processor. It is where we encode and retrieve episodic memories. One can find a damaged hippocampus in patients with Alzheimer's disease and epilepsy, and it has been the topic for researchers to find all its functions and connections to other brain areas in order to understand normal physiological functioning of the hippocampus. The discovery of place cells paved the way for studying the hippocampus as a spatial map (O'Keefe & Dostrovsky 1971)). Place cells make up a high fraction of all cells in the hippocampus (O'Keefe 1976)), and they selectively activate when we move through space (Colgin et al., 2008). As a result, we map our surroundings based on differential activation of place cells in distinct locations, so place cells serve to construct a spatial map that can be the basis of episodic memory (O'Keefe & Nadel 1978).

In the past three decades of investigating place cell activation patterns, researchers discovered remapping, which means that the rate and specificity of place cell activation can be modified by minute changes in the environment (Muller et al., 1991), such as shape of enclosure (Muller & Kubie 1987)), amount of lighting (Quirk et al. 1990), and replacement of an object in the environment (Bostock et al. 1991; Kentros et al. 1998). This evidence suggests that the neural circuit for remapping is dynamic, and that certain associations allow mammals to automatically recognize their location to help spatial navigation. It also suggests that the representation of an environment gets updated, as would be expected during the formation of an episodic memory.

With the development of technology to record physiology of individual neurons in the hippocampus, research looking at remapping of single units has become feasible. Simultaneous recording of multiple neurons was made possible with the use of multi-microelectrode devices implanted into the brain (Krüger & Bach 1981). Using triangulation, tetrodes allow for the recording and separation of distinct neurons detected by the same tetrode, and the 16 tetrodes are inserted throughout the hippocampus to record from different regions. The dorsal region is generally for cognitive and spatial processing, whereas the ventral region relates more to emotions and stress (Fanselow & Dong 2010*).*

**Auditory Stimuli**

In people, there has been evidence that familiar music evokes memories from the past, including information about locations (Greensfelder 2016). There are significant neural connections between the auditory cortex and the entorhinal cortex of the parahippocampal cortex (Chen et al., 2013), suggesting that sensory sound inputs may affect place cell firing in the hippocampus, thus remapping.

Rats emit various behaviorally significant vocalizations in the ultrasonic frequency range (>20 kHz) (Constantini & D'Amato, 2006; Knutson et al., 2002). In aversive situations, rats tend to emit 22 kHz ultrasonic vocalizations (USVs), and in affective situations such as playing and tickling, rats emit short USVs in the 50 kHz range. Previous studies have shown that playback of 22 kHz USV can induce anxiety-like behaviors such as freezing and corresponding neural changes in rats (Brudzynski & Chiu, 1995; Kim et al., 2010; Demaestri et al., 2019). However, stressed rats have been shown to fail to exhibit these responses (Shukla & Chattarji, 2021). On the other hand, playback of 50 kHz USV induce approach behavior in Wistar rats in a radial maze (Wöhr & Schwarting, 2007).

The present study aims to investigate the effect of these USVs in modifying place cell representation of an environment. While there are connections between the auditory and parahippocampal cortices (Chen et al., 2013), it is unknown how auditory stimuli can affect place cell remapping. Rats trained on a familiar runway had their hippocampal cell activity recorded using a 64-channel hyperdrive targeting dorsal and ventral regions. Novel USVs were then introduced to the environment. This project can shed light on how the auditory sensory input experienced during spatial navigation contributes to its representation in the hippocampal spatial map, and ultimately how they are encoded and interpreted.

## Methods

**Subjects**: One male F-344 rat, approximately ten months old, was food restricted to 85% of its *ad libitum* weight. It was single housed, with a 12 hr light/dark cycle, and had *ad libitum* access to water. It was never previously exposed to USV playback recordings. Protocols were approved by the University of Connecticut IACUC.

**Apparatus**: 130cm x 10cm linear track maze with a speaker placed 20 cm away from the midpoint, level and facing the track.

**USV Playback Equipment**: The USV files (.wav) were provided by Dr. Markus Wöhr of the University of Marburg in Germany. They are USVs from Wistar rats. Each is one minute long. The audio is controlled to approximately 70 dBs when measured 20 cm away. Audio was played from a desktop computer through an E-MU 0404 USB 2.0 Audio MIDI Interface to be converted to a digital signal. It was then amplified using an ultrasonic portable power amplifier (Avisoft-Bioacoustics). The signal was finally played using the ultrasonic dynamic speaker (Vifa, Avisoft-Bioacoustics). The frequency and power of the output signal was verified with an ultrasonic microphone using the Wildlife Acoustics Inc Echo Meter Touch 2 Bat Detector.

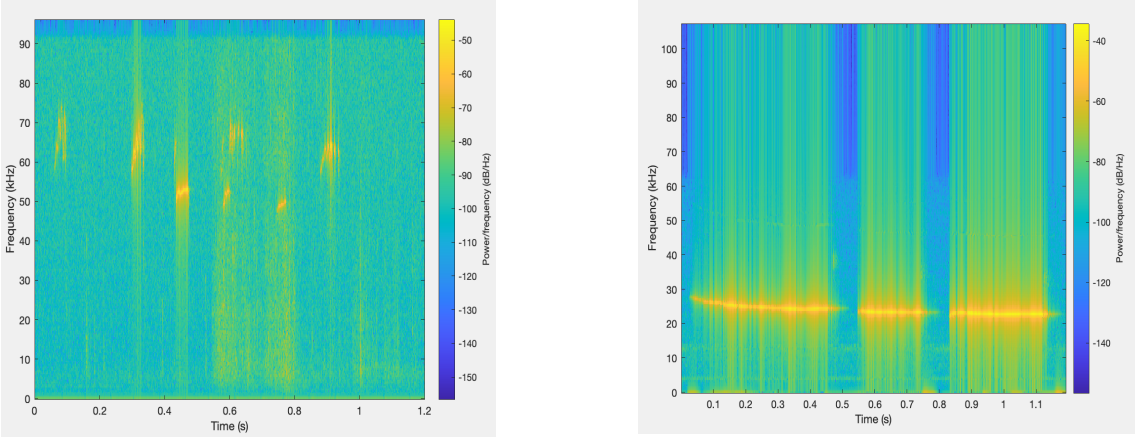


**Figure 1.** Example spectrograms of 50 kHz **(left)** and 22 kHz **(right)** USVs. X-axis represents time from 0 to 1.2s, and Y-axis represents frequency in kHz from 0 to 100 kHz). Warmer colors signify higher power at the specific frequency. 22 kHz USVs are generally longer in duration, possibly

lasting for several seconds. 50 kHz USVs are much shorter in duration, and there is more variability in frequency, ranging from 50 to 65 kHz. Different chirps have different waveforms as well.

**General Procedure:**

*Pretraining* - Before surgery, the animal was trained to run back and forth on a linear maze for a food reward. The rat was considered "ready" for surgery after reaching at least 60 one-way trips in ten minutes for two consecutive days.

*Surgery: Hyperdrive implantation* - After completing the initial training on the maze, the rat underwent open-brain surgery to have a hyperdrive consisting of 16 movable recording tetrodes and two ground electrodes inserted into the hippocampus. Half of the recording tetrodes were inserted into the dorsal hippocampus, and the other half were in the ventral hippocampus. The tetrodes were inserted above the hippocampal cell layers to allow room for them to be advanced. Insertion sites were determined using the rat brain atlas as well as previous experience, at 3.4mm posterior to bregma.

*Place cell recording* - After a 10 day recovery, the rats were retrained on the runway and neuron activity recorded. The tetrodes were advanced into the hippocampus cell layer. Each tetrode were adjusted independently. Observation of theta rhythm and sharp wave ripples distinct to the hippocampus in the raw data recordings signify that the tetrode tip has reached the hippocampal cell layer.

*Experimental Procedure* - Rat position and heading direction (based on two-colored LEDs above the head) on the maze were tracked by a video camera at 30 fps and plotted on a position vs. frame plot. Each day consisted of three sessions. The first and third sessions were set up the same way, and the second session had the auditory stimuli played throughout. Data of all three sessions from one day were recorded as one data file, and the animal underwent three days of

data collection. On the first day, the playback equipment was set up, but no sound was played during the second session in order to control for novelty effects of the equipment in the environment. On the second day, 50 kHz USVs are played during the second session, and on the third day, 22 kHz USVs are played during the second session. Recording begins when the rat is placed in a small cage next to the linear maze for five minutes, after which the rat is placed on one end of the maze. The animal completes 5 ups and downs on the maze for each session, getting a food reward on each end, then placed into the cage for five minutes to rest. Throughout all sessions, the hyperdrive on the head will be plugged into the computer to record neuron firing activity.

**Data Analysis:** Only recordings from neurons which are isolated enough and free of noise signals were kept for analysis. This was done using MATLAB and MountainSort, which is an automated cluster cutting software, from which we get a starting point to further inspect the metrics for each isolated cell. Tracking data was separated to distinguish when the rat is moving up versus down the maze because heading direction can cause differential place cell firing. Then, firing activity from neurons were plotted onto the position vs. time plot. The rate of firing and place field data were calculated, and differences within and between sessions can be analyzed. This analysis takes place in MATLAB as well.

*Histological verification* - By the end of all data collection, the animal was sacrificed, the tetrodes removed, and the brain was perfused and sectioned into 75μm thick slices along the coronal axis and Nissl stained for cell bodies. Sections encompass all of the hippocampus. Because the insertion of tetrodes will leave a thin track of tissue damage, the tetrode tracks were be reconstructed to see from where exactly each tetrode was recording.

# Results

*Behavior*

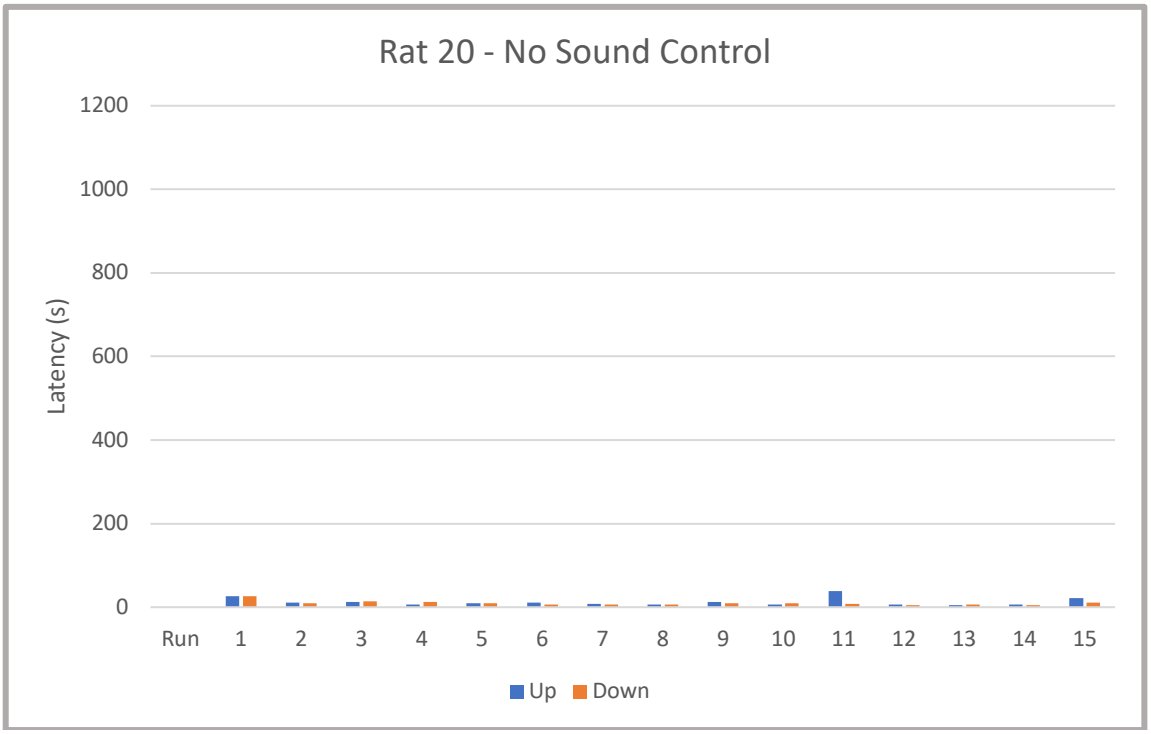


**Figure 2.** Latency (s) of each up and down trial (from one end of the track to the other, bidirectional) of the experiment on Day 1, where the no sound was played throughout all sessions.

This was the first day that the animal ran on the track with the playback equipment set up. There are three sessions, each with five round trip runs, consisting of an up and a down on the track, for a total of 15 up runs and 15 down runs. There was no break in between the sessions, and because this was the control day, no sound was played during any of the sessions, making all of them the same. Latency was low for all up and down runs, which suggests that the novel addition of the speaker to the environment had little behavioral effect on the rat.

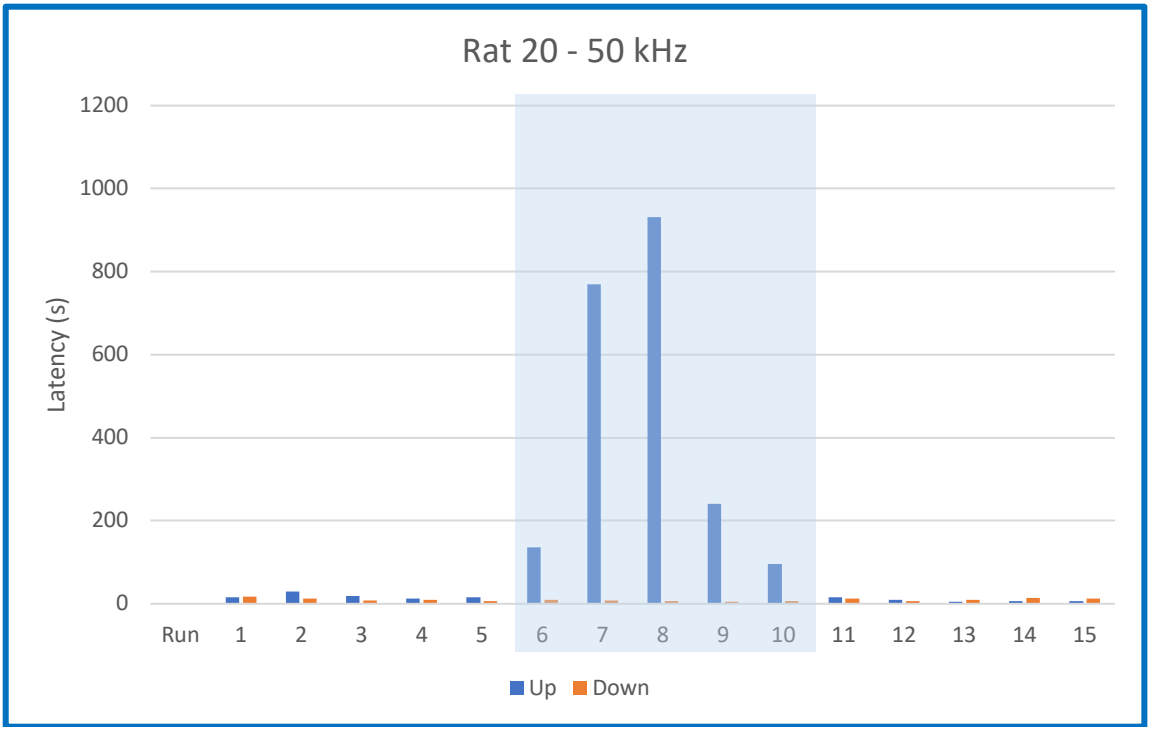


**Figure 3.** Latency (s) of each up and down trial of the experiment on Day 2, where the 50 kHz sound was played throughout session B (highlighted).

The task on this day was the same as the control day except that during the second session (Session B), the 50 kHz vocalizations were played from the speaker. There was a total of 15 round trip runs on the track, with the sound turning on for runs 6 through 10. The animal slowed down significantly during up trials of Session B. This suggests that the animal was behaviorally affected due to the addition of the USVs, not the speaker itself. Though food deprived, the rat's interest in the food rewards seem to decrease when the 50 kHz sound was on.

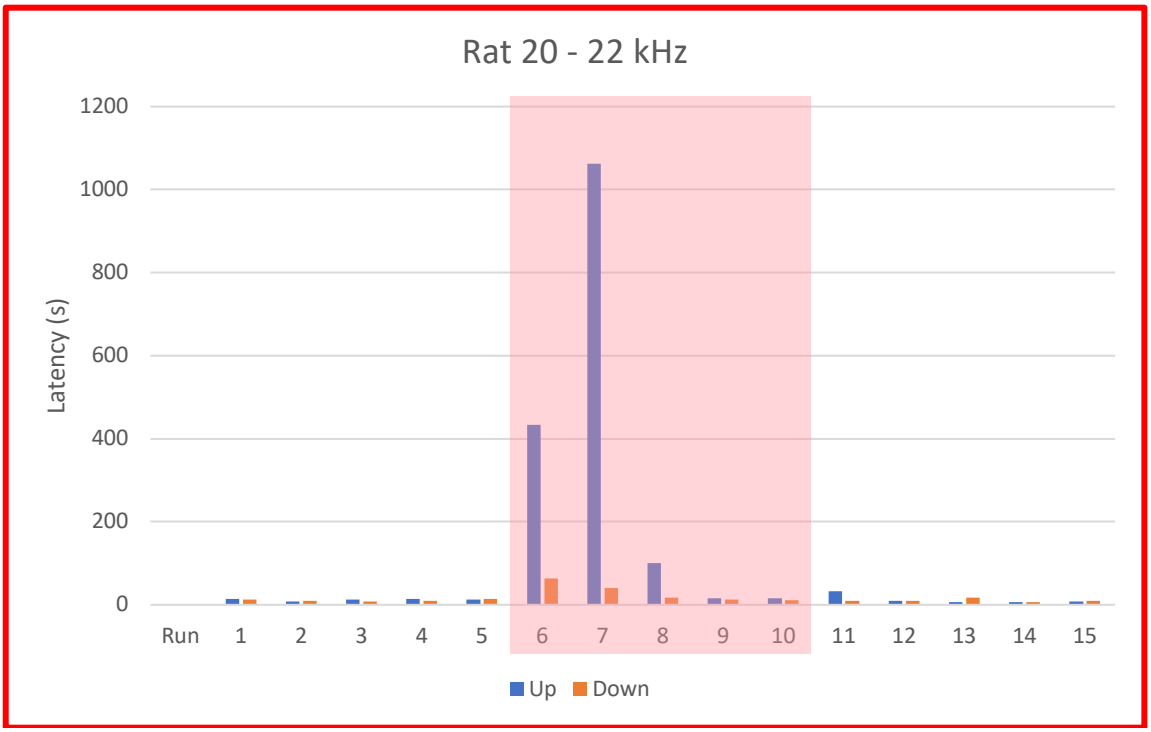


**Figure 4.** Latency (s) of each up and down trial of the experiment on Day 3, where the 22 kHz sound was played throughout session B (highlighted).

The task on this day was the same as the control day except that during the second session (Session B), the 22 kHz vocalizations were played from the speaker. There was a total of 15 round trip runs on the track, with the sound turning on for runs 6 through 10. The animal slowed down significantly during up trials of Session B, though seemingly returning to a normal pace after 3 runs with the sound on. This suggests that the animal was behaviorally affected due to the addition of the USVs, not the speaker itself. Though food deprived, the rat's interest in the food rewards seem to decrease when the 22 kHz sound was on. Compared to Day 2 (50 kHz), the change in latency was not present throughout the entirety of session B up trials, suggesting that the 50 kHz sound may be more behaviorally significant in the long term than 22 kHz.

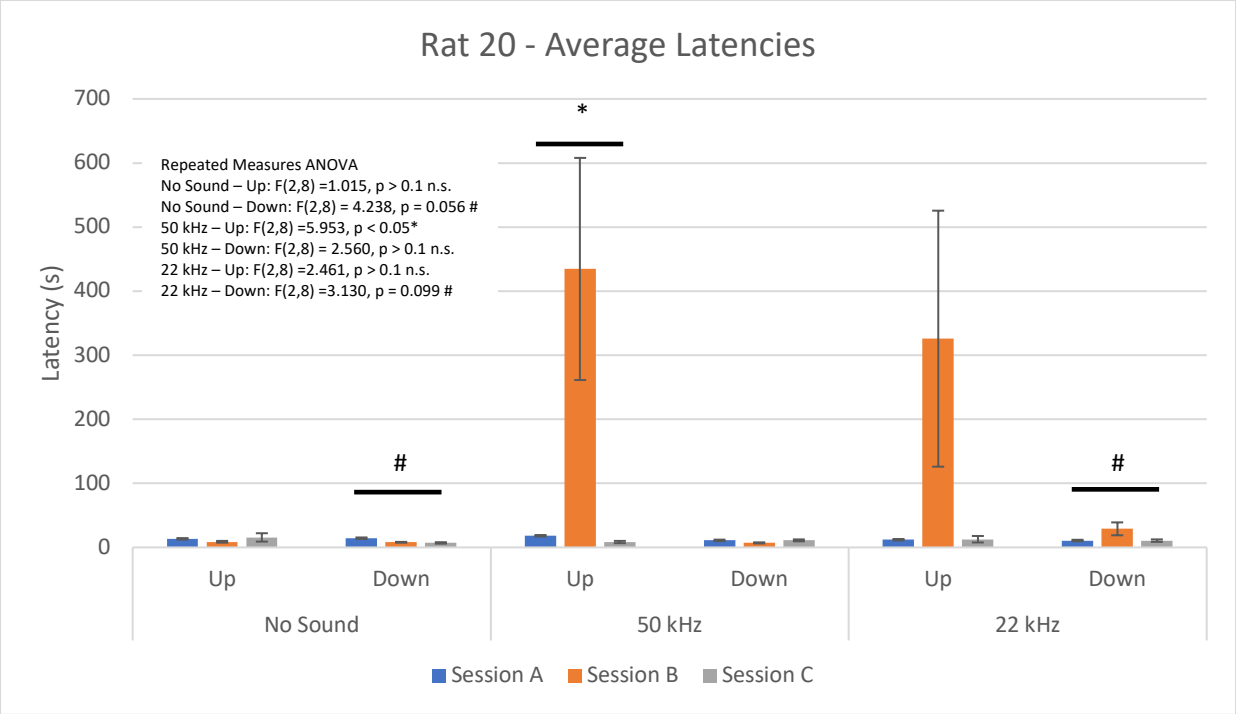


**Figure 5.** Average latencies (±SEM) of all three days of the experiment, separated by ups and downs.

The average latencies combines data from Figures 2, 3, and 4. The up trials on the 50 kHz day differed across the three sessions (repeated measures ANOVA: $F(2,8) = 5.953$, $p < 0.05$), with the second session having a much higher latency than Sessions A and C. The down trials for both day 1 (no sound) and day 3 (22 kHz) were trending to be different (repeated measures ANOVA: no sound down $F(2,8) = 4.238$, $p = 0.056$; 22kHz down $F(2,8) = 3.130$, $p = 0.099$). For day 1 with no sound, this variability may be due to the novelty of the speaker itself since this was the first time that the rat was exposed to it in the track environment. With more trials, this difference is likely to go away. Surpringly, the average latency of up trials for day 3 (22 kHz) were not statistically significant, and this is likely due to the high variability in the data. The trending significance in the down trials for this day is also likely to go away with more trials.

*Firing Rates*

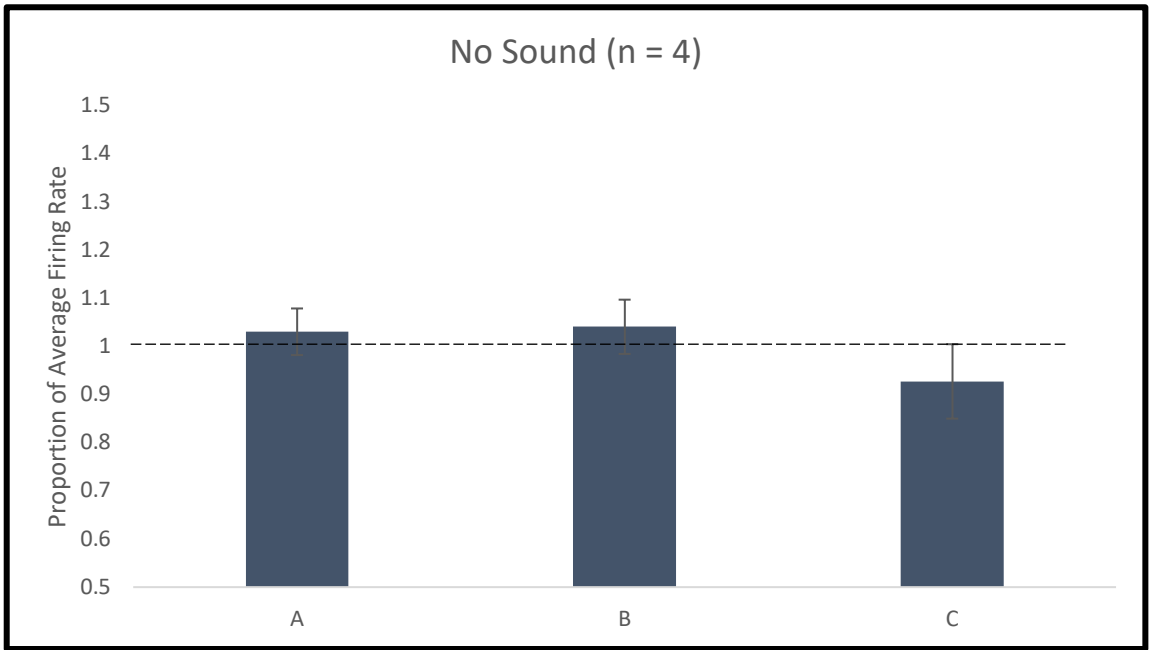


**Figure 6.** Proportion of average firing rate (±SEM) of cells recorded from the hippocampus of the experiment where Session B had no sound introduced.

As the animal traversed the maze, the single unit activity was recorded by the hyperdrive. The proportion of average firing rate was found by dividing the single session rate by the average of all three sessions. A value higher than 1 signifies that the firing rate of that session is higher than the average firing rate of all three sessions, and a value lower than 1 signifies the opposite. There is no difference in the average firing rate in sessions A, B, and C (one way ANOVA, not significant). This is to be expected because the condition and environment is constant across these three sessions.

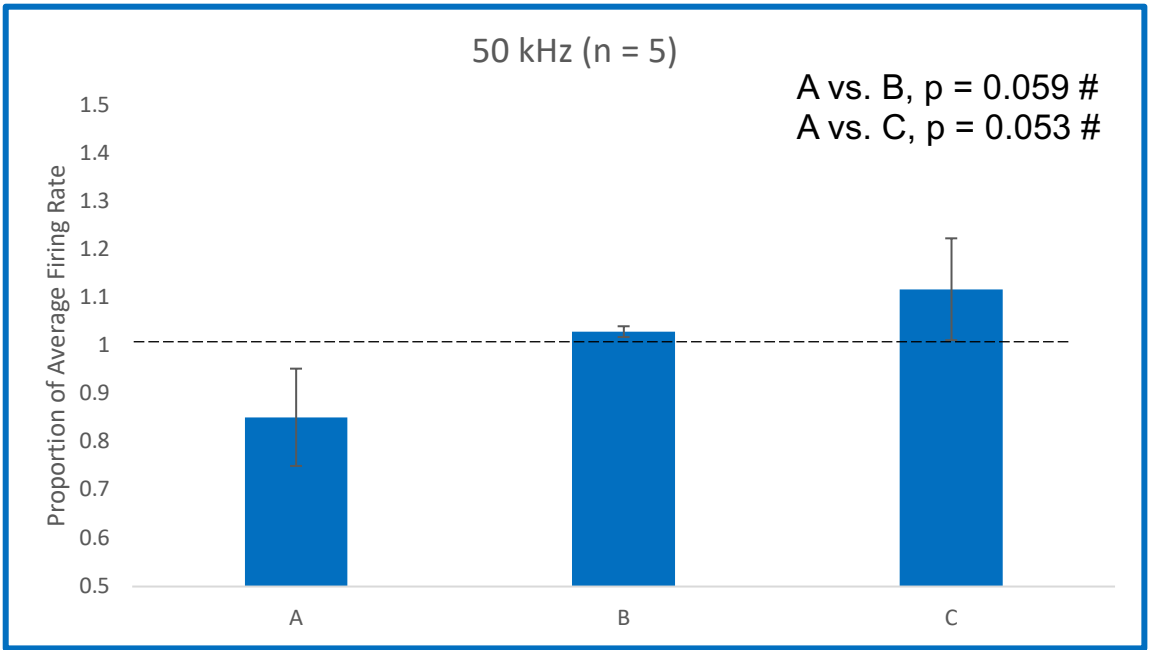


**Figure 7.** Proportion of average firing rate (±SEM) of cells recorded from the hippocampus of the experiment where Session B had 50 kHz USVs ("play" vocalizations) introduced.

No statistical significance was found in ANOVA likely due to the limited number of cells (five) and high variability. There is a trending significance between in sessions A and B and A and C, with average firing rate in session B and C trending to be higher than session A (One-tailed t-test: A vs. B: $t(5,8) = 1.750$, $p = 0.059$ #; A vs. C: $t(5,8) = 1.818$, $p = 0.053$). This general increase in firing rate may become more significant with more cells because rats are suggested to be attracted to the 50 kHz USVs, thus increases in cell activity can be expected.

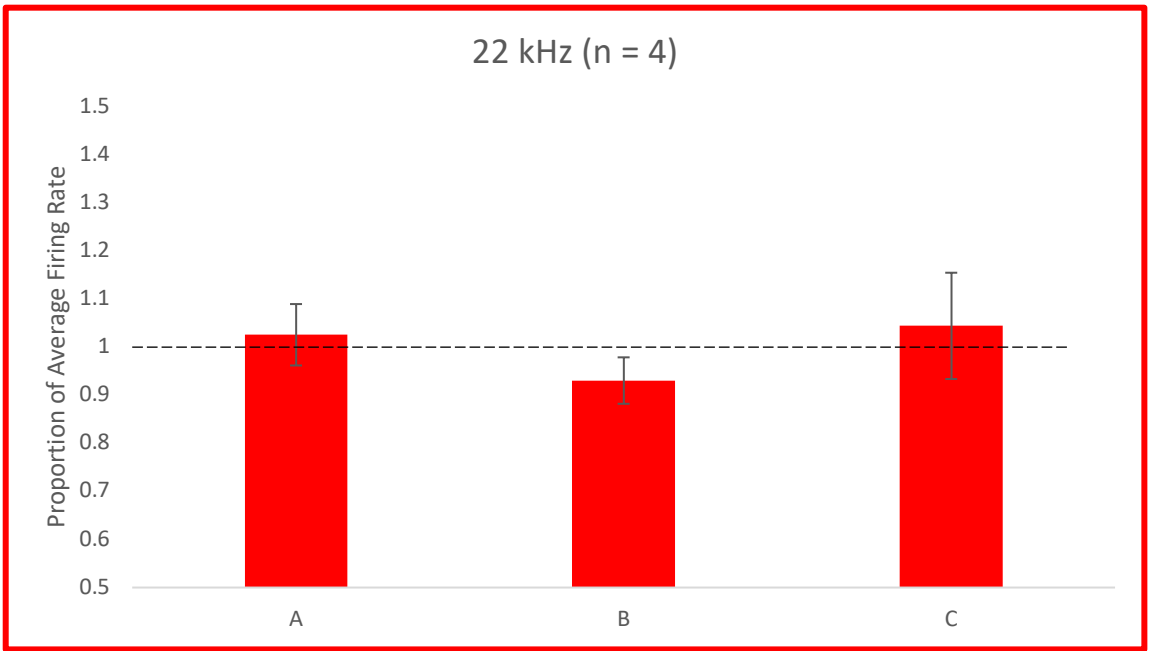


**Figure 8.** Proportion of average firing rate (±SEM) of cells recorded from the hippocampus of the experiment where Session B had 22 kHz USVs ("danger" vocalizations) introduced.

No statistical significance was found in ANOVA due to the limited number of cells (four) and high variability. Overall, average firing rates are relatively consistent throughout sessions. This is surprising since behaviorally, the animal had a much higher latency in the first few runs of session B. These behavioral changes did not translate to the neuronal firing activity level.

*Place Fields*

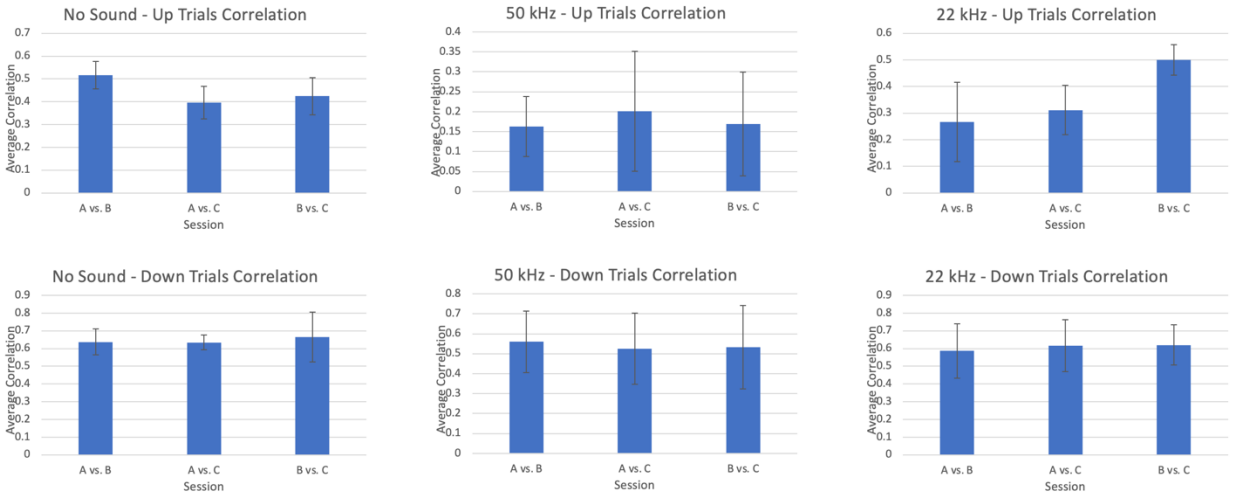


**Figure 9.** Correlations of sessions A vs. B, A vs. C, and B vs. C for all experiments.

A higher correlation value (closer to 1) indicates that two sessions had a more similar rate map. A lower correlation value (closer to 0) indicates that two sessions had a less similar rate map. Place cells create a spatial map of the environment, so differential firing patterns, i.e. changes in the number and location of place fields are expected with a change in the environment. If remapping occurs, correlation values should be lower. Variability was very high in the correlation values, making it difficult to see any significance across sessions.

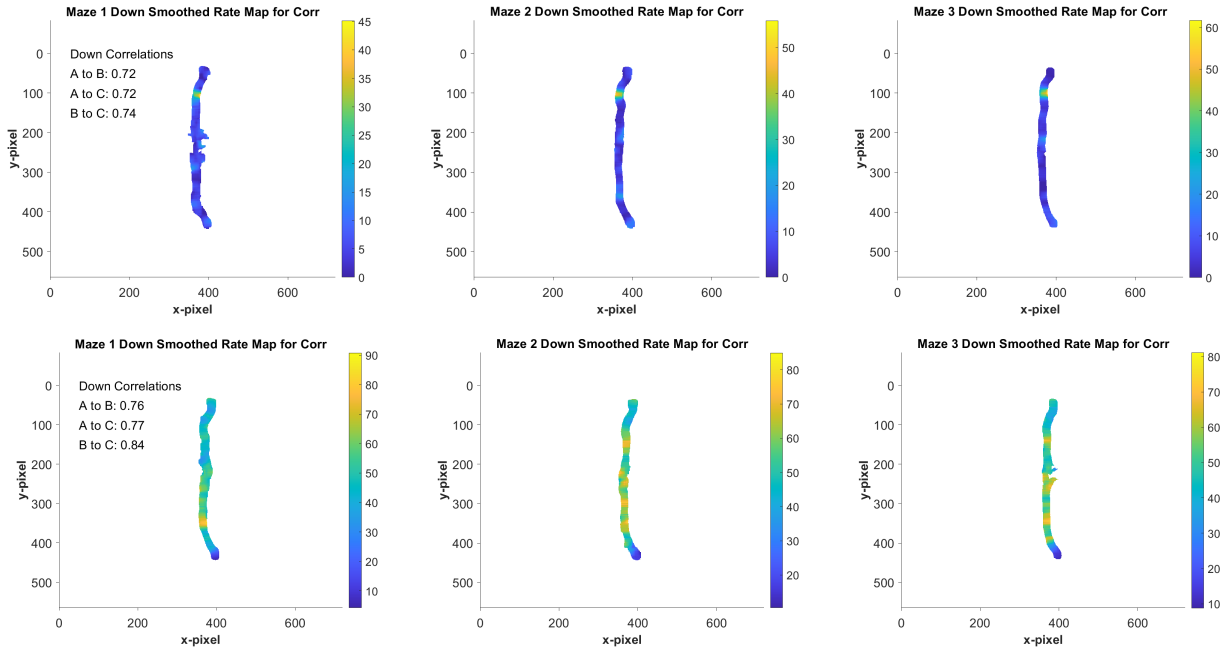


**Figure 10 (Top 3).** Example of a stable and persistent place field of a single unit cell. This is from the No sound control day. The correlation values among all three sessions are very similar, confirming the visual observation. **(Bottom 3).** Example of a new place field appearing during Session B and persisting into Session C. This is from the 50kHz day. Sessions B and C have a higher correlation value than Sessions A to either B or C, confirming the higher level of similarity in firing pattern between Sessions B and C.

Though average correlation values (Figure 9) showed no differences, some individual cells did display remapping, where new place fields showed up or changed locations in session B. This suggests that hippocampal place cells could have the potential to remap due to a novel, emotional, auditory stimuli.

## Discussion

In this study, we examined the behavior of a rat traversing up and down a linear track while recording from single unit cells in its hippocampus. Specifically, the focus was on the effects of auditory stimuli, in the form of playing back behaviorally significant ultrasonic vocalizations from other rats, on rat behavior and place cell firing and remapping. We hope to investigate the connections between the auditory cortex and the hippocampal cortex, along with associated brain areas, in order to further understand the normal physiological processes behind memory processing.

### *Behavioral Effects*

As expected, there were behavioral differences as a result of introducing the novel USVs to the rat on the linear track. The rat took significantly longer to traverse up the track in the presence of both USV frequencies, but there were no latency differences in the down direction. A possible explanation for the lack of effects in the down direction is that the rat's "home cage," which is where it rests during breaks, is at the start of the "up" direction and the end of the "down" direction. There may be more of a motivation to go toward the home cage than away from it upon first hearing the novel sounds. Connecting to previous literature showing rats' approach behavior with 50 kHz USV playback (Wöhr & Schwarting, 2007), an explanation for an increase in latency could be that the rat is more interested in the sound, therefore losing motivation for the food reward and traversing the track. Rats tended to exhibit anxiety-related behaviors with 22 kHz USV playback (Brudzynski & Chiu, 1995; Kim et al., 2010; Demaestri et al., 2019), which may contribute to the increased latency on that day because the rat spends more time freezing and grooming instead of traversing the track. Overall, the increased latency of the

rat when the sounds are on suggests a behavioral response and that the rat has sensed a change in the environment.

*Neuronal Effects*

There was a trend for an increase in firing rate of hippocampal cells between Sessions A (prior to stimuli) and C (after stimuli shuts off) for the 50kHz trials. However, no differences in average firing rate of hippocampal cells between all sessions for control and 22kHz trials. The rate map correlation plots showed no significant differences between any two sessions in any condition, and that was likely due to the high variability and low number of cells. Looking at cells individually, however, we found that some cells have persistent place fields throughout every session, while others remap upon the introduction of USVs, and those changes can either persist or revert. These findings suggest that USV auditory stimuli can change the way that a space is encoded by certain place cells in the hippocampus, thus play a role in encoding episodic memories about location information.

*Limitations of Design and Future Studies*

This was a case study of just one animal, limiting the ability to detect statistical significance. In addition, if there were more than one camera recording the experiments, we may be able to analyze other aspects of behavior such as grooming and freezing, which tell a more complete story about the anxiety of rats. With more animals and more cells recorded, we can also separate the cells in to dorsal and ventral cells, which have shown to have different remapping properties (Fanselow & Dong 2010), to see if there are any regional effects to USVs in terms of firing rate and remapping. In addition, perhaps a similar design but changing the apparatus to an open field may yield more interesting results because this allows the animal to move more freely and generate more spatial information.

## References


Bostock, E. *et al.* (1991) Experience-dependent modifications of hippocampal place cell firing. *Hippocampus* 1, 193–205.

Brudzynski, S. M., & Chiu, E. M. (1995). Behavioural responses of laboratory rats to playback of 22 kHz ultrasonic calls. *Physiology & behavior*, 57(6), 1039-1044.

Chen, X., Guo, Y., Feng, J., Liao, Z., Li, X., Wang, H., ... & He, J. (2013). Encoding and retrieval of artificial visuoauditory memory traces in the auditory cortex requires the entorhinal cortex. *Journal of Neuroscience*, *33*(24), 9963-9974.

Colgin, L. L., Moser, E. I., & Moser, M. B. (2008). Understanding memory through hippocampal remapping. *Trends in neurosciences*, *31*(9), 469-477.

Constantini F, D'Amato F (2006) Ultrasonic vocalizations in mice and rats: social contexts and functions. *Acta Zool Sinica* 52: 619–633.

Demaestri, C., Brenhouse, H. C., & Honeycutt, J. A. (2019). 22 kHz and 55 kHz ultrasonic vocalizations differentially influence neural and behavioral outcomes: implications for modeling anxiety via auditory stimuli in the rat. *Behavioural brain research*, 360, 134-145.

Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures?. *Neuron*, *65*(1), 7-19.

Greensfelder, L. (2016, January 24). Study Finds Brain Hub That Links Music, Memory and Emotion. Retrieved November 23, 2020, from https://www.ucdavis.edu/news/study-finds-brain-hub-links-music-memory-and-emotion/

Kentros, C. et al. (1998) Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science* 280, 2121–2126.

Kim, E. J., Kim, E. S., Covey, E., & Kim, J. J. (2010). Social transmission of fear in rats: the role of 22-kHz ultrasonic distress vocalization. *PloS one*, 5(12), e15077.

Knutson B, Burgdorf J, Panksepp J (2002) Ultrasonic vocalizations as indices of affective states in rats. *Psychol Bull* 28: 961–977.

Krüger, J., Bach, M. Simultaneous recording with 30 microelectrodes in monkey visual cortex. *Exp Brain Res* 41, 191–194 (1981).

Muller, R.U. and Kubie, J.L. (1987) The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J. Neurosci.* 7, 1951–1968.

Muller, R.U. et al. (1991) Spatial firing correlates of neurons in the hippocampal formation of freely moving rats. In *Brain and Space* (Paillard, J., ed.), pp. 296–333, Oxford University Press.

O'Keefe, J. (1976) Place units in the hippocampus of the freely moving rat. *Exp. Neurol.* 51, 78–109.

O'Keefe, J. and Dostrovsky, J. (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 34, 171–175.

O'Keefe, J. and Nadel, L. (1978) *The Hippocampus as a Cognitive Map.* Clarendon Press.

Quirk, G.J. *et al.* (1990) The Firing Of Hippocampal Place cells in the dark depends on the rat's recent experience. *J. Neurosci.* 10, 2008–2017.

Shukla, A., & Chattarji, S. (2021). Stressed rats fail to exhibit avoidance reactions to innately aversive social calls. *Neuropsychopharmacology*, 1-11.

Wöhr, M., & Schwarting, R. K. (2007). Ultrasonic communication in rats: can playback of 50-kHz calls induce approach behavior?. *PloS one*, 2(12), e1365.