Novel Virtual Reality System for Auditory Tasks in Head-fixed Mice

Sibo Gao, James Webb, Zakir Mridha, Anton Banta, Caleb Kemere, Matthew McGinley, *Member, IEEE*

Abstract— **An emerging corpus of research seeks to use virtual realities (VRs) to understand the neural mechanisms underlying spatial navigation and decision making in rodents. These studies have primarily used visual stimuli to represent the virtual world. However, auditory cues play an important role in navigation for animals, especially when the visual system cannot detect objects or predators. We have developed a virtual reality environment defined exclusively by free-field acoustic landmarks for head-fixed mice. We trained animals to run in a virtual environment with 3 acoustic landmarks. We present evidence that they can learn to navigate in our context: we observed anticipatory licking and modest anticipatory slowing preceding the reward region. Furthermore, we found that animals were highly aware of changes in landmark cues: licking behavior changed dramatically when the familiar virtual environment was switched to a novel one, and then rapidly reverted to normal when the familiar virtual environment was re-introduced, all within the same session. Finally, while animals executed the task, we performed in-vivo calcium imaging in the CA1 region of the hippocampus using a modified Miniscope.org system. Our experiments point to a future in which auditory virtual reality can be used to expand our understanding of the neural bases of audition in locomoting animals and the variety of sensory cues which anchor spatial representations in a new virtual environment.**

I. Introduction

It is well established that animals can solve navigation tasks in VR [1], [2], [3], primarily with visual cues. Experiments carried out in VR environments have the advantage of enabling precise and repeatable control of external stimuli while still enabling them to be delivered in ethologically relevant ways. The vast majority of VR systems developed for experiments with head-fixed rodents use visual stimulation, which often involves multiple screens or projection onto large curved surfaces to account for the wide peripheral vision in these species. The critical feature of these VR systems – what sets them apart from other experiments in awake, head-fixed animals – is that the animal's locomotion in virtual space controls the presentation of stimuli. Beyond vision, one recent study presented two olfactory cues with concentrations that changed dynamically in a virtual-location-dependent way [4]. In another study, a rat used a joystick to shift a tone to reach a target frequency [5], which the authors described as behavior in a non-spatial virtual reality. However, despite the ubiquity of spatially-modulated sound in surround-sound systems for motion pictures and video gaming, and the ubiquity of acoustic spatial cues in nature, acoustic VR has not been employed to study rodent behavior or to study neural circuits in head-fixed animals.

Here we present the design of a simple, reconfigurable VR system which allows spatially modulated acoustic stimuli to be presented in free-field configuration and preliminary data demonstrating its utility. We begin by presenting the architecture of our system. We then present evidence that mice are able to process the acoustic VR context, as evidenced by the high performance in a newly developed sparse acoustic landmark (SAL) VR navigation task. Mice appear to interpret novel acoustic stimuli as a different VR environmental context, as evidenced by altered licking behavior. Finally, we report the results of preliminary neural recordings in the CA1 region of hippocampus, a brain area widely implicated in processing spatial information and navigation [6].

II. Method

A. Virtual Reality System Architecture

One of our goals was to develop an affordable VR system with acoustic landmarks that could be easily configured for a variety of sound stimuli, virtual track parameters, landmark positions and characteristics, and reward parameters (e.g., lick-dependence, position, reward volume, repetition rate, etc.). In our design, animals ran on a bearing-supported foam/ fiberglass cylindrical treadmill (15 cm diameter, Public Missiles, Ltd). A quadrature encoder (AMT102, CUI Devices, Inc.) attached to the rotational axis measured the angular

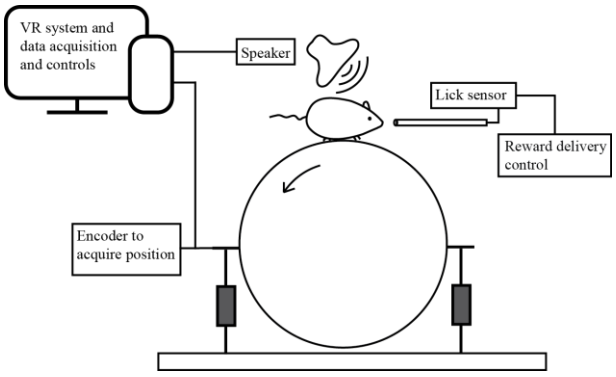

Figure 1. Virtual reality system architecture.

*This work was funded by the Dunn Foundation, NIH RF1NS110501 and R21EY028397A, and DARPA N6600119C.

S. G. and A. B are with the Electrical and Computer Engineering Department, Rice University, Houston, TX 77005 USA (e-mail: sibo.gao@rice.edu, arb17@rice.edu).

J. W and Z. M. are with the Neuroscience Department, Baylor College of Medicine, TX 77030 USA. (e-mail: james.webb@bcm.edu, mridha@bcm.edu).

C.K is with the Electrical and Computer Engineering Department, Rice University, Houston, TX 77005 USA and the Neuroscience Department, BCM, TX 77030 USA (e-mail: caleb.kemere@rice.edu).

M.M is with the Neuroscience Department, BCM, TX 77030 USA and the Electrical and Computer Engineering Department, Rice University, Houston, TX 77005 USA, and Duncan Neurological Research Institute, Texas Children's Hospital, TX 77005 USA (corresponding author: phone: 832-824-3966; e-mail: matthew.mcginley@bcm.edu).

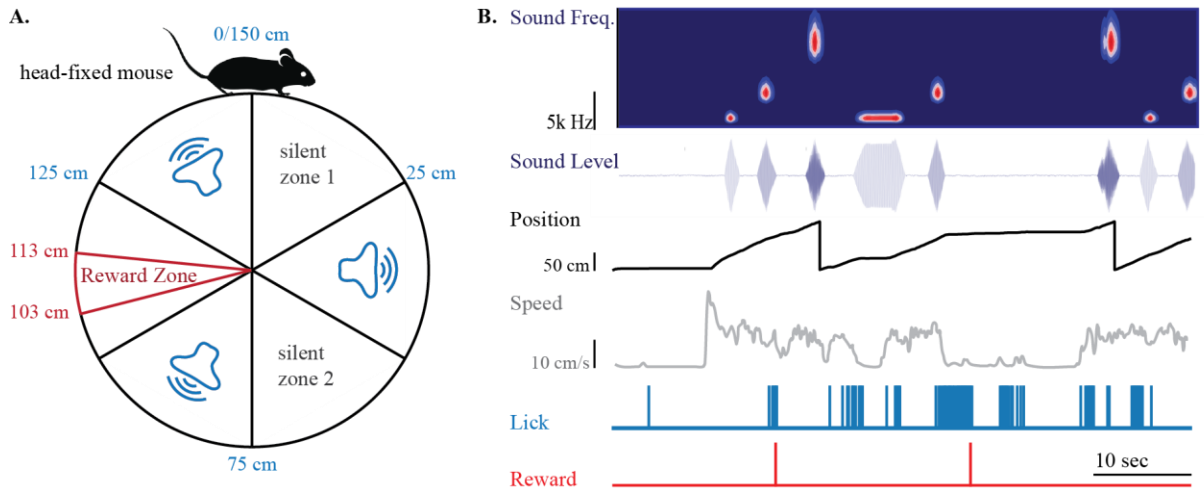

Figure 2. A. Virtual environment track regions. B. Example behavioral data including sound level, position, speed, licks and reward signals for two laps.

position of the wheel movement. Animals were rewarded with drops of liquid (2 µL per triggered reward) delivered through a 26-gauge blunt needle connected to a digitally-triggered syringe pump (New Era Pump Systems #NE-500).

We constructed a capacitive-sensing lick detector using an Arduino (Arduino Uno), which translates instances of tongue contact with the reward spout to digital pulses. We built a custom board (based on the Teensy 3.5) which maintains a master time clock, acquired wheel position, lick signals and other digital inputs, and delivered digital pulses for reward and other purposes. Instructions, schematics, 3D printer files, and source code for firmware and VR-control software can be found at https://github.com/ckemere/TreadmillTracker. The Python-based VR control software uses angular wheel position data to control audio by interfacing to a simple software stack based on the open source JACK Audio Connection Kit low latency audio platform [7]. Specifically, the volume of looped sound stimuli is controlled using open sound control messages sent to a mixer application (JackMiniMix [8]). Miniscope data was acquired using PoMiDAQ [9], with a patch which enables imaging frames to be easily synchronized with behavioral data (https://github.com/ckemere/pomidaq). Calcium traces are extracted using ImageJ. Change in fluorescence (dF) is calculated by subtracting the overall average fluorescence within a session from each video frame.

B. Experimental Virtual Space Defined by Sound

We configured our VR software such that locomotion on the treadmill was mapped to a ring-like virtual environment. As shown in Fig. 2A, the total length of the virtual track is 1.5 meters. Three auditory landmarks (3 kHz, 6 kHz, 12 kHz pure tones) are located at equidistant positions, with the sound loudness increasing and then decreasing as animals approached and then exited each sound zone (25 cm total). In between sound zones, there were three, 25 cm silent regions, with a reward zone located within one of them. Behavior data collected by our VR system (i.e. position, licks, and reward) are shown for an example session (Fig. 2B).

In the "operant conditioning" configuration of our VR control system, the pump was activated if a lick was detected while the animal was in the virtual reward zone. In the "classical conditioning" configuration, rewards were automatically dispensed while the animal was in the virtual reward zone, up to an experimenter-defined maximum number of rewards per passage. Note that this differs from normative classical conditioning in that animals are actively controlling the stimuli that they hear by moving rather than passively receiving them. In a "novel landmark" configuration, one of the sound stimuli (6 kHz) was replaced with a new sound (10 kHz), while keeping other VR parameters the same.

C. Animals, Surgery, and Training Protocol

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Rice University and Baylor College of Medicine and followed the guidelines of the National Institutes of Health. C57BL/6 mice were purchased from Charles River Laboratories. Mice were anesthetized with 1-2% isoflurane and preoperatively administered sustained release Buprenorphine (ZooPharm, 0.5 mg/kg) and, for imaging animals, Dexamethasone (4 mg/kg). Post-operatively analgesia was achieved with Meloxicam (5 mg/kg) and 0.25% Bupivicaine around the headpost implant. The mouse's head was fixed while on the treadmill using a design based on [10]. Briefly, a 3D-printed frame is attached to the skull using C&B Metabond (Parkell, Inc) dental cement, and then a titanium or stainless-steel post is attached to the frame and the collection is reinforced with additional cement.

For calcium imaging, we infect neurons in area CA1 of the hippocampus with the activity-dependent calcium-sensitive protein GCaMP6f by stereotactically infusing an adeno-assoicated virus (0.5 µL to 1 µL at a rate of 0.08 µL/min AAV9.CamKII.GCaMP6f.WPRE.SV40, Penn Vector Core, coordinates -2 AP; +2 ML; -1.5 DV). For imaging neural activity, we use a modified version of the Miniscope [11]. We implant a 0 mm WD gradient-index (GRIN) objective (Edmunds Optics, #64-519) at the same time as the headpost, at least 4 weeks after viral infusion. A large cut is made to expose the skull and match the size of the 3D printed frame. We then use a 2 mm biopsy punch to carefully center a craniotomy over the AAV injection site, and slowly rotate it until a skull window can be lifted away with forceps. Several 30-gauge blunt needles are then used to aspirate the cortex while irrigating regularly with saline, stopping when the medial-lateral fibers become visible. After hemostasis is

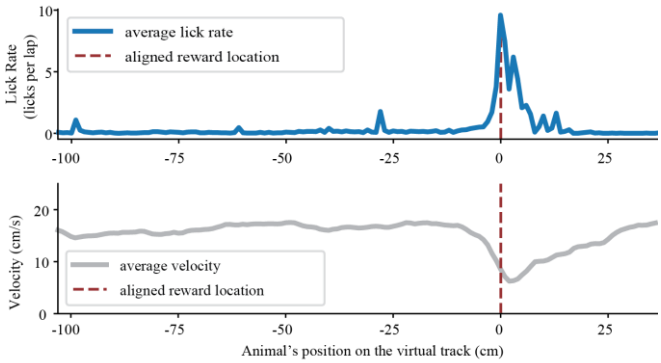

Figure 3. Anticipated licking and slowing preceding the reward zone for one example session.

achieved, we lower the GRIN lens to -1.35 DV from brain surface (zeroed at medial side of the lens). The lens is affixed using UV-cured Flow-It® ALC (Pentron) composite.

Mice were trained in the SAL-VR task in a succession of progressively more complex phases: acclimation, classical conditioning, and finally operant conditioning. The acclimation phase begins after mice reached 85% to 90% of their ad lib weight under water restriction. They were allowed to run freely in a large cage with a mouse wheel inside (Bio-Serv, Mouse Igloo and Fast Tracs) for up to one hour before beginning of each session. For the first 3 days, animals were handled up to 3 sessions per day and slowly introduced and head-fixed to the wheel in the experimental setup. Next, after they were acclimated, mice were trained on the "classical conditioning" (easy) version of the task, with the main goal being to make the animals learn how to lick and adjust to the acoustic of the VR environment. During the initial classical conditioning phase, the maximum rewards per lap was initially high (e.g., 5x) to maximize reward for task engagement (locomotion). The starting position of the reward zone varied from 1-3 cm from the end of the last sound zone. The final "operant conditioning" (hard) version was introduced after animals' performance plateaued in the easy condition. Animals that were not able to reliably receive reward in the classical conditioning phase were removed from further study.

III. System Evaluation Results

A. VR System-- Sound and Reward Delivery Latency Tests

An animal's accurate perception of spatial coordinates in the virtual environment is facilitated by minimizing the latency between movement and changes in sound. Since the sound signal (including silent zone signals) is controlled purely by animal's position on the wheel, we tried to avoid excessive latencies in the system. To measure system latency, we modified our system to deliver a tone in response to licks. The measured latency from the digital lick input to the change in signal from the sound card's output was 6 ± 0.1 milliseconds.

Abrupt sound transitions are highly salient and aversive at high sound levels. Therefore, the JackMiniMix software by default controls the maximum rate at which the volume of a sound can change (measured in dB per second). Specifically, with the default value of 400 dB/s, we measured an increase in our VR system delay from 6 ms to 214 ± 2.091 ms for a transition from full 'off' to full 'on' (113 dB increase). Were we to employ abrupt transients in our VR system, the default value of this parameter would be problematic. However, as the rate of change of sounds is ultimately limited by the slower, animal behavior-controlled change in volume, its effect in practice is less pronounced. We found that VR performance was acceptable with rates as high as 40000 dB/s. In addition, we measured reward delivery latency—the time between animal's lick to the TTL trigger signal sent to the syringe pump—of our system, and found values of 2.62 ± 0.5119 milliseconds. Thus, both sound stimuli and input-controlled digital outputs have sufficiently low latency for robust acoustic VR behavior.

IV. In-vivo Results

A. Anticipated licking behavior in the SAL-VR task

Using our VR system, we found that animals could reliably learn to perform our 'sparse acoustic landmark' (SAL) VR navigation task. Many VR experiments in rodents can require extended training [4], [14]. Similarly, we found that high performance could require up to three months of training. An example of learned behavior in "operant conditioning" mode is shown in Fig. 3. The rate of licks rapidly increases (top) and velocity decreases (bottom) as the animal approaches the reward zone entry point (vertical red dashed line).

B. Animals alter licking with novel spatial acoustic cues

To evaluate whether animals actually processed the auditory stimuli as landmarks, for one animal we changed the frequency of the landmark which preceded the reward zone. In this novel condition, we found that the licking pattern changed profoundly, and the proper reward licking patten also returned as shown in Fig. 4 when the landmark was returned to normal. Note that the animal did not lick indifferently in the altered configuration, but rather tended to run continuously for a long distance (~5 laps, which corresponds to 7.5 meters) without any licking. It is possible that the animal was searching for the familiar sound B (instead, animal can only hear sound D), which was previously associated with the reward location in the familiar acoustic environment.

C. Calcium Imaging using 1P Miniaturized Microscope

Our VR system is compatible with most forms of head-fixed neural recording. Here, we used a micro-endoscope to record neural activity in the CA1 region of dorsal hippocampus, while animal performed the SAL-VR task. Our modified Miniscope uses a customized housing and baseplate [12], which allows for ease of manipulation when head-mounting the animals, in comparison to e.g. the standard UCLA Miniscope setup. Fig. 5 shows the typical calcium transient traces extracted from five CA1 neurons recorded during behavior in the SAL-VR task. We found that our head-fixation resulted in fluorescence signals were stable, and it was not necessary to apply a motion-correct algorithm to our raw Miniscope videos before extracting calcium traces.

Calcium imaging, particularly using microendoscopes, is particularly useful in the context of rodent VR learning and memory experiments; experiments can involve thousands of trials over many weeks (as described above). Endoscopy permits repeated measurement of activity in the same neurons across days [13] in circuits related to learning and memory. These long timescales also are beneficial for experimental paradigms involving high-throughput behavior.

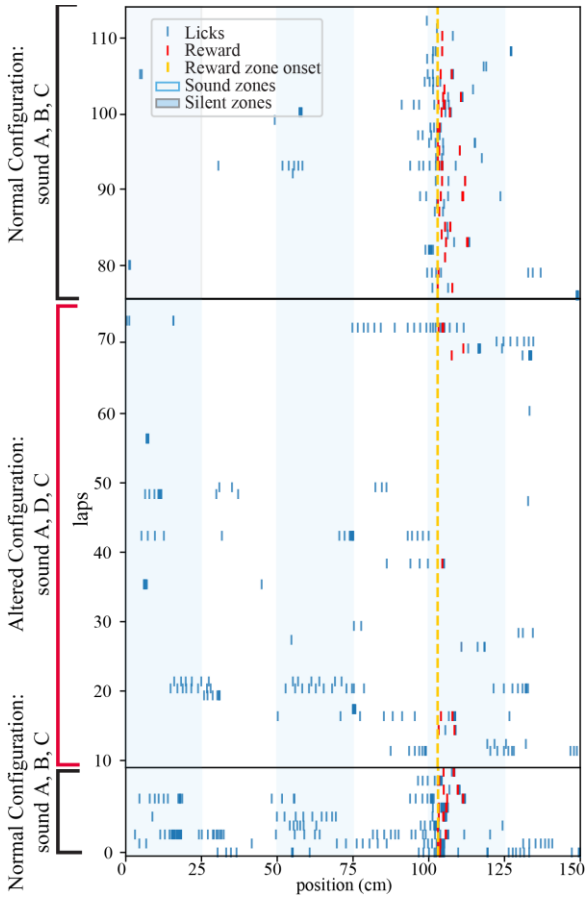

Figure 4. Animal's licking pattern for one example session alternating between normal, altered, and normal VR configuration.

V. CONCLUSION

We have developed a low cost, open source platform for conducting a novel class of VR experiments in head-fixed mice. Specifically, we present acoustic stimuli in a free-field VR in which animals can locomote by running on a treadmill. The licking and slowing demonstrated by mice in our preliminary experiments are evidence that animals can learn to navigate in such environments. Moreover, the fact that they are aware of specific frequencies of the acoustic landmarks (as evidence by introduction of novel cues) strongly point to their awareness of the auditory features. Combined with calcium imaging, as well as diverse electrophysiology approaches, this platform has the potential to enable high-throughput experiments over long periods of time, enabling the circuits which underly auditory processing and navigation in moving animals to be studied in detail. Finally, our approach points to the potential of VR systems – enhanced with proper models of spatial acoustics, including the head/ear and environment – will allow study of the neural circuits linking spatial hearing to navigation in mice. The approach can also be used other rodent species with robust coding of spatial acoustic cues in low-frequency sounds, such as gerbils.

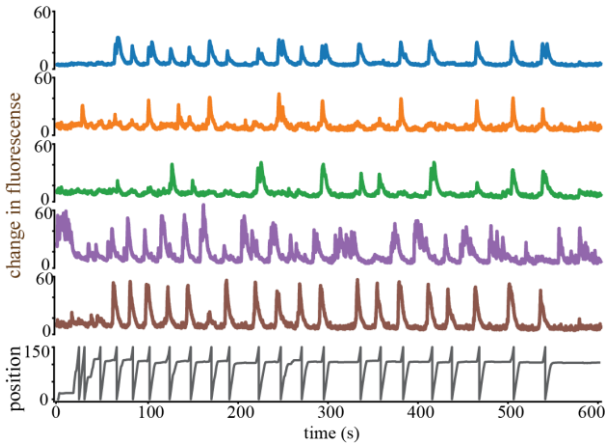

Figure 5. Example of extracted calcium transient traces from five manually identified CA1 neurons, along with animal's position (cm) data shown in the bottom panel. Animal spend more time around 103 cm (reward zone).

ACKNOWLEDGMENT

S. G. thanks H. Ramsaywak, J. Shi, and S. Smith for help with animal training, and J. Juneau, J. Chu, S. Dutta, and Z. Chen for helpful discussions and support.

REFERENCES

[1] C. Schmidt-Hieber, M. Häusser, "Cellular mechanisms of spatial navigation in the medial entorhinal cortex," Nat. Neurosci., vol. 16, pp. 325–331, 2013.

[2] M. Sato, M. Kawano, K. Mizuta, T. Islam, M.G. Lee, and Y. Hayashi, "Hippocampus-Dependent Goal Localization by Head-Fixed Mice in Virtual Reality," eNeuro., vol. 4, no.3, 2017. doi:10.1523/ENEURO.0369-16.2017

[3] L. Pinto et al., "An Accumulation-of-Evidence Task Using Visual Pulses for Mice Navigating in Virtual Reality," Front. Behav. Neurosci., vol. 12, pp. 36, 2018.

[4] B. A. Radvansky and D. A. Dombeck, "An olfactory virtual reality system for mice," Nat. Commun., vol. 9, no. 1, p. 839, 2018.

[5] D. Aronov, R. Nevers, and D. W. Tank, "Mapping of a non-spatial dimension by the hippocampal–entorhinal circuit," Nature, vol. 543, no. 7647, pp. 719–722, 2017.

[6] J. O'Keefe and J. Dostrovsky, "The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely moving rat," Brain Res., vol. 34, no. 1, pp. 171–175, 1971.

[7] "Jack Audio Connection Kit," Accessed on: Jan. 20, 2020. [Online]. Available: https://jackaudio.org/

[8] "JackMiniMix," Accessed on: Jan. 20, 2020. [Online]. Available: https://www.aelius.com/njh/jackminimix/

[9] "Portable Miniscope Data Acquisition," Accessed on: Sep. 2, 2019. [Online]. Available: https://github.com/bothlab/pomidaq

[10] L. Ghanbari et al., "Cortex-wide neural interfacing via transparent polymer skulls," Nat. Commun., vol. 10, no. 1500, 2019. https://www.nature.com/articles/s41467-019-09488-0

[11] "Miniscope.org Wiki Home," Jan., 2016. Accessed on: Jan. 20, 2020. [Online]. Available: http://miniscope.org/index.php/Main_Page

[12] J. Juneau, G. Duret, J. Robinson and C. Kemere, "Enhanced Image Sensor Module for Head-Mounted Microscopes*," 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Honolulu, HI, 2018, pp. 826-829.doi: 10.1109/EMBC.2018.8512387

[13] Y. Ziv et al., "Long-term dynamics of CA1 hippocampal place codes," Nat. Neurosci., vol. 16, no. 3, pp. 264-266, 2013. doi:10.1038/nn.3329

[14] C. Harvey, P. Coen, D. Tank, "Choice-specific sequences in parietal cortex during a virtual-navigation decision task," Nature, vol. 484, no. 7392, pp. 62-68, 2012. doi: 10.1038/nature10918.