Discrimination of partial from whole ultrasonic vocalizations using a go/no-go task in mice

David P. Holfoth, Erikson G. Neilans, and Micheal L. Dent^{a)}

Department of Psychology, University at Buffalo, The State University of New York, Buffalo, New York 14260

(Received 24 March 2014; revised 3 October 2014; accepted 14 October 2014)

Mice are a commonly used model in hearing research, yet little is known about how they perceive conspecific ultrasonic vocalizations (USVs). Humans and birds can distinguish partial versions of a communication signal, and discrimination is superior when the beginning of the signal is present compared to the end of the signal. Since these effects occur in both humans and birds, it was hypothesized that mice would display similar facilitative effects with the initial portions of their USVs. Laboratory mice were tested on a discrimination task using operant conditioning procedures. The mice were required to discriminate incomplete versions of a USV target from a repeating background containing the whole USV. The results showed that the mice had difficulty discriminating incomplete USVs from whole USVs, especially when the beginning of the USVs were presented. This finding suggests that the mice perceive the initial portions of a USV as more similar to the whole USV than the latter parts of the USV, similar to results from humans and birds.

© 2014 Acoustical Society of America. [<http://dx.doi.org/10.1121/1.4900564>]

PACS number(s): 43.80.Lb, 43.66.Gf [AMS]

Pages: 3401–3409

I. INTRODUCTION

Laboratory mice are often used as neurological models of human hearing since their inner ear structure and auditory system organization are similar to that of humans (Henry and McGinn, 1992). While such studies are useful, they can only provide an indirect measure of a mouse's auditory abilities. It is important to understand how mice respond behaviorally to auditory stimuli before using them as a model for human hearing (Fay, 1994). Behavioral studies provide a way of directly assessing an organism's perceptual space (reviewed by Nyby, 2001). One way to behaviorally study the auditory capabilities of mice is to train them, using operant conditioning techniques, to respond to certain auditory stimuli. Several studies have demonstrated that go/no-go procedures can provide reliable measures of auditory sensitivity in mice (Prosen *et al.*, 2003; Klink *et al.*, 2006; Radziwon *et al.*, 2009), and researchers are beginning to use the natural utterances of these mammals in psychophysical studies (Neilans *et al.*, 2014).

Nyby (2001), among others, has stressed the importance of using natural vocalizations in studies of mouse hearing, although these studies are currently limited. Mice produce a wide variety of ultrasonic vocalizations (USVs), which have recently received increasing attention. Several researchers have attempted to classify these USVs; however, the types and numbers of categories differ greatly between studies (Portfors, 2007; Grimsley *et al.*, 2011; Grimsley *et al.*, 2012; Kikusui *et al.*, 2011; Mahrt *et al.*, 2013). So it remains unclear how USVs are processed by mice, although there is growing evidence that these USVs have biological relevance.

Hammerschmidt *et al.* (2009) found that female mice approached speakers playing male USVs, highlighting the potential use of these vocalizations as attraction signals. Shepard and Liu (2011) extended these findings by showing that exposure to males restores this approach behavior after habituation to USVs had occurred. This finding suggests that experience can alter the behavioral meaning of a USV. Mouse pups will also produce isolation vocalizations when they are cold or removed from the nest, even before they have the ability to hear (Ehret, 1976). These USVs elicit search and retrieval behavior in female mice that have experience with pups (Ehret *et al.*, 1987). If these USVs are behaviorally relevant to the mice, then being able to perceive and identify them accurately in the environment, even when portions of the calls are perceptually masked, would be beneficial to an individual's survival.

Previous studies on different species of animals have shown that the beginning of a sound sequence is more important than the middle or the end. Studies with humans, for example, have found that the beginning of a word is the most important for identification. Salasoo and Pisoni (1985) found that this initial portion of a word is a major source of information used in word recognition and that its presence leads to faster recognition times. Marslen-Wilson and Zwitserlood (1989) suggested that word onsets have a special status in spoken word recognition. It is unclear if animals process communication signals in a similar way to human speech, however, evidence for this primacy effect has also been shown in birds. Toarmino *et al.* (2011) used an operant conditioning procedure to train budgerigars (*Melopsittacus undulatus*) to categorize two different contact calls. In probe test trials, only small portions of the calls were presented to the birds. Similar to what has been shown in human studies, Toarmino *et al.* (2011) found that budgerigars were better at recognizing calls when the first portion was present compared to when it was absent. Previous

^{a)}Author to whom correspondence should be addressed. Electronic mail: mdent@buffalo.edu

research with European starlings (*Sturnus vulgaris*) also showed that song recognition improves as more of the song is presented (Knudsen *et al.*, 2010). Finally, Australian sea lion (*Neophoca cinerea*) mothers were significantly more attracted to a pup-call playback containing the first half of a call than one containing the second half of a call (Pitcher *et al.*, 2012). Since these effects occur in several animal species, it stands to reason that mice may also find the initial portions of their USVs more similar to the whole USVs and that discrimination of these USVs will decrease as more of the calls are presented.

The present study investigated how well mice discriminate small portions of a USV from a repeating background containing the whole USV, to determine if they perceive partial calls as similar to the whole calls. Mice were trained to discriminate partial USV targets from a repeating background whole USV, using operant conditioning techniques similar to Neilans *et al.* (2014). Based on the previous human, Australian sea lion, budgerigar, and European starling experiments, it was expected that (1) a mouse's discrimination performance would be poorer for targets containing the initial portions of the background USV since these portions are most important for recognition. That is, if the beginning of the call was more similar to the whole call than the end of the call, discrimination performance would be lower for the beginning than the end. It was also expected that (2) the discrimination performance would be lower for targets containing larger portions of the background USV (two-thirds of the USV) compared to targets containing smaller portions of the background USV (one-third of the USV). Poorer discrimination performance would suggest that the mice perceive the target as more similar to the background whole USV.

Using our planned design, however, we were concerned that the mice might show different discrimination abilities for conditions where an element is added to a stimulus compared to when an element is taken away (somewhat similar to the Feature Positive Effect described by Sainsbury and Jenkins, 1967; Newman *et al.*, 1980, among others). By using the whole USV as the repeating background and small portions of USVs as targets, signal components were being removed from the stimulus. To test for differences in discriminating the addition of call fragments from the subtraction of call fragments, we swapped the background/target conditions and compared performance to the original results.

The current studies were designed to increase our understanding of mouse auditory processing and to learn more about how USVs might be utilized for communication purposes. With the increasing usage of mice as models for acoustic communication in humans, knowing where similarities and differences arise in sensory processing is vitally important. Neilans *et al.* (2014) found, using psychophysical methods, that discrimination of different USVs was possible. Here, we extend those experiments to determine if the mice could discriminate a partial USV from a whole USV. In many naturally occurring instances of communication in the real world of a mouse, portions of a call may be masked by noise or not completely detected by the mouse. We wanted

to know whether communication would still be possible under such a situation. We found that mice had trouble discriminating portions of a USV from a whole USV and had *more* difficulty discriminating partial USVs containing the beginning of the USV from the whole call than partial USVs containing than the end of the USV from the whole call. These results are similar to findings from recognition and playback studies in humans and several other animals. Control experiments comparing the discrimination of USVs from the discrimination of synthetic tonal stimuli revealed, not surprisingly, that the target tones were much less similar to the background calls than the partial USVs. Finally, the mice found it difficult to discriminate a partial call target from a whole call background, no matter which part of the call was present.

II. METHODS

A. Subjects

Five adult, female CBA/CaJ mice (*Mus musculus*) were used as subjects in this experiment. Mice began training at approximately two months of age and the experiments lasted approximately 12 months. The mice were housed separately and kept on a reversed day/night cycle (lights off at 6 am and on at 6 pm). The mice were tested during the dark portion of their cycle. All of the mice were water restricted and maintained at approximately 85% of their free-drinking weight during the course of the experiment. Food was available *ad libitum*, except during testing sessions. The mice were bred at the University at Buffalo, State University of New York (SUNY) and all procedures were approved by University at Buffalo, SUNY's Institutional Animal Care and Use Committee.

B. Apparatus

The mice were tested in a wire cage (23 cm × 39 cm × 15.5 cm) placed in a sound attenuated chamber (53.5 cm × 54.5 cm × 57 cm) lined with 4-cm thick Sonex sound attenuating foam (Illbruck, Inc., Minneapolis, MN). The chamber contained an overhead web camera (Logitech QuickCam Pro, model 4000) and a small 25-W white light to monitor the animals during test sessions. Sounds were played from an electrostatic speaker [Tucker-Davis Technologies (TDT), Gainesville, FL, Model ES1]. The cage also contained two nose-poke holes surrounded by infrared sensors (Med Associates Model ENV-254), and a response dipper [Med Associates Model ENV-302M-UP, see Fig. 1(A)].

The experiments were controlled by Dell Optiplex 580 computers operating TDT modules and software. Stimuli were sent through an RP2 signal processor, an SA1 power amplifier, a PA5 programmable attenuator, and finally to the speaker. Inputs to and outputs from the testing cages were controlled via RP2 and RX6 processors. Power supplies were used to drive the dipper (Elenco Precision, Wheeling, IL, Model XP-603) and infrared sensors (Elenco Precision, Model XP-605). Custom MATLAB and TDT RPvds software programs were used to control the hardware.

FIG. 1. (A) Schematic of the operant apparatus depicting the locations of the nose-poke holes, loudspeaker, and water dipper. (B) Flow diagram of the operant task.

C. Test stimuli

The background stimuli used in this experiment consisted of four different USVs recorded from different CBA/CaJ mice. The 30 kHz Harm, 40 kHz Harm, and 2HarmD USVs were recorded for [Holmstrom et al. \(2010\)](#). The Chevron USV was recorded in our own lab (using an Avisoft UltraSoundGate recorder, model 416H). The 40 kHz Harm USV ranged from 30 to 82 kHz and had a duration of 114 ms. The 30 kHz Harm USV ranged from 30 to 75 kHz and had a duration of 121 ms. The 2 HarmD USV ranged from 35 to 84 kHz and had a duration of 51 ms. Last, the Chevron USV had a frequency range of 66 to 84 kHz and a duration of 56 ms (Fig. 2). All calls were recorded during male-female social interactions by sexually naive mice that were approximately 1 month old. The names of the stimuli matched the spectral characteristics of the calls. The 30 kHz Harm, 40 kHz Harm, and 2HarmD calls had fundamental frequencies at 30, 40, and 40 kHz, respectively, and one harmonic each. The three calls were chosen for their spectrotemporal complexity and because they were known to be discriminable from one another ([Neilans et al., 2014](#)). We additionally used the simpler Chevron call (named for its

spectral shape) for this experiment because it was frequency modulated but did not contain a harmonic. All of the stimuli are readily produced by both male and female mice in social situations (e.g., [Portfors, 2007](#)), although the calls have no known specific “meanings” at this time.

The ten target stimuli included (1) incomplete versions of the repeating background USVs (truncated from the originals using Adobe Audition; also see y axis of Fig. 4 for all testing conditions), (2) 30 kHz pure tones, and (3) synthetic versions of the calls with no frequency modulation (FM). The incomplete USV stimuli contained either one-third (the initial third, middle third, or last third), or two-thirds (initial two-thirds, last two-thirds, or middle third removed) of the whole USV [see Fig. 3(A)]. The 30 kHz tones with the same duration as either one-third or two-thirds of the background USVs were also used as target stimuli [Fig. 3(B)]. These tones served as controls to measure the discrimination performance on targets that were very different from the background USV. Additionally, no FM versions of the background USVs, which were made from pure tones at the mean frequencies of the fundamental and harmonic components (if present), with a duration of either one-third or two-thirds of

FIG. 2. Oscillograms (top) and spectrograms (bottom) of the four mouse USVs (A–D) used as background stimuli in the discrimination task.

the background, were used as controls to test the importance of frequency modulation and duration as cues for discrimination [Fig. 3(C)]. All full and partial stimuli were presented at approximately 65 dB sound pressure level, measured at the position where the mouse's head would normally be during testing. Stimuli were roved by ± 3 dB from presentation to presentation. Sound pressure levels were calculated using an ultrasound recording system (Avisoft Model USG 116-200) and Raven Pro (v 1.3, Cornell University) software.

D. Procedure

The mice were trained using a go/no-go operant conditioning procedure on a discrimination task [Fig. 1(B)]. The

mice were tested in two 30-min sessions/day, 5 to 6 days per week. The mice typically ran between 50 and 100 trials per session. Each mouse was tested on all four background calls in a random order, and a different random order was used for each subject. In each session, subjects listened to just one vocalization (background) presented repeatedly and were required to indicate when they heard any other stimulus type (target).

During testing, the mouse began a trial by nose poking through the observation nose-poke hole two times, which initiated a variable waiting interval ranging from 1 to 4 s. During this time, a repeating background of one vocalization was presented with a silent interstimulus interval of 200 ms. After the waiting interval, a single test stimulus was

FIG. 3. Whole 40 kHz Harm background USV split into partial thirds (A), a 30 kHz pure tone at one and two-thirds the duration of the USV (B), and a no-FM version of the USV at one and two-thirds the duration (C).

presented, alternating with the background stimulus vocalization two times. If the mouse discriminated the change between the background and target, it was required to nose poke through the report nose-poke hole within 2 s of the onset of the target. In this trial type, a “hit” was recorded if the mouse correctly responded within the response window and the animal received 0.01 ml of Ensure or water as a reinforcement. A “miss” was recorded if the mouse failed to nose poke through the report hole within 2 s. If the mouse responded to the report nose-poke hole during the waiting interval, the trial was aborted and the mouse received a 3–5-s timeout, during which no stimuli were presented.

Experimental sessions consisted of multiple randomized blocks of ten trials each, and mice completed between one and ten blocks per session. Within each block of ten trials, seven were target “go” trials, and three were sham “no go” catch trials. Each block was randomly generated so that no more than two sham trials could be presented in a row. In the sham trials, the repeating background continued to be presented during the response phase. These trials were required to measure the false alarm rate and calculate the animal’s response bias. If the subject nose poked to the report hole during a catch trial, a “false alarm” was recorded and the mouse was punished with a 3-s timeout interval. However, if the subject continued to nose poke to the observation hole, a “correct rejection” was recorded and the next trial would begin immediately. In either case, no reinforcement was given. Chance performance was represented by the animal’s false alarm rate. Sessions were excluded from analysis if the percentage of false alarms was greater than 20%. Using this criterion ensures that the mice are under stimulus control. Approximately 25% of sessions were discarded due to high false alarm rate. These sessions were randomly interspersed during the testing, with no discernable pattern to their occurrence.

In the “go” condition, the seven target trial types remained the same for each block in an experimental session (although the trials were presented in a random order, and a different random order of ten trials was generated for each block). Two of the target trials types were experimental trials drawn from the conditions below and the other five were very easy targets (10 kHz pure tones) to keep the motivation levels high for the mice and to ensure that there were no wild fluctuations in reinforcement rate from session to session (since only 20% of trials had response rates that varied with experimental condition). The experimental trials were randomly chosen from the ten types of stimuli: (1) shortened version of original USV (first third, second third, third third, portions 1 and 2, portions 1 and 3, and portions 2 and 3), (2) shortened 30 kHz tone (1/3 duration or 2/3 duration), or (3) no-FM version of original USV (1/3 duration or 2/3 duration). Thus, all targets were shorter than the background and some also differed in other acoustic characteristics.

Testing on each USV background continued until results from 20 trials of each target type comparison were collected (two targets out of the ten possible conditions were randomly chosen and completed, then two more were chosen and completed, and so on until all target types were finished for that background). Different random orders of testing conditions

were chosen for each background and for each mouse. The results were used to calculate percent correct discrimination performance for every experimental condition.

To test for the effects of the discrimination task type, background and target conditions were reversed, where one-third partial USVs were used for the repeating background and the whole USV was used as targets. Using the whole USV as a target added more physical material to the stimulus relative to the partial background, instead of subtracting it. By comparing the hit rate during the reversed condition to the normal testing conditions (whole USV background with partial targets) we can discern if discrimination is easier for the mice when cues are added to the stimuli rather than removed from the stimuli.

A two-way repeated-measures analysis of variance (ANOVA) was used to compare performance across all USVs and target stimulus types. Another repeated-measures ANOVA was used to compare the reversed background/target condition results with the original-condition results. Holm-Sidak *post hoc* analyses were conducted for pairwise comparisons.

III. RESULTS

The mice discriminated the whole USV backgrounds from all of the target stimuli at a rate above chance performance (the mean false alarm rate for these experiments was 10.41%). There was quite a bit of variation between discrimination of the ten target stimulus types. A two-way repeated measures ANOVA showed a main effect for target stimulus type, $F(9,36) = 30.20$, $p < 0.001$, as well as a main effect for USV background, $F(3,12) = 36.51$, $p < 0.001$. There was also a significant interaction between target stimulus and USV background, $F(27,95) = 4.47$, $p < 0.001$. Overall, the mice discriminated a partial USV target from the whole USV background at a lower rate than when discriminating a tone from the whole USV (Fig. 4, compare the six bars on the left with the four bars on the right). This suggests that the mice perceive partial USVs as more like the whole USVs compared to pure tones, which are perceived as different.

Post hoc analyses revealed that the mice showed significantly lower discrimination performance when presented with the first third of the USV compared to when they received the second ($p < 0.05$) or last third ($p < 0.001$) of the USV as a target (Fig. 4, three left bars). Additionally, they had significantly lower discrimination performance on the initial two-thirds compared to last two-thirds ($p < 0.01$, Fig. 4, three middle bars). These findings indicate that it was harder for the mice to discriminate a partial USV from the whole background when the initial portion of the USV was present compared to when it was absent, suggesting that the mice perceive the beginning portion of the USV as more similar to the whole USV than the end portion.

The synthetic tonal stimuli were easier to discriminate from the whole USVs than the partial USVs were (Fig. 4, four right bars). Discrimination of the 30 kHz tones was significantly higher than the discrimination of any other targets ($p < 0.05$). Variation in performance between background

FIG. 4. Mean discrimination performance for each target stimulus type, averaged across all four USV backgrounds. Error bars represent between-subject standard errors. A “*” indicates a significant pairwise comparison (pairs denoted by the bracket). A “***” indicates the two target stimuli where discrimination performance was significantly different than performance on all other targets. Dashed line indicates mean false alarm rate.

USVs lowered the performance for the “no FM” conditions (see Fig. 5), but generally, discrimination for these stimuli was also higher than for the partial USVs.

The significant interaction effect in the ANOVA was most likely driven by the differences in responses when the Chevron USV was the background (Fig. 5). Here, the only easy discrimination for the mice was between the Chevron background stimulus and the shorter 30 kHz tones ($p < 0.05$). The Chevron calls had, by far, the smallest bandwidth of all of the stimuli, probably accounting for this difference.

Duration of the stimuli could also have been used as a cue for discrimination, but *post hoc* analyses showed that this was not the case when comparing discrimination of the first third to the first and second-thirds of the stimuli (first third vs portions 1 and 2, $p > 0.05$) and when comparing discrimination of the second third to the second and third-thirds of the stimuli (second third vs portions 2 and 3, $p > 0.05$). Thus, duration of the target did not significantly impact discrimination in these mice since adding more of the USV did not change performance.

A two-way repeated measures ANOVA was also conducted to examine any influence of the discrimination procedures (Fig. 6), in an attempt to determine whether discrimination differed for trials where portions of the targets were longer than or shorter than the repeating background. There was a main effect for the location of the partial USV (the initial third, middle third, or last third), $F(2,8) = 57.88$, $p < 0.001$, matching the above results. There was also a main effect for the repeating background/target condition, $F(1,4) = 33.32$, $p < 0.01$. This finding suggests that the discrimination is easier when the targets are longer than the background than when the targets are shorter than the background. The interaction was also significant $F(2,8) = 32.08$, $p < 0.01$. This result is most likely due to the differences in discrimination performance on the partial USVs in the normal condition compared to the reversed background/target condition (white vs black bars). Discrimination performance did not vary much across partial USV conditions in the new stimulus configuration, possibly due to ceiling effects.

IV. DISCUSSION

As far as we know, there have been only two psychoacoustic studies measuring USV perception in mice. The first revealed that mice could discriminate between USVs differing in spectrotemporal characteristics (Neilans *et al.*, 2014). The present study showed that mice have difficulty discriminating partial USVs from a whole USV. Discrimination performance across all combinations of partial USVs was significantly below performance with pure tones. This suggests that the information content in these small segments of USVs is preserved since the tone and partial USV stimuli had similar frequency and duration characteristics. Scientists are still unsure about how mouse USVs are exactly used in acoustic communication and whether the USV “types” such as those we separated ours into even have different meanings for the mice. The results reported here, along with those from Neilans *et al.* (2014), suggest that spectral and temporal cues could be important for acoustic communication in mice. An important caveat, however, is that just because the mice are *able* to perceive differences between USVs does not mean that these differences actually carry any meaning or importance to the communicating animal. Much more research is needed to determine exactly how USVs are being used by mice in communication, and to further probe the limits of their perception of these acoustic stimuli.

Another important finding of this experiment is that discrimination is more difficult for mice when an initial portion of the background USV is presented instead of the last portion. This poor discrimination performance suggests that the mice perceive the initial portions of a USV as more similar to the whole USV than the later parts of the USV, which is similar to the results of human (Salasoo and Pisoni, 1985; Marslen-Wilson and Zwitserlood, 1989), sea lion (Pitcher *et al.*, 2012), and bird recognition and playback studies (Toarmino *et al.*, 2011). Even though the studies used different methodologies, each could be interpreted as showing that the beginning of the word or call is the most important part of the signal. Recognition paradigms, like those used in humans and birds, require both the discrimination and then the classification of stimuli. Our task was slightly easier,

FIG. 5. Mean discrimination performance for each target stimulus type for all four individual USV backgrounds. Error bars represent between-subject standard errors. Dashed lines indicate mean false alarm rate.

involving the discrimination of the stimulus, but not a long term memory of it to place it into a specific category. Conducting this experiment using a recognition task in the future would further clarify similarities in auditory processing across species.

However, it is still unclear why these initial portions of a USV hinder discrimination. It is possible that the call recognition system works in a similar way to the cohort model of human word recognition proposed by Marslen-Wilson and Zwitserlood (1989). This model suggests that, as the first sections of a word are heard, all non-matching words are eliminated until only one word remains; at that point, it is recognized. It is possible that mice could use similar mechanisms to eliminate possible USV types as more of the USV is heard. Again, more research on the use and categorization of call types by mice, and experiments matching the methodologies of those used in the human and bird studies would illuminate whether this type of recognition process is

possible. Pitcher *et al.* (2012) simply believe that the vocal signature of Australian sea lions is contained in the beginning of the call, and this is a possible explanation for the current results as well.

Another possible explanation for the poor discrimination for the beginning of the call relative to the end of the call is that only the beginning of the calls are processed and remembered, making the first third target identical to the background, and making it extremely difficult to discriminate from the background. This does not seem likely for two reasons. First, performance is still above chance for all calls. Second, performance for the second and third thirds of the calls is still lower than for our control stimuli. If these were perceived as completely different from the background, performance would be higher.

It was surprising that there were no differences in discrimination performance as stimulus duration increased (one-thirds partials vs two-thirds partials). These results

FIG. 6. Mean discrimination performance for the reversed background/target condition where each partial USV is a background “call portion,” and whole USVs are targets. Error bars represent between-subject standard errors. Dashed line indicates mean false alarm rate.

contradict previous findings in birds, where budgerigars showed higher identification performance on targets containing longer portions of bird calls (Toarmino *et al.*, 2011). Aside from possible species differences, an alternate explanation could be differences in the stimuli used. The present experiment split the USVs into three portions rather than the four employed by Toarmino *et al.* It is also possible that the durational differences between the stimuli could explain the differences in results. The bird calls used in the Toarmino *et al.* (2011) study were 500 ms in duration, compared to the mouse USVs in the present study, which were only 51–120 ms. Consequently, it could have been more difficult for the mice to perceive the extra stimulus length added to the much-shorter USVs. It is also possible that separating the USV into additional portions could have produced more pronounced differences in discrimination performance.

We did not expect to find the differences in discriminability between the targets and the four background USVs that we discovered. The mice easily discriminated the “40 kHz harm” call from the first third of the call at a much higher rate than they did for the other three calls. Conversely, the mice had great difficulty discriminating the “Chevron” USV background from almost all of the targets. One possible explanation for this result is that the Chevron USV may be harder for the mice to accurately perceive than the other USVs, while the 40 kHz harm call is easier to perceive. Unlike the other three USVs, the Chevron USV has no complex harmonic structure. It also is at a higher frequency range and contains a lot less frequency modulation than the other three calls. This entire USV falls within 66 and 84 kHz, so sensation levels for this call would be 10+ dB higher than those for the other calls. Although frequency discrimination performance for pure tones at differing sensation levels is not robustly different (Radziwon and Dent, 2014), it is possible that they hindered performance here. The differences in performance do match those found by Neilans *et al.* (2014) using a similar discrimination

paradigm and similar stimuli. They discovered that mouse discrimination performance was lower when an up-sweep vocalization (similar in duration and frequency range as the Chevron USV) was used as a background compared to a harmonic vocalization background. Thus, when studying discriminability of vocal signals by mice, characteristics such as frequency range, peak frequency, duration, and the presence or absence of harmonics should all be taken into consideration when identifying the cues used by the mice. Calls with harmonic structure are also emitted less often than the Chevron, upsweep, and downsweep calls (Mahrt *et al.*, 2013), which could be a function of any number of things, including increased repetition of harder-to-perceive calls, different meanings for the different call types, and ease or difficulty of producing the different calls.

Finally, discrimination performance was higher when stimulus duration was added than when it was removed. This suggests differences in the processing of auditory information under different experimental configurations, similar to that seen in birds and humans in the Feature Positive Effect, and highlights the importance of good experimental design and considering the animals’ tendencies in studies of auditory processing.

Overall, this study adds to the growing body of literature on mouse acoustic communication and auditory perception. Similar to findings in both humans and birds, the initial portions of mouse USVs seem to be important for vocal communication, suggesting that USVs could possibly be analogous to human words and bird calls, although testing with many other signals needs to be conducted. Because mice are often used as a model of human hearing, it would be beneficial to continue to look at both the similarities and differences between mice and humans in how auditory signals are perceived. Future research in this subject could provide further information on mouse USV perception and improve how we use mice as models for human hearing and communication.

ACKNOWLEDGMENTS

This work was supported by NIH DC009483 and DC012302 to M.L.D. Thanks to Dr. Christine Portfors, Dr. Paul Luce, Dr. Richard Salvi, Dr. Matthew Xu-Friedman, Dr. Kelly Radziwon, and Dr. Michael Dygert for assistance.

- Ehret, G. (1976). “Development of absolute auditory thresholds in the house mouse (*Mus musculus*),” *J. Am. Audiol. Soc.* **1**, 179–184.
- Ehret, G., Koch, M., Haack, B., and Markl, H. (1987). “Sex and parental experience determine the onset of an instinctive behavior in mice,” *Naturwissenschaften* **74**, 47.
- Fay, R. R. (1994). *Comparative Hearing: Mammals* (Springer-Verlag, New York), pp. 1–17.
- Grimsley, J. M., Gadziola, M. A., and Wenstrup, J. J. (2012). “Automated classification of mouse pup isolation syllables: From cluster analysis to an Excel-based mouse pup syllable classification calculator,” *Front. Behav. Neurosci.* **6**, 89.
- Grimsley, J. M., Monaghan, J. J., and Wenstrup, J. J. (2011). “Development of social vocalizations in mice,” *PLoS One* **6**, e17460.
- Hammerschmidt, K., Radyushkin, K., Ehrenreich, H., and Fischer, J. (2009). “Female mice respond to male ultrasonic ‘songs’ with approach behavior,” *Biol. Letters* **5**, 589–592.
- Henry, K. R., and McGinn, M. D. (1992). “The mouse as a model for human audition: Review,” *Int. J. Audiol.* **31**, 181–189.

- Holmstrom, L. A., Eeuwes, L. B., Roberts, P. D., and Portfors, C. V. (2010). "Efficient encoding of vocalizations in the auditory midbrain," *J. Neurosci.* **30**, 802–819.
- Kikusui, T., Nakanishi, K., Nakagawa, R., Nagasawa, M., Mogi, K., and Okanoya, K. (2011). "Cross fostering experiments suggest that mice songs are innate," *PLoS ONE* **6**, e17721.
- Klink, K. B., Bendig, G., and Klump, G. M. (2006). "Operant methods for mouse psychoacoustics," *Behav. Res. Methods* **38**, 1–7.
- Knudsen, D., Thompson, J. V., and Gentner, T. Q. (2010). "Distributed recognition of natural songs by European starlings," *Learn. Motiv.* **41**, 287–306.
- Mahrt, E. J., Perkel, D. J., Tong, L., Rubel, E. W., and Portfors, C. V. (2013). "Engineered deafness reveals that mouse courtship vocalizations do not require auditory experience," *J. Neurosci.* **33**, 5573–5583.
- Marslen-Wilson, W., and Zwitserlood, P. (1989). "Accessing spoken words: The importance of word onsets," *J. Exp. Psychol. Human.* **15**, 576–585.
- Neilans, E. G., Holfoth, D. P., Radzwon, K. E., Portfors, C. V., and Dent, M. L. (2014). "Discrimination of ultrasonic vocalizations by CBA/CaJ mice (*Mus musculus*) is related to spectrotemporal dissimilarity of vocalizations," *PLoS ONE* **9**, e85405.
- Newman, J., Wolff, W. T., and Hearst, E. (1980). "The feature-positive effect in adult human subjects," *J. Exp. Psychol. Hum. Learn.* **6**, 630–650.
- Nyby, J. G. (2001). *Handbook of Mouse Auditory Research* (CRC Press, Boca Raton, FL), pp. 3–18.
- Pitcher, B. J., Harcourt, R. G., and Charrier, I. (2012). "Individual identity encoding and environmental constraints in vocal recognition of pups by Australian sea lion mothers," *Anim. Behav.* **83**, 681–690.
- Portfors, C. V. (2007). "Types and functions of ultrasonic vocalizations in laboratory rats and mice," *J. Am. Assoc. Lab. Anim.* **46**, 28–34.
- Prosen, C. A., Dore, D. J., and May, B. J. (2003). "The functional age of hearing loss in a mouse model of presbycusis. I. Behavioral assessments," *Hear. Res.* **183**, 44–56.
- Radziwon, K. E., and Dent, M. L. (2014). "Frequency difference limens and auditory cue trading in CBA/CaJ mice (*Mus musculus*)," *Behav. Processes* **106**, 74–76.
- Radziwon, K. E., June, K. M., Stolzberg, D. J., Xu-Friedman, M. A., Salvi, R. J., and Dent, M. L. (2009). "Behaviorally measured audiograms and gap detection thresholds in CBA/CaJ mice," *J. Comp. Physiol. A* **195**, 961–969.
- Sainsbury, R. S., and Jenkins, H. M. (1967). "Feature-positive effect in discrimination learning," *P. Ann. Conv. APA.* **2**, 17–18.
- Salasoo, A., and Pisoni, D. B. (1985). "Interaction of knowledge sources in spoken word identification," *J. Mem. Lang.* **24**, 210–231.
- Shepard, K. N., and Liu, R. C. (2011). "Experience restores innate female preference for male ultrasonic vocalizations," *Genes Brain Behav.* **10**, 28–34.
- Toarmino, C., Neilans, E. G., and Dent, M. L. (2011). "Identification of conspecific calls by budgerigars (*Melopsittacus undulatus*)," *Yale Rev. Undergraduate Res. Psychol.* **2011**, 38–44.