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Frequency Difference Limens and Auditory Cue Trading in CBA/CaJ Mice (*Mus musculus*)

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Abstract

Mice are emerging as an important behavioral model for studies of auditory perception and acoustic communication. These mammals frequently produce ultrasonic vocalizations, although the details of how these vocalizations are used for communication are not entirely understood. An important step in determining how they might be differentiating their calls is to measure discrimination and identification of the dimensions of various acoustic stimuli. Here, behavioral operant conditioning methods were employed to assess frequency difference limens for pure tones. We found that their thresholds were similar to those in other rodents but higher than in humans. We also asked mice, in an identification paradigm, whether they would use frequency or duration differences to classify stimuli varying on those two dimensions. We found that the mice classified the stimuli based on frequency rather than duration.

Keywords

Cue trading; Frequency difference limens; CBA/CaJ mice; psychoacoustics; mouse ultrasonic vocalizations; operant conditioning

1. Introduction

Researchers have long known that mice produce various types of sonic and ultrasonic vocalizations (USVs; Whitney et al., 1973). However, the abilities of mice to discriminate vocalizations have only recently been investigated. Neilans et al. (2014) found that CBA/CaJ mice discriminate USVs well above chance, and that discrimination performance declines as spectrotemporal similarity between the vocalizations increases. The mice could have used frequency, duration, or any combination of auditory dimensions to distinguish the vocalizations. Therefore, we sought to add to the psychophysical literature on the CBA/CaJ mouse strain (May et al., 2006; Radziwon et al., 2009), a mouse known to retain its hearing into middle age, making it a good model for hearing and acoustic communication (Zheng et al., 1999).

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We first measured simple frequency difference limens for pure tones. We used a modified go/no-go paradigm where the mice were required to discriminate a change in frequency between a repeating background reference tone and various target tones of higher frequencies. The purpose of this experiment was to determine whether the CBA/CaJ mouse could resolve high frequencies adequately enough to use frequency to discriminate between vocalizations.

In a second condition, we tested the mice in a novel cue trading study. The cue trading condition made use of an identification paradigm where the mice placed tones into two categories. For instance, if a mouse heard a 70 kHz tone with a 25 ms duration it made one response, and if it heard a 30 kHz tone with a 120 ms duration, it made a different response. Once the mouse was able to identify these two categories, a 70 kHz tone with a 120 ms duration was presented. If the mouse used frequency to identify the tones then it made the first response; however, if duration was more important, then it made the second response. Using this type of controlled paradigm, it was possible to determine whether frequency or duration was the more salient feature for mice when differentiating tones, potentially clarifying the feature(s) the mice use when identifying USVs.

2. Methods

2.1 Subjects

Three CBA/CaJ mice were used in the FDL condition, and four CBA/CaJ mice were used in the cue trading condition. Subjects began training at three months of age (sexually mature) and were tested until 12 months of age. They were bred at the University at Buffalo, SUNY and all procedures were approved by the University at Buffalo, SUNY’s Institutional Animal Care and Use Committee. All mice were housed separately and were kept on a reverse day/night cycle. They were water restricted and kept at approximately 85% of their free-drinking weight.

2.2 Apparatus, procedure, and data analysis for FDL condition

2.2.1 Apparatus—The mice were tested in a cage containing an ultrasonic speaker, a response dipper, and nose poke holes surrounded by infrared sensors. TDT’s RPvds software and a MATLAB interface were used to control the hardware and monitor the animals’ responses (see Neilans et al., 2014 for more detail).

2.2.2 Procedure—The test stimuli used in this condition were 500 ms pure tones with cosine rise/fall times of 20 ms. The frequencies tested were 12, 16, 24, and 42 kHz presented at 10 and 30 dB sensation level (SL), as determined by a previously obtained audiogram (Radziwon et al., 2009), and roved by ±3 dB between presentations to eliminate level cues. The mice were trained using a go/no-go operant conditioning procedure to discriminate a higher-frequency comparison tone (target) from a reference tone (repeating background).

The mouse began a trial by nose poking the *observation nose-poke hole*, which initiated a variable waiting interval of 1 to 4 s. During this time, the reference tone played at a rate of 1 tone every 700 ms. After the waiting interval, a single target was presented. In the *go* condition, the target was the comparison tone. If the mouse detected a change between the reference and target, it poked the *report nose-poke hole*. In this trial type, a *hit* was recorded if the mouse correctly responded to the comparison tone within 2 s. The animal received a reinforcement of 0.01 ml of water (morning session) or Ensure® (afternoon). A *miss* was recorded if the mouse either failed to poke the report hole or if it did not respond within 2 s.

Thirty percent of all trials were *catch* trials, where the repeating background continued through the response interval. These constituted the *no-go* part of the procedure. If the mouse poked the report hole during a *catch* trial, a *false alarm* was recorded and the mouse received a 20 s timeout. However, if the subject continued to nose-poke to the observation nose-poke hole, a *correct rejection* was recorded. No reinforcement was given for a *correct rejection* but the next trial began immediately. Sessions were excluded from analysis if the percentage of false alarms was greater than 20%.

Targets were presented according to the psychophysical Method of Constant Stimuli, where stimuli both above and below the presumed threshold were presented to the subjects in a random order in blocks of ten trials (7 targets and 3 catch trials).

2.2.3 Data analysis—Mean *hit* and *false alarm* rates were used to calculate thresholds. Thresholds for each frequency were calculated using the last 200 trials out of at least 400 trials. If thresholds varied by more than 3% Weber fraction between the first 200 and last 200 trials, testing continued until thresholds stabilized.

2.3 Apparatus and procedure for cue trading condition

2.3.1 Apparatus—The apparatus for this study is identical to the first study except there are three nose pokes instead of two.

2.3.2 Procedure—The test stimuli were 30 or 70 kHz pure tones, with 25 or 120 ms durations. These features were chosen because they were within the range of naturally-produced USVs (Neilans et al., 2014) and because they were easily discriminable (Ehret, 1975; Klink and Klump, 2004). All tones had cosine rise/fall times of 5 ms, were presented at 30 dB SL, and were roved ±3 dB to eliminate level cues (rise/fall times were shorter in this study because the tone durations were much shorter). After the mice reached 85% correct identification performance for at least 100 trials, untrained probe stimuli were added. The probe stimuli tested whether the mice were using frequency or duration to classify the endpoints. For instance, if the mice were trained with the 70 kHz/25 ms and 30 kHz/120 ms endpoints, then the probes were the 70 kHz/120 ms and 30 kHz/25 ms tones.

As in the first study, the mice were trained to nose poke to initiate trials and make responses to the stimuli. If the mouse made a correct response, a *hit* was recorded and the animal received either water or Ensure®. A *miss* was recorded if the mouse poked to the incorrect hole, resulting in a 25 s timeout. During testing, the untrained probe stimuli were randomly interspersed with the endpoint stimuli on 20% of trials. The responses to the probe stimuli were always rewarded regardless of how the mouse responded. The mice received 20 presentations of each probe stimulus, and their responses were counted to determine whether they were categorizing the probes based on frequency or duration.

3. Results and Discussion

3.1 Frequency difference limens

The FDLs (Figure 1) for these mice were similar to those found in other rodents, but were higher than those found in humans and cats (~200 Hz higher at lowest frequencies; Heffner et al., 1971). In general, thresholds increased with increasing frequency. Their FDLs, though unsurprising given the known physiological (Saunders and Crumling, 2001) and psychophysical (Ehret, 1975; May et al., 2006) data, were useful in determining which features the mice can use when discriminating conspecific USVs. Since their thresholds fell within the frequency boundaries of pup and adult USVs (Grimsley et al., 2011), our results suggest that frequency can be used to identify pups from adults. However, more research is needed to determine whether mice *actually* differentiate calls based upon frequency alone.

3.2 Cue trading

For the first time here, mice were trained on an auditory identification task. The mice identified the two endpoint stimuli with at least 87% accuracy. Further, every mouse classified the untrained probes based on frequency instead of duration (Figure 2). Responses to the probes were not different than the responses to the endpoint stimuli [$t(6)=1.17$, $p=0.29$, Cohen's d (effect size)=0.83], suggesting that the mice were only using frequency to classify the stimuli.

Mouse USVs, like other complex stimuli, differ by more than frequency, and to understand mouse communication, researchers must take into account their perceptual strategies. Therefore, cue trading experiments like this one are useful in examining interactions among auditory dimensions (Melara and Marks, 1990). Given that duration *and* frequency differences were well above threshold, the mice could have used either dimension to classify the probes (Klink and Klump, 2004) but they mainly chose frequency. These results highlight the reliance the mice have on frequency, and suggest that frequency can be the dominant feature mice use to discriminate among various auditory stimuli, including, possibly, vocalizations.

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HIGHLIGHTS

- Frequency difference limens were measured in CBA/CaJ mice
- Frequency difference limens were similar to those in other rodents
- Mice were also trained, for the first time, on an auditory identification cue trading task
- Mice overwhelmingly chose frequency over duration during identification

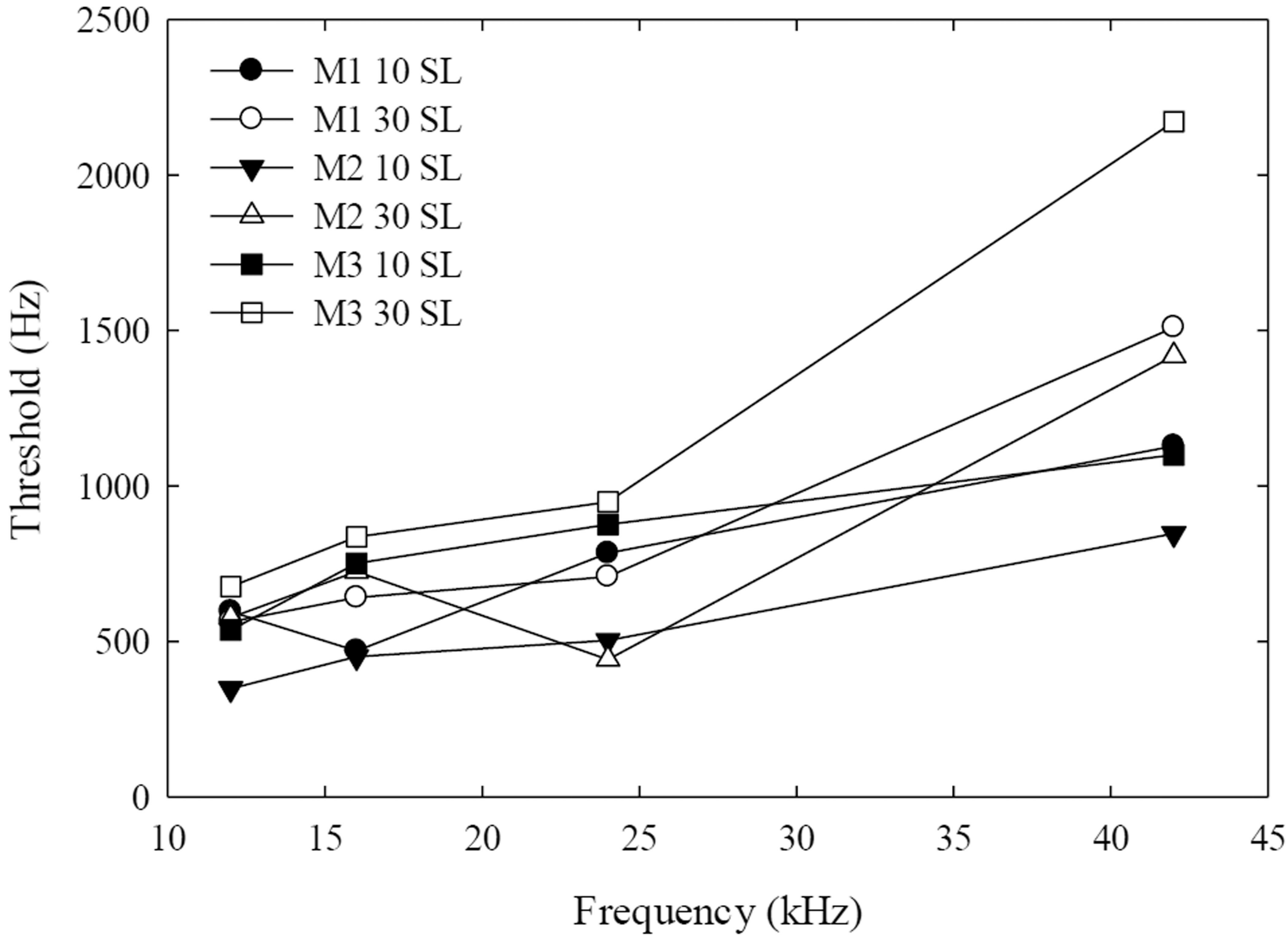

Figure 1.

FDLs for each mouse (M1, M2, and M3) at 12, 16, 24, and 42 kHz for two sensation levels (10 and 30 dB).

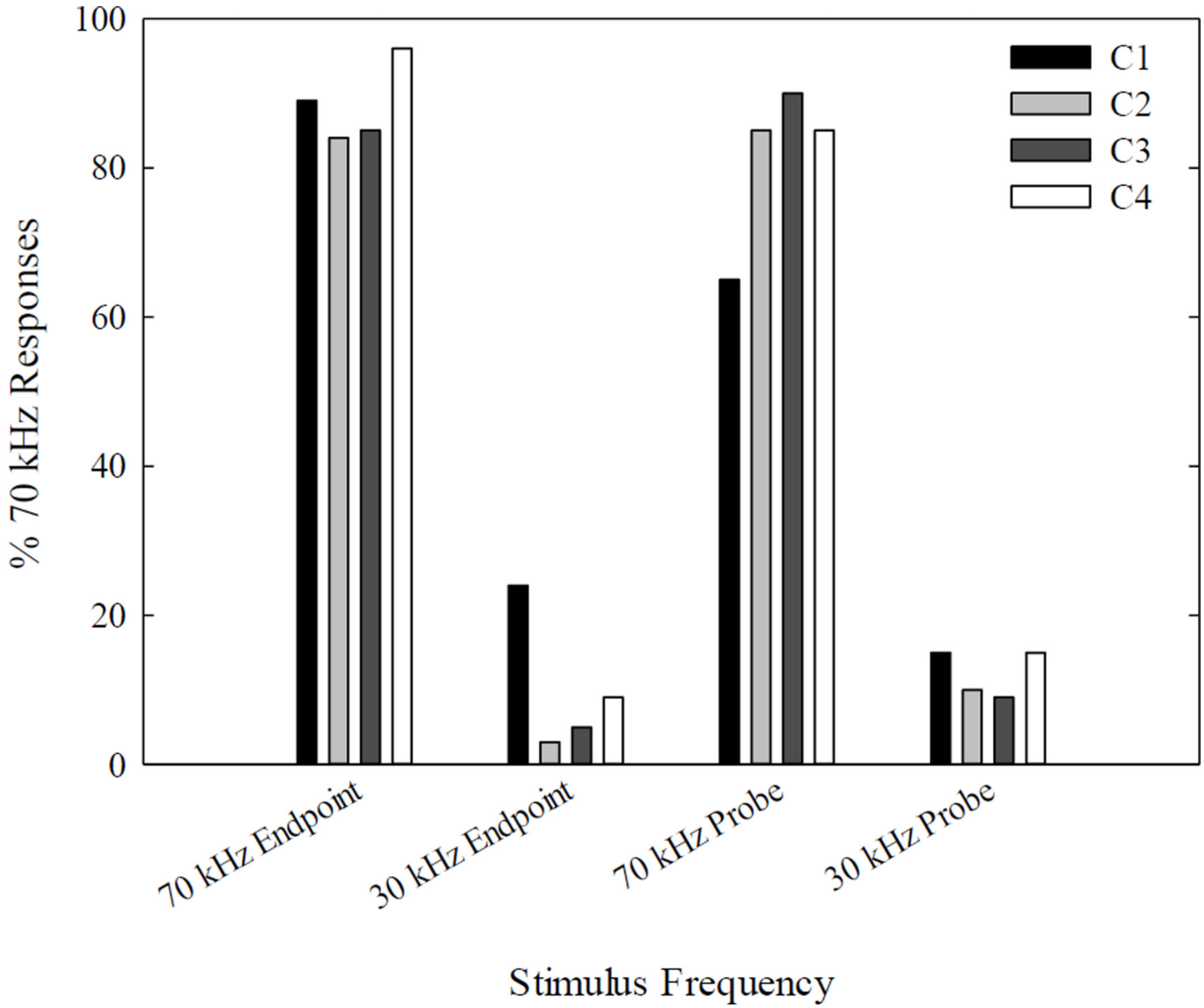

Figure 2.

Percentage of 70 kHz responses for trained endpoints and untrained probes collapsed across duration for each mouse (C1, C2, C3, and C4).