

Discrimination of Functionally Referential Calls by Laboratory-Housed Rhesus Macaques: Implications for Neuroethological Studies

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Key Words

Discrimination · Rhesus · Mammals · Habituation · Vocalization · Auditory

Abstract

Prior to examining the neural correlates of auditory cognition with ethologically relevant stimuli, it is first necessary to establish that laboratory-housed animals respond to these stimuli with species-typical responses. Here, we report the results of experiments on laboratory-housed rhesus monkeys using both species-typical vocalizations and band-pass noise. Paralleling the approach used in field studies of this species, we used a habituation-discrimination paradigm in which auditory stimuli were presented and a monkey's orienting responses to the stimuli were quantified. In parallel with the results obtained in field studies, we found that laboratory-housed rhesus classified species-typical vocalizations according to their putative referent properties as opposed to similarities in their acoustic morphology. In control experiments, monkeys oriented to band-pass noise but did not categorize differences in the spectral composition of the noise stimuli. These findings support the hypothesis that laboratory-housed rhesus classify, in the absence of training, species-typical vocalizations in a manner comparable to rhesus monkeys living under more natural conditions. As such, species-typical vocal-

izations are an appropriate and necessary class of stimuli in experiments that explore the neural correlates of auditory cognition in rhesus monkeys from a neuroethological perspective.

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Introduction

For non-human primates, species-typical vocalizations are one of the most important classes of auditory stimuli. These signals convey information about the identity and age of the caller and often provide information about sex and emotional or motivational state [Cheney and Seyfarth, 1990; Hauser, 1997]. Some species-typical vocalizations have also been described as functionally referential. That is, based on signal morphology alone, and regardless of the mechanisms that might underlie call production in vocalizers, listeners are able to extract information about a call's putative meaning. Examples of such referents include different kinds of predators, social relationships, and food [Struhsaker, 1967; Seyfarth et al., 1980; Dittus, 1984; Gouzoules et al., 1984; Cheney and Seyfarth, 1988, 1992; Elowson et al., 1991; Macedonia, 1991; Pereira and Macedonia, 1991; Benz, 1993; Hauser and Marler, 1993a, b; Fischer, 1998; Hauser, 1998; Zuberbuhler, 2000a, b; for a review, see Seyfarth and Cheney 2003].

Because species-typical vocalizations play an important role in the socioecology of most non-human primates, the auditory system appears to have evolved specialized mechanisms to process these auditory stimuli [Wordon and Galambos, 1972; Hauser, 1997; Ghazanfar and Hauser, 1999]. Indeed, several studies have demonstrated that neurons in different areas of the central auditory pathway respond preferentially to species-typical vocalizations [Ploog, 1981; Rauschecker et al., 1995; Wang et al., 1995; Le Prell and Moody, 1997; Tian et al., 2001; Wang and Kadia, 2001; Romanski and Goldman-Rakic, 2002]. For instance, neurons in the auditory cortex are modulated more strongly by species-typical vocalizations than by spectrally similar stimuli or other artificial auditory stimuli [Rauschecker et al., 1995]. Although these studies have identified neurons that respond selectively to the unique spectrotemporal profiles of species-typical vocalizations, there have not been any studies, to date, that have identified the cortical loci involved in coding the meaning or other abstract qualities of a species-typical vocalization [Newman, 1978].

As a first step in a series of studies focused on examining how the auditory system codes the perceptual and cognitive attributes of species-typical vocalizations in the rhesus monkey, we present the results of behavioral experiments with laboratory-housed rhesus monkeys designed to show how they spontaneously (i.e., without training) categorize species-typical vocalizations. We tested rhesus in a habituation-discrimination paradigm, analogous to that used in previous investigations of free-ranging primates [Cheney and Seyfarth, 1988, 1990; Hauser, 1998] and in classic studies of infant perception [Eimas et al., 1971; Spelke, 1985]. Paralleling studies of free-ranging rhesus monkeys [Hauser, 1998], laboratory-housed monkeys participated in a habituation-discrimination paradigm in which species-typical vocalizations were the habituation and test stimuli. Additionally, in control experiments, band-pass noise was presented as either the habituation stimulus or the test stimulus.

Materials and Methods

Subjects

Twenty-six laboratory-housed adult rhesus monkeys (*Macaca mulatta*; 20 males and 6 females) were used in this study. These monkeys were housed in vivariums at Yale University Medical School (n = 7), Harvard University Medical School (n = 12), and Dartmouth College (n = 7).

The monkeys in this study participated in different numbers of experimental sessions. Each of the seven monkeys from Yale University participated in one experimental session. Five of the twelve mon-

keys from Harvard University participated in two experimental sessions, each of which was separated by at least 30 min, and seven monkeys participated in one experimental session. The seven monkeys from Dartmouth College participated in six experimental sessions, each of which was separated by at least 4 days (and typically several weeks). None of the monkeys that participated in more than one session was exposed to the same pair of habituation and test-discrimination stimuli (see below). That is, if during one experimental session a monkey was habituated with stimulus *A* and exposed to test-discrimination stimulus *B*, she would not be exposed to this stimulus pairing in subsequent experimental sessions.

A few important points about the relationship between the rhesus monkeys and the auditory stimuli used in this study are worth noting. First, none of the monkeys had been operantly conditioned to associate any of the auditory stimuli (see *Auditory Stimuli*) used in this experiment with a behavioral task and a subsequent reward. Second, our rhesus did not have any experience with the stimulus exemplars as (1) they were not exposed to any of the auditory stimuli until the experimental session was underway and (2) they were not familiar with the individuals from which the species-typical vocalizations originated. Finally, some of the species-typical vocalizations used in this experiment (e.g., 'harmonic arch' and 'warble'; see below) occur rarely, if at all, in captivity (M.D. Hauser, unpublished observation) and, thus, may be experimentally novel to our test subjects.

Experimental Set-Up

Each session took place in a sound-attenuated room that was similar to the one in which each rhesus monkey normally participated in experiments. At the beginning of each session, a monkey was placed in a primate chair. While in the primate chair, the monkey was permitted to freely move her head and body. A speaker was placed 90° to the left of the front of the primate chair, concealed out of view. The monkey's response (i.e., a directed head and eye movement) toward the location of the auditory stimulus was recorded with a video camera. The video camera was placed 45° to the left of the front of the primate chair to ensure that a clear image of the monkey's behavioral response was obtained. Additionally, a red light-emitting diode (LED) was placed within view of the camera but out of the monkey's line of sight. This LED was connected in parallel with the speaker so that it was illuminated when the auditory stimulus was present in the environment; the illumination assisted with data analysis by providing a visual cue to auditory-stimulus onset (see below). Finally, the output of the video camera was attached to a video monitor that was placed outside of the sound-attenuated room. The video monitor allowed the experimenters to monitor the monkey during an experimental session.

Habituation-Discrimination Paradigm

After entering the sound-attenuated room, the monkey was allowed 3–5 min to acclimate before data collection started. After this acclimation period, a trial was initiated when the monkey's head was perpendicular to the sound source; this orientation maximized the magnitude of the monkey's response and mimicked field conditions [Hauser, 1998].

A trial was initiated by introducing a 'habituation' stimulus into the environment of the sound-attenuated room. The habituation stimulus was an exemplar from a specific class of auditory stimuli (see *Auditory Stimuli* below). Different exemplars of the habituation stimulus were presented until the monkey habituated to the auditory stimuli. The inter-stimulus interval was 5–60 s. This presentation

rate approximates the vocalization rate in free-ranging rhesus monkeys [Hauser, 1998], and it allowed enough time for the monkey to return her head to a position that was perpendicular to the speaker.

A monkey was classified as habituated if, following two consecutive presentations of a habituation stimulus, she did not orient her head toward the sound-source location within 2 s of habituation-stimulus onset. The advantage of this criterion was that it placed all of the monkeys in the same relative behavioral state before the presentation of a 'test-discrimination' stimulus [Hauser, 1998].

After habituating, the test-discrimination stimulus was presented, and the monkey's response to this stimulus was recorded. The test-discrimination stimulus was an exemplar from a different class of auditory stimuli (see *Auditory Stimuli* below).

If the monkey did not orient her head toward the location of the speaker within 2 s of onset of the test-discrimination auditory stimulus, a third type of auditory stimulus (see *Auditory Stimuli* below) was presented. The response of the monkey to this third stimulus allowed us to differentiate between whether the monkey (1) remained habituated to the test-discrimination stimulus or (2) had habituated to the testing environment. If the monkey did not respond to this third class of auditory stimulus, it was assumed that she had habituated to the test environment itself and her data were not analyzed further [Hauser, 1998].

An experimental session was aborted prior to the monkey habituating if one of two conditions were not met. First, a session was aborted if a monkey did not return her head to a position perpendicular to the speaker within 60 s of the offset of a habituation stimulus. Second, a session was aborted if a monkey did not respond (i.e., orient) to at least the first two presentations of the auditory stimulus.

After completing her participation in the experiment, the monkey was removed from the sound-attenuated room, given food rewards, and returned to the vivarium.

Auditory Stimuli

Species-typical vocalizations and band-pass white noise were presented as either habituation or test-discrimination stimuli. Spectrograms of exemplars from each of these stimulus classes are shown in figure 1.

Three classes of species-typical vocalizations, 'harmonic arches', 'warbles', and 'grunts', were used as habituation and test-discrimination stimuli. In free-ranging rhesus macaques, harmonic arches and warbles are produced when individuals encounter food items of high value [Hauser and Marler, 1993a; Hauser, 1998]. Grunts are produced when free-ranging rhesus encounter food items of low value and in non-food contexts (e.g., during grooming or group movement) [Hauser and Marler, 1993a; Hauser, 1998]. Within a given experimental session, exemplars of harmonic arches, warbles, or grunts came from the same monkey. This restriction eliminates a potential source of bias because changes in the monkey's response cannot be attributed to trial-by-trial changes in the specific individual that elicited the vocalization. The mean duration of these vocalizations was 429 ms with a standard deviation of 330 ms. These vocalizations were recorded and digitized as part of an earlier series of studies; see Hauser [1998] for more details.

Two classes of band-pass noise were used as habituation and test-discrimination stimuli. 'High-pass' noise had a pass-band between 9.75 and 15.25 kHz, and 'low-pass' noise had a pass-band between 0.55 and 2.8 kHz. The duration of the noise stimuli was matched to the distribution of rhesus vocalization duration (mean = 429 ms and

standard deviation = 330 ms). The fall and rise times of the noise bursts were 10 ms. In a digital-signal-processing environment that is based on the AP2 DSP card (Tucker Davis Technologies), the noise bursts were generated from broadband gaussian white noise that was filtered to achieve the correct spectrum.

A fourth type of species-typical vocalization, a 'shrill bark' was also used; a spectrogram of the shrill bark is shown in figure 1. This vocalization is elicited by free-ranging rhesus during alarm [Bercovitch et al., 1996]. The shrill bark helped us determine whether a laboratory-housed rhesus monkey had habituated to the test-discrimination stimulus or to the testing environment (see *Habituation-Discrimination Paradigm*).

For each class of auditory stimuli used as habituation or test stimulus, we had multiple exemplars. We had recordings of 4 exemplars from two individuals for the harmonic arches and warbles and recordings of 5–9 exemplars from three individuals for the grunts. We generated three sets of low-pass and high-pass noise, each of which contained 10 exemplars. On a session-by-session basis, the individual from which species-typical vocalizations were chosen or the set from which noise exemplars were chosen was random.

All of the auditory stimuli were pre-recorded to disk and presented during an experimental session through a D/A converter (DA1, Tucker Davis Technologies), an amplifier (SA1, Tucker Davis Technologies, and MPA-250, Radio Shack), and a speaker (Pyle, PLS32). Because the temporospectral structure (see fig. 1) of rhesus' vocalizations is critically related to rhesus monkeys' behavioral responses [Ghazanfar et al., 2001], we did not control for differences in the frequency range, duration, onset, etc. of the different vocalization classes. However, to control for differences in loudness between different vocalization classes, all of the stimuli, both the species-typical vocalizations and band-pass noise, were presented at the same sound level: 65 dB SPL (sound pressure level, relative to 20 μ Pa, A-weighting).

Data Analysis

Behavioral data were recorded with a video camera (Panasonic, AG-456) onto videotape at a frame rate of 30 frames/s. Video-editing equipment (BR-S500U, BR-S800U, and RM-G800U, JVC Inc.) was used to analyze the videotapes on a frame-by-frame basis. From these videotapes, we quantified the time that the monkey spent looking at the speaker (i.e., 'looking time'). Looking time was defined as the interval between the end of a monkey's orienting response and the time when the orientation of her head moved away from the sound-source location. Four observers, who were blind to the treatment conditions, quantified the videotapes. One-hundred percent of the quantified data was tested for inter-observer reliability using a correlation analysis. Because there were not any systematic differences ($r = 0.97$), data from the observers were pooled for statistical analysis. Statistical analyses (analyses of variance with post-hoc Tukey tests and t tests) were used to test for differences in looking time using the Excel (Microsoft Inc.) and SPSS (SPSS Inc.) environments.

The original research reported herein was performed under guidelines established by the National Institutes of Health and the recommendations of the Dartmouth College Animal Care and Use Committee. The experiments were also performed under the guidelines of the Animal Care and Use Committees at Yale University Medical School and Harvard University Medical School.

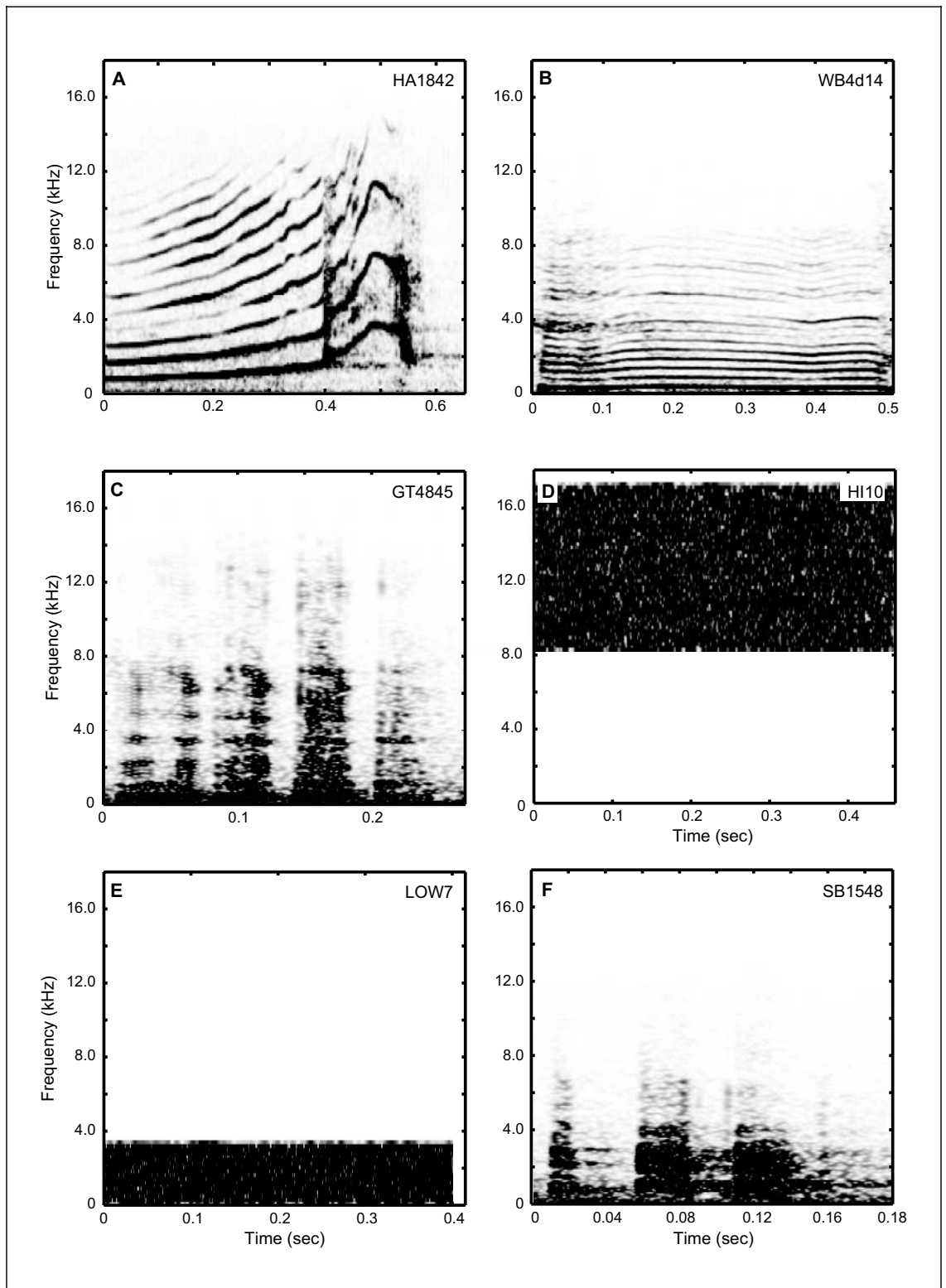
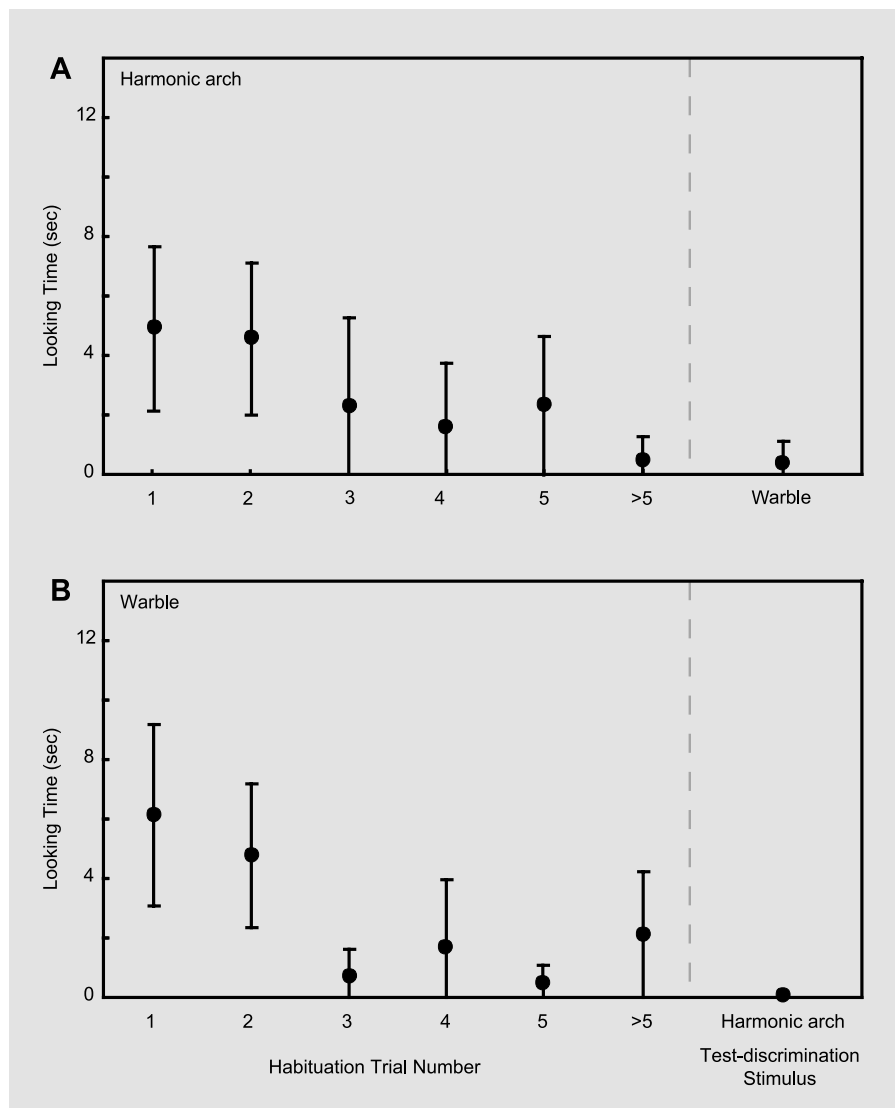


Fig. 1. Sound spectrograms of the six classes of auditory stimuli used in the study. **A** Harmonic arches, **B** warbles, and **C** grunts were used as habituation and test-discrimination stimuli. **D** High-pass and **E** low-pass were used as control stimuli and also served as habituation and test-discrimination stimuli. Finally, the shrill bark (**F**) was used as a post-test-discrimination stimulus. Note that the time scale on the abscissa is different in each panel.

Fig. 2. Habituation and test-discrimination responses. In each panel, the mean (\pm standard deviation) looking time for a habituation stimulus is plotted as a function of trial number, and the mean looking time (\pm standard deviation) for the test-discrimination stimulus is shown to the right of the dotted line. Because on average for each of the habituation stimuli, most monkeys habituated within five trials, looking time for trials >5 are grouped together. **A** The mean looking time for habituation to harmonic arches and the mean looking time for the test-discrimination warbles. For trials 1–4, the mean looking time was based on 5 presentations of the habituation stimulus; for trial 5, it was based on 4 presentations; and for trial >5, it was based on 18 presentations; and for the warble test-discrimination stimulus, 5 trials. **B** The mean looking time for habituation to warbles and the mean looking time for the test-discrimination harmonic arches. For trials 1–4, the mean looking time was based on 6 presentations of the habituation stimulus; for trial 5, it was based on 3 presentations; for trial >5, it was based on 12 presentations, and for the harmonic-arch test-discrimination stimulus, 6 trials.



Results

Data were collected from 26 monkeys, who participated in a total of 66 experimental sessions. Of these 66 experimental sessions, 59 were analyzed and are presented below. We excluded 7 experimental sessions because either (1) the monkey did not respond to the first two presentations of the habituation stimulus or (2) the monkey habituated to the testing environment. As discussed previously, a monkey was classified as ‘habituated to the testing environment’ when she did not meet two criteria. First, she did not orient her head toward the location of the speaker within 2 s of onset of the test-discrimination auditory stimulus. Second, she did not orient within 2 s of

onset of the shrill bark; the shrill bark was only presented when the monkey failed to orient to the test-discrimination stimulus.

Twelve monkeys participated in more than one experimental session of the habituation-discrimination paradigm (see *Subjects*). To test whether repeated experimental sessions influenced the monkey’s behavior, we compared the looking time of monkeys participating in their first session with the looking time of monkeys participating in subsequent sessions. We found that there were not any systematic differences in looking time between the first and subsequent experimental sessions ($F(1,44) = 1.51, p > 0.05$). As a result, data from the first and subsequent sessions were combined into the same data set.

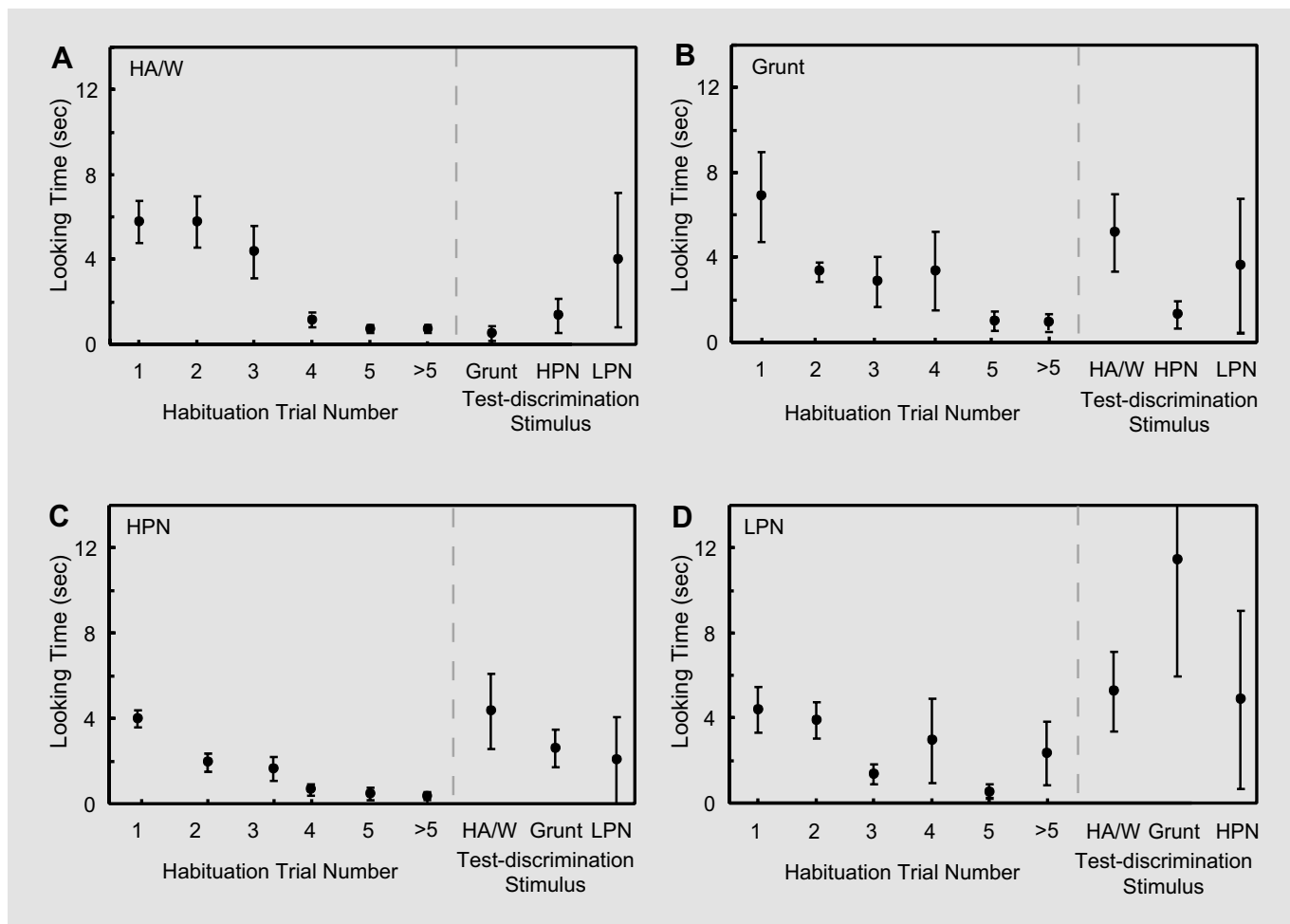


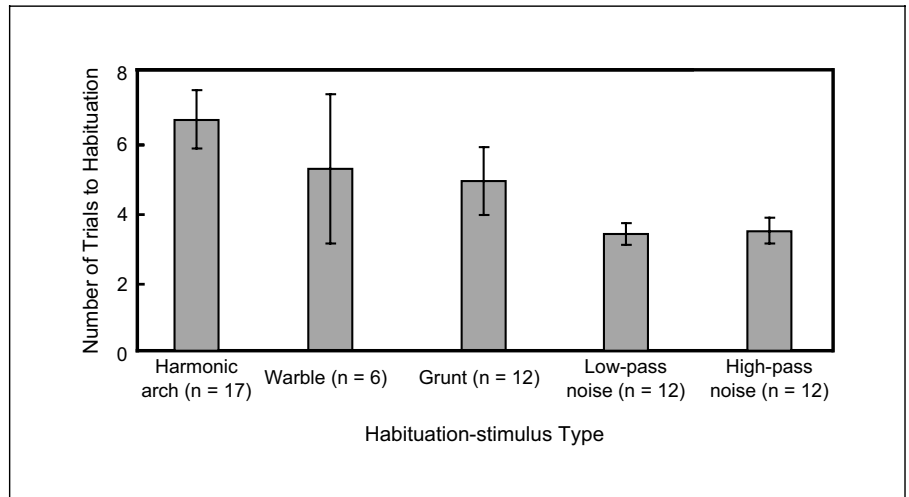
Fig. 3. Habituation and test-discrimination responses. In each panel, the mean (\pm standard deviation) looking time for a habituation stimulus is plotted as a function of trial number, and the mean looking time (\pm standard deviation) for each of the test-discrimination stimulus is shown to the right of the dotted line. Because on average for all of the habituation stimuli most monkeys habituated within five trials looking time for trials >5 are grouped together. **A** The mean looking time for habituation to harmonic arches/warbles (HA/W) and the mean looking times for the test-discrimination grunts, high-pass noise (HPN), and low-pass noise (LPN). For trials 1–5, the mean looking time was based on 12 presentations of the habituation stimulus (i.e., 4 monkeys participated in each of the 3 test-discrimination trials); for trial >5 , it was based on 35 presentations; and for each test-discrimination stimulus, 4 trials. **B** The mean looking time for habituation to grunts and the mean looking times for the test-dis-

crimination HA/W, HPN, and LPN. For trials 1–4, the mean looking time was based on 12 presentations of the habituation stimulus; for trial 5, it was based on 10 presentations; and for trial >5 , it was based on 24 presentations; and for each test-discrimination stimulus, 4 trials. **C** The mean looking times for habituation to HPN and the mean looking time for the test-discrimination HA/W, grunts, and LPN. For trials 1–4, the mean looking time was based on 12 presentations of the habituation stimulus; for trial 5, it was based on 9 presentations; and for trial >5 , it was based on 8 presentations, and for each test-discrimination stimulus, 4 trials. **D** The mean looking times for habituation to LPN and the mean looking time for the test-discrimination HA/W, grunts, and HPN. For trials 1–4, the mean looking time was based on 12 presentations of the habituation stimulus; for trial 5, it was based on 9 presentations; and for trial >5 , it was based on 17 presentations; and for each test-discrimination stimulus, 4 trials.

Figures 2–3 illustrate the average looking time as a function of trial number and habituation-stimulus class. As can be seen, subjects initially responded robustly to all 5 classes of habituation stimuli (3 classes of species-typical vocalizations and 2 classes of band-pass noise). They

oriented to the stimuli and fixated their gaze at the sound-source location for several seconds. On average, their looking time on the first trial was 3–6 s. However, with repeated presentations looking time decreased and by the fifth or sixth trial averaged less than 1 s. We tested the

Fig. 4. Number of trials to habituation. The mean (\pm standard deviation) number of trials required to habituate a monkey as a function of auditory-stimulus class. Habituation was defined as the first of two consecutive presentations of the habituation stimulus in which a monkey did not orient within 2 s of auditory-stimulus onset. Data from all the habituation-discrimination trials are combined in this figure.



reliability of this change in looking time with a repeated-measures ANOVA in which habituation-trial number was the repeated measure (trials 1–5) and habituation-stimulus class was the between-groups factor. Only data collected on the first five trials were analyzed statistically because most of the monkeys habituated to all of the stimulus classes within 5 trials (see fig. 4), a pattern that mirrors field studies [Hauser, 1998]. A significant effect ($F(4,164) = 9.92, p < 0.05$) for the repeated measure of trial number was found, which indicated that looking time differed as a function of trial number. Post-hoc tests further indicated that looking time decreased significantly ($p < 0.05$) between the first and third trials. However, habituation-stimulus class was not a significant ($F(4,41) = 1.98, p > 0.05$) between-group factor, nor was there a significant ($F(16,164) = 1.02, p > 0.05$) interaction between trial number and habituation-stimulus class. These results in total suggest that although a monkey's looking time decreased with trial number, her looking time was not dependent on habituation-stimulus class.

A regression analysis in which trial number was the independent variable and looking time was the dependent variable was also employed to examine the relationship between trial number and habituation-stimulus class. This regression analysis was carried out as a function of habituation-stimulus class and for data collected during the first five trials. The slopes of the regression line were negative indicating that looking time decreased with increasing trial number. However, consistent with the aforementioned ANOVA analysis, the rate of the decrease was not dependent on habituation-stimulus class ($F(4,54) = 0.71, p > 0.05$).

Habituation was defined as the first of two consecutive presentations of the habituation stimulus in which a monkey did not respond (see Materials and Methods). Monkeys habituated to a stimulus class, on average, after 5 trials (fig. 4). An ANOVA indicated that the number of trials required to habituate a monkey differed ($F(4,54) = 3.64, p < 0.05$) as a function of habituation-stimulus class. Post-hoc pairwise tests indicated that there were reliable differences between harmonic arches and the two classes of band-pass noise: monkeys habituated to low-pass noise (mean = 3.4 trials) and high-pass noise (mean = 3.3 trials) in significantly (high-pass noise: $HSD = 3.82, p < 0.05$; low-pass noise: $HSD = 3.74, p < 0.05$) fewer trials than they did to harmonic arches (mean = 7.2 trials).

In the first set of experiments, we examined how monkeys responded to harmonic arches and warbles. Monkeys were either (1) habituated to harmonic arches and then presented a warble as a test-discrimination stimulus or (2) habituated to warbles and then presented a harmonic arch as a test-discrimination stimulus. These two stimulus classes were used because in free-ranging rhesus monkeys they have the same referential (but different acoustic; see figure 1) properties: harmonic arches and warbles are produced when free-ranging rhesus encounter food items of high value [Hauser and Marler, 1993a; Hauser, 1998].

Figure 2 shows the results of these experiments. The mean looking time in response to each test-discrimination stimulus is shown in the right-hand portion of each panel in the figure. As can be seen, the duration of the monkeys' looking time in response to the test-discrimination stimulus was short and substantially different than the looking time following the first trial of the habituation stimulus.

Table 1. Summary of t-statistics of comparison between fifth trial of presentation of habituation stimulus and test-discrimination stimulus*

Habituation stimulus	Dishabituation stimulus			
	HA/warble	grunt	HPN	LPN
HA/warble		T(14) = 0.56 p > 0.05	T(14) = -1.11 p > 0.05	T(14) = -1.9 p > 0.05
Grunt	T(12) = -2.88 p < 0.05		T(12) = -0.11 p > 0.05	T(12) = -1.49 p > 0.05
HPN	T(11) = -2.59 p < 0.05	T(11) = -2.45 p < 0.05		T(11) = -1.02 p > 0.05
LPN	T(11) = -3.36 p < 0.05	T(11) = -3.07 p < 0.05	T(11) = -1.54 p > 0.05	

* Different degree-of-freedom values in each cell reflect the fact that some monkeys habituated prior to the fifth trial. This was especially true when the band-pass noise was the habituation stimulus. Harmonic arch (HA), HPN (high-pass noise), and LPN (low-pass noise).

To test the reliability of these observations, we compared the looking time following the fifth trial presentation of the habituation stimulus with the looking time following the test-discrimination stimulus. We found that, after a monkey habituated to harmonic arches (fig. 2A), the looking time following the fifth presentation of a harmonic arch was not significantly different ($T(8) = 1.78$, $p > 0.05$) than the looking time in response to a warble as the test-discrimination stimulus. Similarly, we found that after a monkey habituated to warbles (fig. 2B), the looking time following the fifth presentation of a warble was not significantly different ($T(10) = 1.00$, $p > 0.05$) than the looking time in response to a harmonic arch as the test-discrimination stimulus. We interpreted these results to suggest that, following habituation to harmonic arches, habituation transfers (i.e., no significant change in response duration) to a warble when it is presented as a test-discrimination stimulus. Similarly, when habituated to a warble, rhesus monkeys transfer habituation to a harmonic arch.

In the second set of experiments, we examined how monkeys responded to harmonic arches/warbles and grunts. Because habituation was transferred between harmonic arches and warbles (see above; fig. 2), data from these two classes of vocalizations were grouped together [Hauser, 1998]. Monkeys were either (1) habituated to harmonic arches/warbles and then presented a grunt as a test-discrimination stimulus or (2) habituated to grunts and then presented a harmonic arch/warble as a test-discrimination stimulus. These stimulus classes were used because in free-ranging rhesus they have different referential (and different acoustic; see fig. 1) properties: har-

monic arches and warbles are produced when free-ranging rhesus encounter food items of high value, and grunts are produced when free-ranging rhesus encounter food items of low value and in other non-food contexts [Hauser and Marler, 1993a; Hauser, 1998].

Figure 3A, B shows the results of this experiment. The mean looking time in response to each test-discrimination stimulus is shown in the right-hand portion of each panel in the figure. Although the monkeys oriented to the test-discrimination stimulus, the duration of their looking time differed as a function of habituation and test-discrimination stimulus class. To test the reliability of these observations, we compared the looking time following the fifth presentation of the habituation stimulus with the looking time following the test-discrimination stimulus. We found that after a monkey habituated to harmonic arches/warbles (fig. 3A) the looking time following the fifth presentation of a harmonic arch/warble was not significantly different (see table 1; $p > 0.05$) than the looking time in response to a grunt as a test-discrimination stimulus. In contrast, we found that after a monkey habituated to grunts (fig. 3B) the looking time following the fifth presentation of a grunt was significantly shorter (see table 1; $p < 0.05$) than the looking time in response to a harmonic arch/warble as a test-discrimination stimulus. We interpreted this result to suggest that, following habituation to harmonic arches/warbles, monkeys transferred habituation (i.e., no significant change in response duration) to a grunt as the test-discrimination stimulus. However, after habituating to grunts, rhesus monkeys responded to the harmonic arch/warble when it was the test-discrimination stimulus.

Finally, in a series of control experiments we examined how monkeys responded when two classes of band-pass noise (high pass and low pass; see fig. 1) were incorporated into the habituation paradigm. Band-pass noise was (1) used as both the habituation and test-discrimination stimuli, (2) used as a habituation stimulus that preceded a species-typical vocalization (either a harmonic arch/warble or a grunt) as a test-discrimination stimulus, or (3) used as a test-discrimination stimulus that followed habituation to species-typical vocalizations (either harmonic arches/warbles or grunts).

As shown in figure 3, the results of this experiment were dependent on both the habituation and test-discrimination stimulus classes. To test the reliability of these observations, we compared the looking time following the fifth presentation of the habituation stimulus with the looking time following the test-discrimination stimulus. Three results were apparent from these comparisons. First, after habituating to one class of band-pass noise, the looking time following the fifth presentation of band-pass noise was not significantly different (see fig. 3C,D and table 1; $p > 0.05$) than the looking time in response to an exemplar from a different class of band-pass noise as a test-discrimination stimulus. Second, after habituating to species-typical vocalizations, the looking time following the fifth presentation of a species-typical vocalization was not significantly different (see fig. 3C, D and see table 1; $p > 0.05$) than the looking time in response to a band-pass noise burst as a test-discrimination stimulus. Finally, after habituating to band-pass noise, the looking time following the fifth presentation of band-pass noise was significantly shorter (see fig. 3C, D and table 1; $p < 0.05$) than the looking time in response to a species-typical vocalization as a test-discrimination stimulus. We interpreted these results to suggest that, following habituation to species-typical vocalizations or band-pass noise, monkeys transferred habituation (i.e., they did not respond) to band-pass noise when it was the test-discrimination stimulus. However, following habituation to band-pass noise, monkeys responded to species-typical vocalizations when they were the test-discrimination stimuli.

Discussion

The results of this study demonstrated that laboratory-housed rhesus monkeys initially oriented and responded to both species-typical vocalizations and band-pass noise. With repeated presentations of the same class of auditory stimuli, however, the magnitude of their response de-

creased, and eventually they did not respond to the stimulus (i.e., they habituated). Following habituation to one class of auditory stimulus, monkeys were presented with a stimulus from a different class. We found that their response to this test-discrimination stimulus was dependent on the classes of both the habituation and test-discrimination stimuli. Below, we discuss the pattern of responses observed.

Habituation and Test-Discrimination Responses to Species-Typical Vocalizations

The responses of the monkeys to the test-discrimination stimuli were dependent on the class of both the habituation and test-discrimination stimuli (see fig. 2–3 and table 1). When rhesus monkeys were habituated to harmonic arches, they did not respond (i.e., habituation transferred) to the warble that was the test-discrimination stimulus. Similarly, when habituated to warbles, habituation transferred to the harmonic arch that was the test-discrimination stimulus. In contrast, when habituated to harmonic arches/warbles or grunts, rhesus responded asymmetrically. When rhesus monkeys were habituated to grunts, they responded to a harmonic arch/warble that was the test-discrimination stimulus. In contrast, when rhesus monkeys were habituated to harmonic arches/warbles, they did not respond (i.e., habituation transferred) to the grunt that was the test-discrimination stimulus.

One potential interpretation of these results stems from the literature on categorical perception [Harnad, 1987; Kuhl, 1989]. This interpretation hypothesizes that the monkeys responded to perceptual differences that were based on differences in the spectrotemporal profiles of the different classes of species-typical vocalizations. Indeed, as illustrated in figure 1, there are considerable differences in the spectrotemporal profiles of the species-typical vocalizations that were used in this study. For instance, the fundamental frequencies of harmonic arches are generally higher than those of warbles. Also, although harmonic arches and warbles have continuous spectrotemporal profiles, the spectrotemporal profile of a grunt is discontinuous. Finally, the duration of harmonic arches and warbles tend to be longer than those of grunts.

Given the response patterns in our data this hypothesis seems unlikely. Consider the asymmetric response pattern of harmonic arches/warbles and grunts illustrated in figure 3A, B. Although the acoustic differences between the two call types are the same whether subjects are habituated to harmonic arches/warbles or grunts, the strength of their response in the test-discrimination trial varied depending on which vocalization type was used during

habituation. This asymmetric response pattern is unlike that seen in most studies of categorical perception. In these types of studies, responses are usually symmetrical: if a subject habituates to *A* and then responds to *B*, the subject will habituate to *B* and respond as strongly to *A* [Eimas et al., 1971]. Also, the spectrotemporal profiles of harmonic arches and warbles are quite different, but rhesus monkeys did not respond to the test-discrimination stimuli (see fig. 2) even though its acoustic morphology was substantially different than the morphology of the habituation stimulus. Together, these observations indicate that spectrotemporal differences were not the main factor underlying their responses to species-typical vocalizations and that other factors contributed [Tversky, 1977].

We suggest that the factors that influenced the monkeys' response patterns were the functional properties of the species-typical vocalizations [Cheney and Seyfarth, 1988, 1990; Hauser, 1998]. This interpretation posits that habituation transferred between harmonic arches and warbles (see fig. 2) as these two classes of species-typical vocalizations have the same functional properties. The asymmetric response pattern observed in the experiments that used harmonic arches/warbles and grunts (see fig. 3A, B) occurred because these classes of species-typical vocalizations have relatively different functional properties.

Field studies of free-ranging rhesus monkeys suggest that these functional properties may be based, in part, on the referential properties of the species-typical vocalizations [Hauser and Marler, 1993a; Hauser, 1998]. Both a harmonic arch and a warble functionally refer to high-quality/rare food items. On the other hand, a grunt has a different functional referent signaling low-quality/common food items as well as other non-food related behaviors. Free-ranging rhesus spontaneously categorize harmonic arches, warbles, and grunts in a manner consistent with our response patterns [Hauser, 1998], so it is reasonable to assume that these vocalizations functionally pick out the same referents in laboratory-housed rhesus. However, this assumption needs to be directly tested as laboratory-housed and free-ranging rhesus monkeys live in different sensory and social environments, and these differences might affect how they shape their perceptual and classificatory systems. Furthermore, more experiments are needed to confirm this referential hypothesis and rule out the possibility that the monkeys responded based on more subtle differences in acoustic morphology.

Habituation to and Discrimination of Band-Pass Noise

Whereas there was an asymmetric response pattern when the habituation and test-discrimination stimuli were vocalizations, when a monkey habituated to one class of band-pass noise, habituation transferred to the exemplar from other class of band-pass noise as the test-discrimination stimulus (compare fig. 3C, D; see table 1). That is, the subjects' responses to the test-discrimination low-pass (high-pass) noise burst were statistically indistinguishable from their responses to high-pass (low-pass) noise burst on the fifth habituation trial. This response pattern suggests, as one would postulate a priori, that low-pass and high-pass noise are not meaningfully different. Moreover, although psychophysical tasks [Serafin et al., 1982; Moody, 1994; Wienicke et al., 2001] would presumably show that rhesus could discriminate low-pass noise from high-pass noise (i.e., the acoustic differences are greater than the just noticeable difference limens), these detectable differences are insufficient to cause an orienting response using the habituation-discrimination technique (i.e., they are less than the just meaningful differences).

Habituation to and Discrimination of Vocalizations and Band-Pass Noise

Finally, when vocalizations and band-pass noise were used as the habituation or test-discrimination stimulus, an asymmetric response pattern was observed (compare fig. 3A, B with fig. 3C, D; see table 1). When monkeys were habituated to vocalizations, habituation transferred to band-pass noise as the test-discrimination stimulus. In contrast, when band-pass noise was presented during the habituation phase, subjects responded both to harmonic arches/warbles and grunts as the test-discrimination stimuli.

This pattern is consistent with our interpretation of the results presented above. That is, the switch from a non-relevant stimulus, such as band-pass noise, to one with substantial functional properties, such as a species-typical vocalization, captured the monkey's attention, leading to a robust orienting response. In contrast, the switch from a vocalization to a distinctively different burst of noise was insufficient to elicit an orienting response.

As in the case when the habituation and test-discrimination stimuli were vocalizations, it is possible that the asymmetric response pattern is due to factors other than the auditory stimulus' referential properties. This is an important issue given the significant acoustic differences between band-pass noise and rhesus vocalizations. To address these issues, our future experiments, both in the

wild and in captivity, will use artificial stimuli with acoustic complexities comparable to vocalizations, such as ripple noise [DePireux et al., 2001], synthesized rhesus calls, and the vocalizations of other familiar and unfamiliar primates.

Conclusion

The findings of this study provide a preliminary basis by which to guide neurophysiological studies that probe, in the awake, behaving primate, the correlates of the perceptual and cognitive attributes of vocalizations. Of significance, our results show that in the absence of significant experience with the full range of species-typical vocalizations – captive rhesus rarely, if at all, elicit harmonic arches or warbles [M.D. Hauser, unpublished observation] –, laboratory-housed rhesus monkeys nonetheless classify these vocalizations the same as free-ranging rhesus. Furthermore our results show that such perceptual abilities can be tapped using techniques that bypass some of the well-known difficulties associated with training procedures. This last claim is not intended as a criticism of operant techniques, but rather as a comment on the importance of using complimentary methods that tap spontaneous abilities.

We are now beginning to examine the neural mechanisms that transform representations of the spectrotemporal properties of an auditory stimulus into representations based on the monkey's perception and the referent of a vocalization. Two potential cortical loci for such

representations might be the parietal or prefrontal cortices. These areas seem likely candidates because auditory neurons in these areas respond to the meaning of an auditory stimulus and not just its spectrotemporal properties. For instance, parietal neurons code the presence of an auditory stimulus in the environment as it relates to a learned association (i.e., its 'meaning') between it and a behavioral task [Grunewald et al., 1999; Linden et al., 1999]. Similarly, when both auditory and visual stimuli have the same task-related meaning (e.g., both stimuli predict the location of a future target), parietal and prefrontal neurons code the task-related meaning of the stimuli and not their morphological properties [Fuster et al., 2000; Cohen et al., 2002]. The results of studies that combine behavioral measures with neurophysiological measures will lead to a greater understanding of auditory processing and cognition in the primate brain.

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