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7. I thank Dr. J. C. Fentress for helpful discussion. Supported by PHS predoctoral traineeship 5T01GM0036 and Health Science Association Award 1504FR0627 to the University of Oregon Neurobiology Group.

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Discrimination of Intermediate Sounds in a Synthetic Call Continuum by Female Green Tree Frogs

Abstract. *Male* Hyla cinerea *produce two distinctive calls. Acoustically intermediate calls are rare. Females discriminate between synthetic intermediates that differ by one cycle of amplitude modulation (50 per second). Processing appears to be continuous. The tree frog's auditory system thus provides a wide margin for the discrimination of its two principal signals.*

Most animals produce a relatively small number of discrete signals for intraspecific communication (*1*). The existence of repertoires of signals that intergrade (*2*) thus poses some important questions: To what extent, if any, do these intergraded signal systems increase the amount of information communicated? Do the animals using them possess some special sensory capabilities? Or, more generally, do the limited, discrete repertoires of most species indicate the inability of their nervous systems to produce and efficiently distinguish among a larger number of more similar signals? The answers to these questions depend on learning how animals deal with intergraded signals. How finely does an animal with a graded repertoire divide the continuum? How does an animal with discrete signals respond to intermediate signals?

Human speech is graded in its acoustic structure, and humans tend to perceive speech sounds that are intermediate along certain dimensions (for example, voice-onset time) in a nearly categorical manner (*3*). This means that intermediate sounds are usually identified ("labeled") as one or the other of the two end points of a continuum. Intermediates on the same side of a so-called phonetic boundary are very difficult to distinguish; intermediates on opposite sides of a boundary are readily discriminated even though the physical differences between them may be less than between intermediates on the same side the boundary (*4, 5*). At least one nonhuman primate with a graded vocal repertoire may treat some of its intermediate sounds in a similar fashion (*6*). Kuhl and Miller (*7*) showed that chinchillas, trained to distinguish the end points of a synthetic speech continuum and then presented with intermediate tokens, labeled these sounds in a manner similar to that of human subjects. Kuhl and Miller have suggested that categorical perception may simply reflect general psychophysical properties (constraints) of the (mammalian) auditory system rather than "special processing" mechanisms possessed by a species for the perception of its own signals (*8*). This issue remains unresolved not only because of the paucity of comparative data but also because differences in the methods of signal presentation and response criteria often preclude direct comparisons of the available studies. More specifically, we need to distinguish between an animal's ability to differentiate signals on the basis of some single property under "ideal" conditions and its performance in natural, complex contexts where redundant cues, noise, and speed of communication may become relevant (*9*). In any event, sensory mechanisms may restrict the number of discriminable signals (or phonetic segments) even in species with highly intergraded repertoires (*10*). It is thus tempting to speculate that the differences in the discrete signals of most animals reflect to some extent the resolving powers of their sensory systems. This report deals with the margin for error provided by the auditory system of a simple vertebrate for the discrimination of two of its discrete signals.

The vocal repertoires of most frogs and toads consist of unusually small sets of stereotyped signals (*1, 11*). The two principal vocal signals in the green tree frog (*Hyla cinerea*) are the mating and the pulsed calls, both of which are produced by males in breeding aggregations (*12*). Females are attracted to both kinds of calls in playback experiments, but they prefer mating calls. Pulsed calls are produced almost exclusively during agonistic interactions among males (*13*). Males occasionally produce transitional, acoustically intermediate calls (*14*). In comparison with pulsed calls, which are strongly amplitude-modulated (depth of modulation $\geq$ 75 percent) at about 50 per second for all or most of their duration, mating calls have a smooth amplitude-time envelope (*15*). Intermediate calls are strongly modulated for only part of their duration (Fig. 1A). These natural calls served as models for the generation of the synthetic call continuum (Fig. 1B) (*16, 17*). In one series of experiments females were given choices between the synthetic mating call [unmodulated (UM)] and the intermediates, and, in another series, between the modulated (M = +7) call and two of the intermediates (+4 and +5). Finally, females were given choices (four stimulus pairs) between intermediates. The sounds were recorded and played back with a stereophonic recorder (Nagra) (one kind of sound per channel); they were presented alternately (every 0.4 second), each from a separate speaker (Nagra DH) located 2 m apart. Gravid females were released individually from a small hardware-cloth cage located midway between the speakers where the intensities of the two sounds were equalized (75 dB with reference to 2×10^{-4} μbar). Experiments took place in a quiet (C-weighted ambient noise < 50 dB sound pressure level), dimly lit auditorium. Anechoic wedges (acoustic foam) minimized sound reflections in the test area. The criterion for a response was a female's touching a speaker or approaching it (to within 10 cm). This behavior reflects the natural courtship pattern; females typically initiate sexual contact with a calling male. Animals (fewer than 10 percent) that attempted to escape or failed to respond within 15 minutes were discarded. With two exceptions, the 122 animals that responded were tested in a single experiment in one of the three series described above (*18*). All animals were captured in amplexus on Wilmington Island, Georgia (20 km east of Savannah), between 1 June and 8 July 1977; they were tested within 24 hours of capture.

The synthetic mating call was preferred to +2, +3, +4, and +5 but not to +1 (Table 1). Females responded exclusively to +4 and +5 when the M (+7) call was the alternative stimulus (Table 1). The M call attracted some females in the absence of a competing stimulus. Thus most acoustically intermediate

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stimuli were also intermediate in their relative attractiveness. In a third series of experiments (Table 1) females given choices between intermediate calls preferentially responded on the basis of a difference of one cycle of modulation (at 50 per second). Furthermore, they did so across the entire synthetic call continuum. These animals appear to be processing these sounds in a continuous fashion, in the same way that humans process most nonspeech and some speech sounds (*3, 19*).

In one respect the interpretation of these results is straightforward. The resolving power of the tree frog's auditory system is more than adequate to distinguish between its two principal vocal signals; indeed, it is capable of distinguishing among graded intermediates to which the animal is rarely exposed. Nevertheless, the large margin for error may be very important for effective discrimination under noisier, more natural conditions (*20*). Preliminary experiments with a synthetic call continuum similar to the one used in the experiments reported here indicated that as many as five cycles of 50-per-second modulation are necessary for discrimination (in favor of an unmodulated call) when the depth of modulation is reduced to 50 percent (*21*). Furthermore, a call that was entirely pulsed at a depth of 50 percent was less attractive than one that was pulsed at a depth of 10 percent when both sounds were played back at 75 or 85 dB but not when they were played back at 65 dB (*17*). Lowering the playback level and decreasing the depth of modulation are equivalent in some ways to lowering the signal-to-noise ratio.

Table 1. Responses of 122 female *H. cinerea* in discrimination experiments with a synthetic call continuum. Stimulus designations are as shown in Fig. 1B (*18*). Data were analyzed with two-tailed binomial tests. Abbreviation: N.S., not significant.

Alternatives		Responses to		*P*
A	B	A	B	
Synthetic mating call versus intermediates				
UM	+1	5	5	N.S.
UM	+2	20	5	.005
UM	+3	8	0	.008
UM	+4	8	0	.008
UM	+5	8	0	.008
Synthetic pulsed call versus intermediates				
M	+4	0	8	.008
M	+5	0	9	.004
Choices between intermediates				
+1	+2	12	1	.003
+2	+3	14	2	.004
+3	+4	8	0	.008
+4	+5	10	1	.011

The significance of the female tree frog's apparent processing of intermediate calls in a continuous rather than in a categorical fashion is more difficult to assess. One relevant observation is that both ends of the synthetic continuum elicit the same qualitative response, that is, attraction to the sound source. More nearly categorical response patterns might have been observed if one call attracted and the other repelled the animals (*22*). But could the categorization then be considered a sole function of the sensory system? I suggest that categorical response patterns may reflect the activity of a higher integrative center, where other contingencies influence the fineness of the sensory analysis or its utilization in a decision-making process (*4*). In any event, the fact that both ends of the continuum attract females does not preclude response patterns that could be labeled "categorical" (*23*). Furthermore, it would have been possible to present synthetic signals one at a time to female tree frogs. I would expect a smaller proportion to have responded to the more pulsed signals and that their responses would have been slower in comparison with those to the unmodulated call (*24*). By arbitrarily choosing a cutoff time for the definition of a response, I could have obtained data that would have indicated that some sounds attracted and other sounds failed to attract. But the point along the continuum where this "categorization" occurred and its sharpness would be largely dependent on the cutoff time and level of statistical significance chosen beforehand. The resolving power of the tree frog's auditory system would almost certainly be underestimated. The two-choice experiment is not only a more rigorous experimental procedure (*4, 25*), but also it is more realistic in terms of the animal's natural history. A female enters a breeding aggregation very few times during her reproductive life-span. She is then faced with a wide choice of vocal signals. It is unlikely that she merely responds to the calls of the first male she encounters (*26*). Indeed, my field observations indicate that some females spend hours in the vicinity of calling males before responding to one of them.

In conclusion, the differences in the discrete vocal signals of the green tree frog do not reflect the resolution limits of its auditory system, at least in a quiet environment where the different sounds can be readily compared. The margin for error provided by its auditory system may be reduced, however, in more natural situations. In animal studies, categorical response patterns may well be a function of the methods and contexts of signal presentation and response criteria rather than stimulus categorization by the animal's sensory system.

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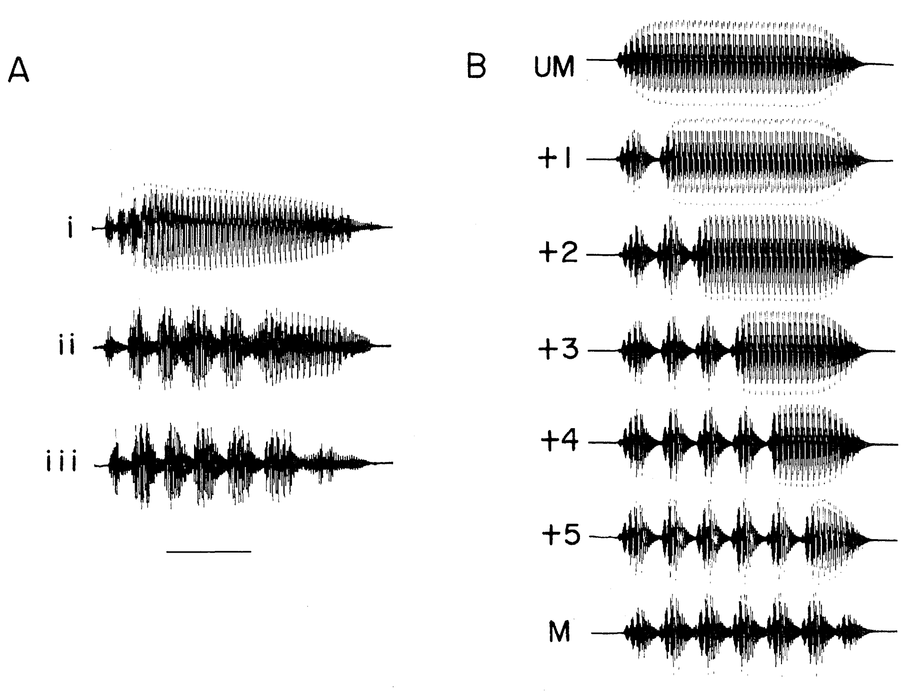

Fig. 1. (A) Oscillograms of the natural calls of a male *H. cinerea* from Georgia: (i) a mating call, (ii) an intermediate call, and (iii) a pulsed call. (B) Oscillograms of the synthetic call continuum used in the experiments described in the text. Each call consisted of three phase-locked sinusoids (0.9 + 2.7 + 3.0 kHz) of equal relative amplitude (*16*). The bars indicate time periods of 50 msec.

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3. A. Liberman, F. Cooper, D. Schankweiler, M. Studdert-Kennedy, *Psychol. Rev.* **74**, 431 (1967); I. Mattingly, *Am. Sci.* **60**, 327 (1972). The introduction of concepts from the field of human speech perception should not be interpreted as an attempt to make direct analogies with animal studies. One very important difference is that speech tokens (phonetic segments) are without meaning (semantic reference) in isolation whereas the end points, at least, of continua of animal sounds may convey a message in isolation. The existence of fine gradations in vocal signals is not necessarily matched by the perceptual ability to distinguish among these gradations.
4. The presence of a peak in a discrimination function is, in my opinion, the most reliable operational definition of categorical perception. However, experimental procedures (methods and contexts of the signal presentation and response criteria) can influence human discrimination of intraphonemic tokens within the voicing continuum. In some situations, intraphonemic discrimination is comparable to interphonemic discrimination. Thus, other criteria for categorical perception in humans are currently being used and debated [see, for example, D. Pisoni and J. Lazarus, *J. Acoust. Soc. Am.* **55**, 328 (1974); Sinnott *et al.* (*5*); N. MacMillan, H. Kaplan, C. Creelman, *Psychol. Rev.* **84**, 452 (1977)].
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8. See D. Pisoni [*J. Acoust. Soc. Am.* **61**, 1352 (1977)] and Sinnott *et al.* (*5*) for relevant data from human and monkey studies.
9. Presenting signals in close temporal proximity to trained animals in a quiet environment may enhance their selective responsiveness relative to animals tested under more natural conditions. Failures to discriminate under "ideal" conditions probably reflect limitations (or "special processing") of the auditory system. However, many species fail to respond at all under highly artificial conditions, and we need more comparative data. C. Snowdon (*Brain Behav. Evol.*, in press) discusses the difficulties of comparing the results of animal studies and human studies, which often have different goals as well as methods.
10. Acoustic signals are, however, usually graded in more than one way. They are often produced at the same time as other kinds of signals (for example, visual) especially in close-range communication in primates. Differences in properties which, in isolation, are hard to discriminate may, in consistent combinations with other cues, contribute to the efficiency of discrimination of the whole (*2, 3*).
11. W. Smith, *The Behavior of Communicating* (Harvard Univ. Press, Cambridge, Mass., 1977).
12. R. Oldham and H. C. Gerhardt, *Copeia* **1975-II**, 223 (1975).
13. If a male produces mating calls near another calling male, one or both animals frequently switch to bouts of pulsed calls until one of the animals either leaves or stops calling.
14. In a preliminary analysis, I found that among 560 pulsed and mating calls recorded in close temporal proximity, about 7 percent could be classified as acoustically intermediate. Calls classified as pulsed had as few as four and as many as seven cycles of modulation. Three-fourths of the intermediate calls had four or more cycles of modulation. They were so classified because the pulsed part of the call was followed by a relatively long unpulsed part. I do not believe that the variations observed in the number of cycles of modulation in pulsed and intermediate calls are correlated with gradations in the level of male aggressive behavior. In other words, a male that produces pulsed calls with seven cycles of modulation is not necessarily more aggressive than a male that produces pulsed calls with four cycles of modulation. A more likely index of male aggression seems to be the frequency with which a male produces bouts of pulsed calls.
15. After the initial pulsatile beginning [the rate of pulsing is much higher than in pulsed calls (Fig. 1A)], the rate of modulation in mating calls is typically around 300 per second. Thus, the amplitude-time envelope appears smoother in comparison with pulsed calls when the same time base is used for oscillographic analysis. The ~50-per-second modulation of pulsed calls appears to be superimposed on the ~300-per-second quasi periodicity of the mating call (*12*).
16. A synthetic call similar to the one illustrated in Fig 1B (UM) attracted female green tree frogs as effectively as a typical, natural mating call [H. C. Gerhardt, *J. Exp. Biol.* **61**, 229 (1974)]. Another study with synthetic calls established that amplitude-modulation of such a synthetic call at rates of about 50 per second and depths of at least 50 percent significantly reduced its relative attractiveness (*17*).
17. H. C. Gerhardt, *J. Exp. Biol.*, in press.
18. One female was first tested with UM versus +5; she later chose +4 over M. Another female chose +2 over +3; she later chose +3 over +4.
19. In a strict methodological sense, these data fail to demonstrate continuous processing because the smallest difference that females discriminate was not determined. Thus, it is possible that if intermediates differed by less than a complete cycle of modulation, then discrimination might differ at different points along the continuum. Nevertheless, in terms of the kinds of gradations that occur in natural signals, it seems reasonable to interpret these results as reflecting more nearly continuous rather than categorical processing. The results of these experiments should be generalized to lower and higher playback levels.
20. A male typically produces pulsed calls (and occasional intermediates) in short bouts of 3 to 25 calls (mean = 10). Other frogs in the immediate vicinity may also be producing mating and pulsed calls at the same time. In the experimental situation, the female was exposed to two kinds of calls without interruption until she responded. The response patterns of the females in the experiment were similar to those of females captured in the field (in amplexus) and released near calling males. Some responded within a few seconds and others took many minutes. The choices made in the discrimination experiments were seemingly independent of the response latency.
21. H. C. Gerhardt, unpublished data.
22. It should be possible to test this hypothesis. S. Perrill, H. Gerhardt, and R. Daniel (in preparation) found that about 16 percent of the calling males in a breeding site had noncalling satellite males associated with them. Preliminary observations indicate that satellite males are attracted (up to a point) by mating calls and repelled by pulsed calls.
23. Suppose, for example, that the UM call were favored only when an intermediate call had at least four cycles of modulation and that the +3 but not the +4 call were more attractive than the M call. If females discriminated between +3 and +4 or even between +2 and +5, these results could be interpreted as demonstrating categorical processing.
24. A greater proportion of nonresponding females were among those tested with the highly pulsed intermediates (+4 and +5) and the M call in this study and other pulsed signals in another study (*17*). The animals usually took longer to respond than females tested with calls from the UM end of the continuum. However, the animals discriminated between +5 and the M call, but failed to discriminate between UM and +1 (*20*).
25. In a two-stimulus test, a nonresponding animal merely fails to provide any information about the relative attractiveness of the two sounds. In a single-stimulus test, a nonresponding animal provides equivocal information about stimulus quality. The failure of an animal to respond in a playback experiment does not necessarily indicate that the stimulus is unattractive.
26. This statement would probably not hold for many other American tree frogs or for other anurans that tend to be opportunistic or explosive breeders. The breeding season of *H. cinerea* lasts from April to mid-August in the southern United States. Males call almost every night during this period, and I have found females night after night, even under drought conditions. The animals usually breed in permanent ponds and lakes. The preference of the female for the mating call over the pulsed call may support the hypothesis that the female assesses call qualities. If she responds to a mating call, there is a much higher probability that she will achieve amplexus with the male producing it than if she responds to a pulsed call which indicates the presence of at least two males. Alternatively, the female may be avoiding aggressive male behavior associated with the production of pulsed calls. Once a female has ovulated, however, she must oviposit on the same night. This probably explains the attractiveness of pulsed calls in the absence of mating calls. Indeed, some female *H. cinerea* respond to the calls of another species, *Hyla gratiosa*, when conspecific calls are unavailable (*12*). The "motivation" of females taken from amplexus may be abnormally high (relative to when they first become responsive to mating calls) and, thus, responses to pulsed calls may be an artifact of the experimental procedure. Nevertheless, the results of discrimination (two-choice) experiments (this study) indicate that these same females make consistent choices between signals with very subtle differences.

27. I thank W. Sherman for designing the special circuitry used to synthesize the stimuli and G. Williamson and G. McClung for the use of Savannah Science Museum facilities. I thank R. Daniel, S. Perrill, S. Hopkins, D. Yaeger, and J. Rheinleander for field assistance and W. Oliver for technical help. I thank R. Dooling, R. Kiester, P. Kuhl, P. Marler, J. Miller, and C. Snowdon for valuable discussions and comments on the manuscript. This work was supported by the NSF grant BNS 73 00795 and NIH career development award NS 00217-02.

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An Ultrastructural Correlate of the Membrane Mutant "Paranoiac" in *Paramecium*

Abstract. *The highly organized array of intramembranous particles, the ciliary plaque, varies from the wild type in size and organization in two stocks of the* Paramecium *behavioral mutant, paranoiac. In one of these stocks, the alteration is dramatic.*

The behavior of paramecia is governed by the membrane potential. When the membrane is at rest, the cilia beat posteriorly and the paramecia swim forward. When the membrane becomes depolarized, the cilia beat in a reversed direction (ciliary reversal) and the cell backs up (*1*). Recent experiments suggest that the ciliary membranes are the specific components which control the direction of ciliary beat because these membranes contain ion channels important in active depolarizations (*2*). A specific structure of the ciliary membrane, the intramembranous plaque consisting of an ordered matrix of membrane particles, is regularly found at the base of the cilia and may contain important sites for ion transport (*3*). One class of behavioral mutants in *Paramecium*, the paranoiacs, is characterized by long periods of continuous ciliary reversal, hence greatly prolonged backward swimming, as an overreaction to sodium. Mutations at any of five loci can result in the paranoiac behavior (*4*). This behavior is cor-

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