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Directionality of the Auditory System and Call Pattern Recognition During Acoustic Interference in the Gray Tree Frog, *Hyla versicolor*

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3

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Males of many species of frogs and toads use amplitude modulated advertisement calls to attract females. However, because males often call in choruses, advertisement calls of males can overlap and so obscure the pulse rate structure of their signals from females. Females of Hyla versicolor discriminate against overlapping calls timed to degrade the amplitude modulation structure of the advertisement calls relative to those that do not interfere. Here, we tested whether spatial separation of the sources of overlapping calls could facilitate pattern recognition during phonotaxis. When given a choice between overlapping calls broadcast at 83 dB sound pressure level (SPL) from adjacent speakers and those separated by 120°, females discriminated in favor of the separated call sources. However, they did not discriminate when the separation of speakers was only 45°. A reduction by 3 dB in the intensity of broadcasts from one of the adjacent speakers was sufficient to eliminate the preference for call sources separated by 120°. Multiunit recordings from the torus semicircularis obtained during broadcasts of calls from speakers located contralateral and ipsilateral to the recording site were used to estimate the magnitude of auditory system directionality in females. At 83 dB SPL, the difference between the neural response measured contralaterally and ipsilaterally was approximately 9 dB SPL when the call sources had an angular separation of 120°. Differences were smaller at lower stimulus intensities or with a speaker separation of 45°.

Key *words:* anuran, frog, pattern recognition, directional hearing, phonotaxis, acoustic interference

MANY ANIMALS FACE THE problem of discriminating among different sound patterns in a noisy environment. Females of many species of frogs face this situation after they enter a chorus to select a mate. Large numbers of males may call at high rates (Wells, 1988) and intensities (Gerhardt, 1975) in these aggregations, and females must be able to detect and discriminate biologically relevant elements of their vocalizations under conditions of high ambient noise (Gerhardt and Klump, 1988). In many species, advertisement calls provide the only information about conspecific males that females use to choose a mating partner (Gerhardt, 1994a). In multispecies assemblages of frogs, females also rely on signal features to facilitate discrimination of males of their own species from those of heterospecific males, a task crucial to females' reproductive success (Gerhardt, 1982; Gerhardt and Klump, 1988).

In humans, a variety of information, including cues provided by the directionality of the auditory system (that is, direction-dependent intensity, temporal or spectral information) enhanced by sound input from two ears, assists in the segregation of concurrent sounds (Durlach and Colbum, 1978; Moore, 1989; Bregman, 1990). The ability to use directional information to attend selectively to a particular sound or sounds among many separate sound sources, such as a conversation at a noisy party, is often referred to as the cocktail party effect (Cherry, 1953). In nonhuman taxa, there is evidence of similar capabilities (Fay and Feng, 1987; Schwartz and Gerhardt, 1989). For example, auditory directionality can help in the discrimination of species-specific temporal features in crickets if the songs of two individuals overlap (Pollack, 1986). The auditory system of anuran amphibians is directionally sensitive (Feng, 1981; Michelsen et *al.,* 1986; Eggermont, 1988; Jerrgensen, 1991; Jerrgensen and Gerhardt, 1991; Jerrgensen et *al.,* 1991; Schmitz *et al.*, 1992; Gooler *et al.,* 1993; Xu et *al.,* 1994), and this property may facilitate signal detection and selective responsiveness in noisy choruses. For example, in females of the green tree frog, *Hyla cinerea,* Schwartz and Gerhardt (1989) demonstrated that separation of speakers broadcasting calls and broadband noise resulted in a release from mask-

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*Corresponding author: Joshua Schwartz, Division of Biological Sciences, University of Missouri, Columbia, MO 65211 ing, an effect presumably mediated by the directional characteristics of the frogs' auditory system.

In many species of anurans, the rate of amplitude modulation (AM), or pulsing, within advertisement calls is a feature important to species discrimination by females (Schwartz, 1987a; Gerhardt, 1988, 1994a). Advertisement and aggressive calls may also be differentiated by patterns of AM as in the green tree frog, *H. cinerea* (Allan and Simmons, 1994; Gerhardt, 1994a). In choruses, when the calls of males overlap, this temporal information can be obscured or degraded unless calls are synchronized. Indeed, degradative interference (overlapping calls are out-of-phase so that their pulses are not in temporal alignment; see Fig. 2) reduces the attractiveness of overlapping pulsed calls relative to nonoverlapping calls in choice tests with female frogs (Schwartz, 1987b). Schwartz (1993) recently conducted a phonotaxis experiment with the neotropical tree frog *Hyla microcephala* to determine whether directional cues might contribute to pattern recognition when pulsed calls overlap (see von Helverson, 1984 and Pollack, 1986, for similar studies on insects). Females of this species use differences in pulse rate to discriminate the calls of conspecific males from those of heterospecific males (Schwartz, 1987a). When *H. microcephala* females were given a choice between degradatively interfering, equal intensity, conspecific calls broadcast from two speakers separated by 120" and two speakers separated by just 5°, however, they failed to show a preference (Schwartz, 1993). Although directional effects would be expected to be stronger coming from the more widely separated call sources, favoring phonotaxis toward them, the experimental results suggest that such effects did not improve significantly the females' ability to discern the fine temporal pattern in individual calls.

The gray tree frog, *Hyla versicolor,* also uses **an** amplitude modulated advertisement call (mean pulse rate, 20/s at 20°C; bimodal call spectrum with mean frequencies of harmonics at approximately 1100 and 2200 **Hz;** Gerhardt, 1991). Females of this North American species are very sensitive to deviations from the pulse rate of the conspecific advertisement call and use this temporal feature to discriminate conspecific males from males of *Hyla chrysoscelis* in mixed-species choruses (Gerhardt and Doherty, 1988). As did females of H *microcephala,* females of *H. versicolor* exhibited strong discrimination against overlapping (out-of-phase) pairs of advertisement calls relative to alternating calls (Schwartz, 1987b). The purpose of this study was to learn whether females of the gray tree frog could use directional information during phonotaxis to extract fine temporal features (AM) in overlapping calls, and thus test the generality of the results obtained with the much smaller tree frog, *H. microcephala* (mean female Snout-to-vent length is 25 mm versus 53 mm for H. *versicolor).* Moreover, we obtained from the auditory midbrain measurements of neural response to calls from different directions to estimate the potential contribution of directional cues to the task of pulse rate extraction from overlapping calls. Such data are not yet available for *H. microcephala.*

METHODS

Behavior

Gravid adult female gray tree frogs captured in amplexus at a pond in Ashland, Missouri, were returned to the laboratory at the University of Missouri, refrigerated, and tested in phonotaxis experiments the following day (experiments 1 and 2; for protocol of experiment 3, see later). The frogs were warmed to 20°C in an incubator prior to use. Females were tested individually in an indoor semianechoic chamber. The chamber (Mr. Winter, Inc.; inside dimensions: 7.0 x **2.7** x 3.7 m) walls were covered with 35.5 cm long wedges of acoustic foam (Soundcoat, Inc.) and the floor with carpet. The temperature (20°C) within the chamber was conditioned externally, and silencers (Acoustic Systems, Austin, TX) attenuated noise created by air moving to and from a remote air conditioner. The testing area was illuminated using an infrared light source (for a Panasonic model WV-CD810 CCTV Camera), and the responses of the subjects monitored (Sanyo VDC-2524 CCTV Camera) on closed circuit television. Additional details about the chamber acoustics are presented by Gerhardt (1994b). For all tests, four centrally facing speakers (Realistic Minimus 0.3) were located at the perimeter of a circle 1 m in diameter within the arena. Differences in the amplitude-frequency responses of the four speakers (measured at the females' release point) at the frequencies of the two harmonics of the advertisement call were small. Over the frequency range of interest, the playback system was flat within ± 2 dB. Before each test, we placed individual females in a small screen cage located at the center of this circle. After 30 s exposure to the test stimuli, we remotely raised a lid on the cage using a cord that extended out of the chamber. We scored a positive response if the subject came within 10 cm of a sound source within 10 minutes after showing phonotactic orientation movements, such as head and body scanning (Rheinlander *et al.,* 1979). Positions of the speakers were switched between testing days to cancel the effect of possible biases of the females for particular speakers on the test results.

The same synthetic call was used in all broadcasts. It was synthesized and saved on floppy diskette using a Commodore Amiga 2000 computer and custom software (sample rate, 20 kHz, 8 bits sample). The call was formed from a train of 18 pulses and was constructed to resemble a natural advertisement call in temporal (pulse period, 50 ms; pulse duration, 25 ms; pulse rise: linear, 20 ms duration; pulse fall: nonlinear and concave down, 5

TABLE I
Responses of Females of *H. versicolor* to Advertisement Calls Broadcast from Speakers with Different Angular Separations*

	Angle of Separation of Speakers Number of Females Choosing		Number Tested	P Value	
Degradative interference					
A	5 degrees	120 degrees			
	1	19	21	<0.001	
B	5 degrees	45 degrees			
	9	11	27	NS	
C	Single speaker	5 degrees			
	16	4	22	0.012	<0.001⁺
D	Single speaker	120 degrees			
	14	6	24	0.116	0.002⁺
Degradative interference One *5* degree speaker attenuated					
E	5 degrees (-6 dB)	120 degrees			
	12	8	23	NS	
F	5 Degrees (–3 dB)	120 degrees			
	13	7	23	NS	
Alternating calls					
G	5 degrees	120 degrees			
	15	*5*	25	0.042	

*Tests C, D, and ***G*** are control tests. NS = not significant; P = probability (two-tailed binomial or tchi-square).

ms duration; call duration, 875 ms; call rise: linear, 50 ms duration) and spectral (1100 Hz [–6.0 dB] + 2200 Hz [0.0 dB]) characteristics.

Calls were broadcast using an Amiga 1000 computer (low-pass filtered at approximately **5** kHz using the Amiga's built-in filter), two Realistic SA-10 stereo amplifiers, and a custom-built circuit incorporating digitally controlled analog switches. Pairs of calls, timed to overlap (see later), were output in stereo using the left and right channel of the computer, and these stimuli could be directed to the four speakers by sending the apporpriate bit pattern to the switching circuit via the parallel port.

Broadcast intensities for each speaker were adjusted with a calibrated (Gen Rad 1562A) Gen Rad 1982 precision sound level meter.

Experiment 1. This experiment consisted of seven tests **(A** through G of Table **1)** and was conducted to assess the effect of angular separation of degradatively interfering sound sources on the phonotaxis of females of *H. versicolor.* The interfering calls, broadcast by a pair of speakers, were time shifted by 50% of the pulse period. Thus, the pulses of one call coincided with the interpulse intervals of the other call and, to a human listener, the pulse rate of the calls appeared to have doubled during their simultaneous broadcast. This, in effect, simulated the pulse rate of the congener, *H. chrysoscelis.* In all tests using four speakers, one pair of speakers had a large angular separation (either 120" or 45") and the other pair of speakers had a smaller angular separation (5"; Fig. 1, left arena). The interfering calls from one pair of speakers (for example, speakers S1 and S3 in Fig. 1) were alternated with those broadcast from the other pair (S2 and S4 in Fig. 1, left arena). In five tests (A, B, C, D, G; Table 1), intensities of all broadcast calls at the release point of the females were 83 dB ± 1 dB (root mean square [RMS] sound pressure level [SPL]; C-weighting, fast response; dB re 20 µPa). In tests E and F, the two speakers separated by 120" broadcast calls at 83 dB SPL while one of the speakers separated by 5" was attenuated by either **3** or 6 dB SPL. This was done to estimate the magnitude, in decibels, of the contribution made by directionality of the auditory system to call pattern recognition at the wide angular separation. The rationale was that discrimination against the adjacent (5" separation) sound sources should be eliminated when the attenuation of one of these sources was close in strength to the contribution of directionality with the wide (120") separation of sound sources.

We conducted two types of control test. In the first type (tests C, D), to verify that degradation of the interfering calls could be detected by females, subjects were exposed to individual calls broadcast from a single speaker alternating with interfering calls broadcast from a pair of speakers separated by 5" (test C) or 120" (test D). The single speaker was located 180" from the line bisecting the other two speakers. Based on previous data (Schwartz, 1987b, 1993), we expected females to discriminate against the sources of interfering calls.

To determine whether females have an inherent preference for sound sources with wide angular separation (Telford, 1985), we conducted a second type of

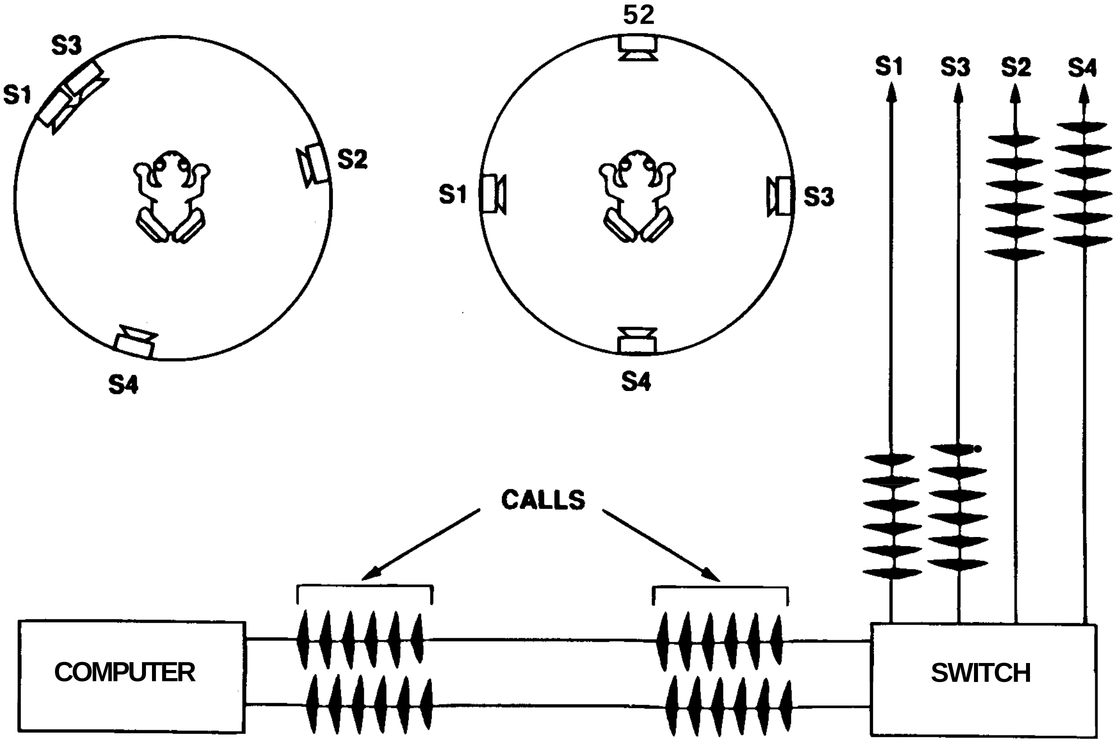

FIGURE 1 **Diagram of equipment and speaker arrangement used for female choice experiment 1 (left) and experiment 2 (right).** For experiment **1,** angular separations of 5" versus 120° are shown. Note that the amplifiers are not illustrated and the actual stimulus calls contained 18 rather than six pulses.

TABLE II

Responses of Females to Advertisement Calls Broadcast at Different Intensities*

	Intensities of Broadcasts Number of Females Choosing			Number Tested	P
Degradative interference—all speakers (90° separation)					
A	83–83 dB	83–80 dB			
	9	4	1	14	NS†
B	83–83 dB	83–77 dB			
	2	10	0	13	<0.002‡

*The intensities of broadcasts for each pair of speakers are given in dB SPL (fast RMS). NS = not significant; P = probability (tChi-square test assuming equal probabilities of approach to the speakers, $chi-square test under the conservative assumption that no females would approach the speaker broadcasting at 77 dB).

control test (test G). It was necessary to rule out the possibility of such a preference before invoking improved detectability of pulse structure of widely separated interfering calls as an explanation for female discrimination in test **A** or test **B.** Subjects were presented with calls from two pairs of speakers separated by 5" and 120". However, unlike the previous tests described, calls from each pair of speakers were timed so that they alternated rather than interfered (intercall interval of 1 s).

***Experiment* 2.** Experiment 2 consisted of two tests **(A, B** of Table 2) and was conducted to determine the effect of relative intensity on temporal degradation of interfering calls. In contrast to tests performed in experiment 1, these tests were conducted using a design with four speakers equally spaced (90° separation; Fig. 1, right arena). This geometric arrangement was identical to that used previously by Schwartz (1987b) with this species. Females were presented with advertisement calls interfering degradatively with those broad-

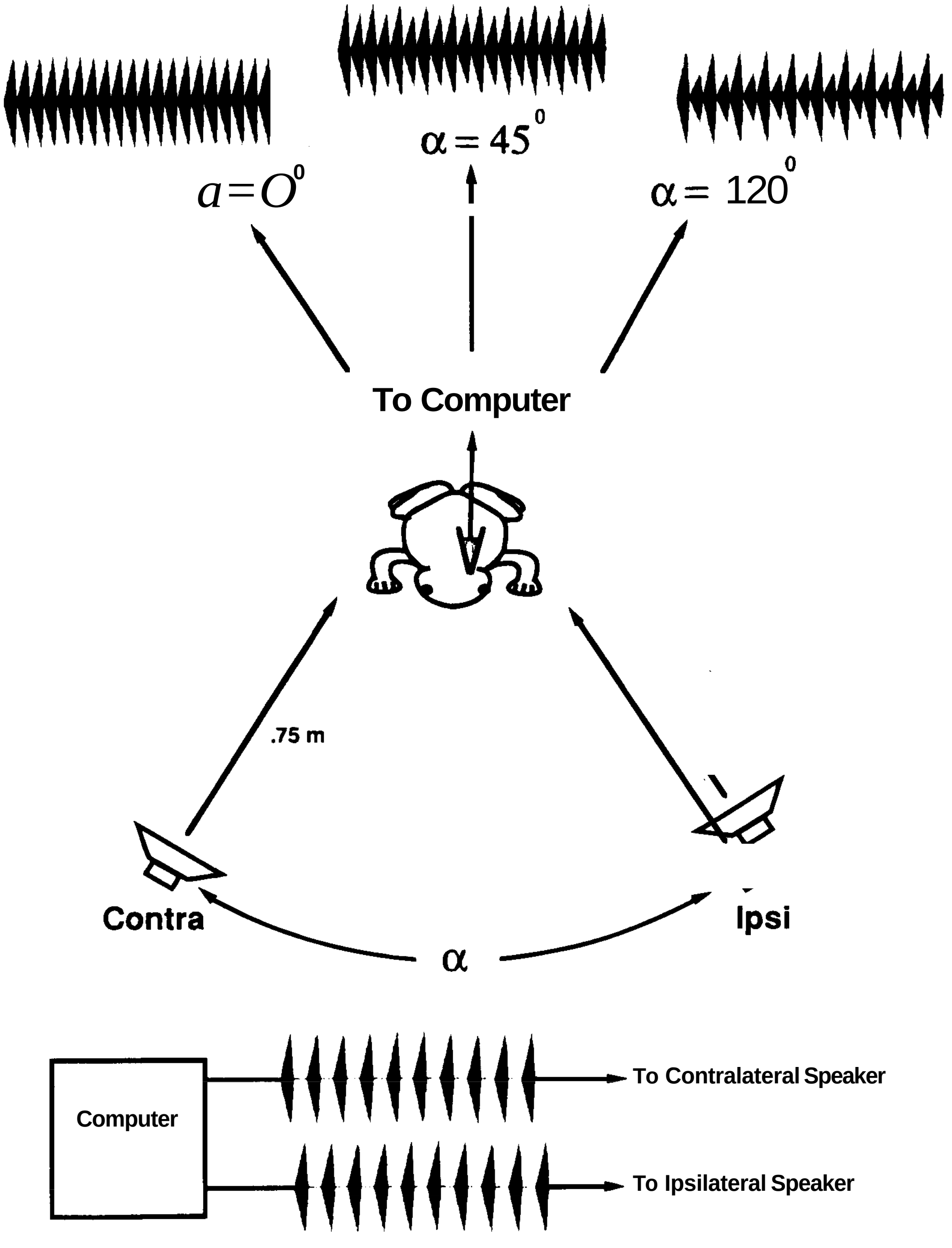

FIGURE 2 Schematic diagram **of** the stimulus presentation design used during neurophysiological tests. Shown below is the delivery of two overlapping (out-of-phase) amplitude modulated advertisement calls each to a speaker located either contralateral or ipsilateral to the brain hemisphere in which the electrode is placed. Above is shown the predicted qualitative effect of speaker separation on the temporal pattern of the multiunit recording of the neural response. Namely, with increased angular separation of the sound sources, the weaker the relative amplitude of the response to the ipsilateral source.

cast from the speaker on the opposite side of the arena (for example, calls from speaker S1 interfered with calls from speaker S3, and calls from speaker S2 interfered with calls from speaker S4 in Fig. **1,** right arena). Broadcast intensity from one source was lowered in 3 dB steps until discrimination against the constant-intensity sources occurred. Therefore, in this experiment we tested for the intensity difference among calls at which out-of-phase overlap fails to impact negatively on female phonotaxis. The stimulus intensities from three speakers were adjusted to 83 dB, and, in separate tests, the attenuated stimulus speaker was adjusted to broadcast at either 80 (test A) or 77 dB SPL (test B).

***Experiment* 3.** A final test was conducted in an attempt to explain a discrepancy between our neurophysiological and behavioral estimates of auditory system directionality. We modified the design of test F by placing the speakers separated by 120" together and attenuated stimulus broadcasts from one of them by 9 dB. The specific rationale for this experiment is given in the Discussion. Most (16) of the 19 females we used were tested 1 to 2 months after capture. Phonotactic receptivity was induced in these animals with injections of progesterone and prostaglandin (protocol modified from Schmidt, 1985).

Neurophysiology

Adult female gray tree frogs (SVL, 42 to 66 mm; weight, 5.6 to 18.5g; n = 9), captured in Ashland, Missouri, and housed in plastic tanks until use later within the year, were anesthetized in a buffered (sodium bicarbonate, pH 7.0) 0.2% solution of 3-aminobenzoic acid ethyl ester (MS-222; pH 7.2) for surgery. The midbrain was exposed using a dorsal approach through an aperture cut in the frontoparietal bone and a small tear in the dura, arachnoid, and pia. A small drop of mineral oil was placed on the exposed brain tissue and lidocaine applied to the region around the wound.

Following a recovery period of at least 30 minutes, animals were immobilized with an intramuscular injection of 0.005 mg/g of tubocurarine chloride. The frogs were placed upright on a vibration isolation table (Kinetic Systems) in an air conditioned (18" to 20°C), semianechoic chamber (Industrial Acoustics) lined with 10 cm thick acoustic foam (UNX4, NRC rating, 1.0; Illbruck, Inc.). To facilitate input of sound to the auditory system in a normal fashion, the lungs of the subjects were inflated by blowing through a small straw inserted part way down the throat (Jerrgensen, 1991; Jerrgensen *et al.,* 1991). Throughout each experiment, the animals were kept moist with a wet piece of paper towel draped over the back to aid cutaneous respiration. These procedures were approved by the Animal Care and Use Committee of the University of Missouri.

Multiunit activity was recorded with tungsten microelectrodes (0.5 to 1.0 MR; Micro Probe, Inc.) lowered into the torus semicircularis using a Burleigh 6000 microdrive. We used brain surface landmarks and the range of microdrive depths that previous neurophysiological and histological studies of this species established as guides for placing a microelectrode in the torus semicircularis (Diekamp, 1990). Neural responses were first amplified (WPI DAM-5A Differential Preamplifier, high-pass filtered, 150 Hz; Krohn-Hite 3202) and then amplified again (HP 461A). Neural responses were digitized (10 kHz) using an accelerated Amiga 500 computer equipped with a GVP A530 Turbo (40 MHz 68030) and an Applied Visions FutureSound sound digitizer (8 bits/sample).

Acoustic stimuli (10 pulses, duration of 500 ms) were synthesized as already described, amplified (Realistic SA-10 stereo amplifier) and broadcast from two speakers (Realistic Minimus-2.5) mounted on mobile (0.78 m tall) pedestals 0.75 m from the subject (Fig. 2) The playback system was flat within 2 dB over the frequency range of interest (1100 to 2200 Hz). Prior to each recording session, sound levels were calibrated using the Gen Rad 1982 precision sound level meter.

Six of nine females were each presented with a broadcast of a synthetic conspecific advertisement call at 12 intensities of 50 to 83 dB SPL (in 3 dB steps from lowest to highest intensity). The stimulus was alternated (every 2 s) between the speakers (total spatial separation 120° corresponding to ± 60" azimuth) located contralateral and ipsilateral to the recording electrode in the torus. This procedure was repeated twice for each frog, giving two repetitions of the contralateral and ipsilateral broadcasts at each intensity, and provided a neural response versus intensity function for ipsilateral and contralateral broadcasts. The sampled neural responses were rectified and summed during the stimulus intercall intervals using custom software. Responses for each female were subsequently normalized relative to each female's maximum response so that the possible range of values for neural response was between zero and 1.0 (Fig. 3). A function relating normalized neural response to stimulus intensity was obtained using linear regression on arcsine transformed responses from this data set. The regression equation was subsequently used to assign intensity (dB SPL) equivalents to differences in normalized responses to additional ipsilateral and contralateral stimulus broadcasts (Fig. **4).**

These six, plus an additional three females, were presented with 20 broadcasts of alternating advertisement calls (10 ipsilateral, 10 contralateral) at 63, 73, and 83 dB SPL. We also presented calls (83 dB SPL) from the 10 ipsilateral and 10 contralateral speakers, which were overlapped and shifted by 180° (that is, 50% of the pulse period; Fig 2). Broadcasts were made using speaker separations of 120° and 45". Thus, the relative stimulus timing and speaker orientations mimicked those used during tests of female behavior. Digitized neural re-

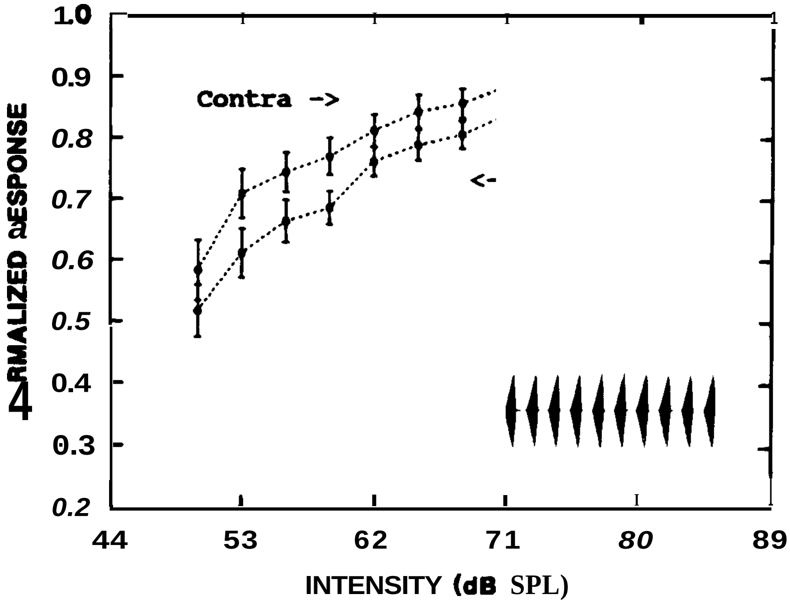

FIGURE 3 **Normalized multiunit neural responses (relative to the maximum per frog) to advertisement calls presented at 12 intensities (n = 9 frogs). The data are presented as means ± SE and a separate curve is shown for contralateral and ipsilateral broadcasts. Oscillogram of a single stimulus call is shown in the lower right corner.**

sponses were stored to disk and subsequently full-wave rectified, summed, and normalized relative to each female's strongest response during presentation of the stimulus (either alternating or overlapping). For the data obtained during presentation of overlapped calls, neural responses to the ipsilateral and contralateral broadcasts were separated by summing rectified activity in successive time windows equal to the pulse duration of the advertisement call. The summed response from each of these windows was alternately totaled to give the entire neural response to the ipsilateral stimulus and the contralateral stimulus. Because of the variability in the response latencies among single units in the torus semicircularis, perfect separation of multiunit responses to call pulses from overlapping contralateral and ipsilateral stimuli was not possible (Fig. 5). Therefore, measurements of directionality obtained from this part of the experiment should be regarded as relatively crude estimates.

Data files were statistically analyzed using SAS (SAS Institute, Cary, NC).

RESULTS

Behavior

Experiment 1. Control tests C and D demonstrated that interference of call sources with a separation of either *5"* or 120" results in a significant degradation for females of the pulse pattern in the overlapped calls (Table **1;** chi-square test). However, the results of tests **A** and B indicate that increasing the angular separation of such call sources mitigated the degradation problem. In test **A,** females discriminated in favor of sources of interfering calls separated by 120" relative to those separated by *5".* However, when the separation was reduced to 45" in test B, females failed to discriminate (Table 1).

Control test G indicated that there was no inherent preference by females for widely spaced relative to narrowly spaced sound sources, in the absence of degradative call interference. Rather, there was discrimination in favor of more closely spaced speakers.

Evidently, only a small decrement **(3** dB) in the relative sound intensity of one of the interfering call sources separated by *5"* was sufficient to counteract the effect of the wider angular separation on pulse rate extraction by females. In tests **E** and F, females no longer preferred sources separated by 120".

Experiment 2. When both pairs of interfering call sources were separated by 180°, it was necessary to attenuate one call source by ***6*** dB SPL to elicit discrimination by females (Table 2). Discrimination was not demonstrated when the difference in intensity of interfering calls was only ***3*** dB SPL.

Neurophysiology

During alternating broadcasts with a separation of 120" neural responses to the contralateral stimuli were stronger than those to the ipsilateral stimuli (Figs. 3, 4),

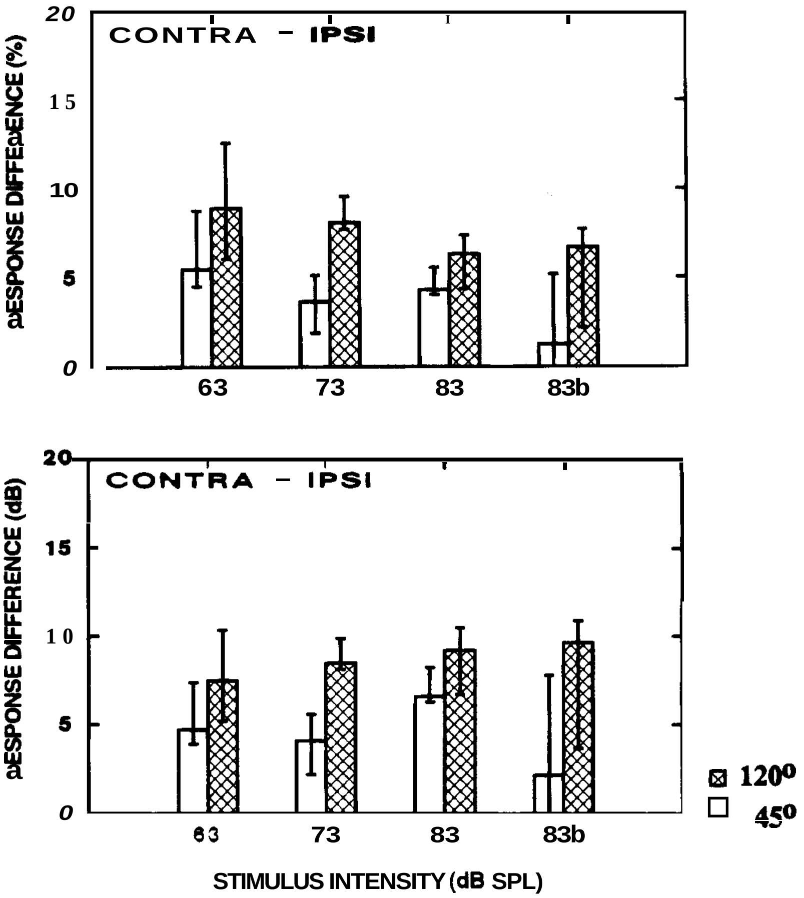

FIGURE *4* Top: The difference (percent) in normalized multiunit neural responses to advertisement calls broadcast from speakers located contralateral and ipsilateral to the recording site in the midbrain. Data are given for stimulus intensities of 63, 73, and 83 dB SPL and speaker separations of 45" and 120°. Histogram bins above 83b give responses during overlapping rather than alternating broadcasts from the speakers. Data are presented as medians and interquartile ranges (n = 9 frogs). Bottom: Same as above but with the differences in normalized responses converted to their decibel equivalents using the data presented in Figure 3 (see text).

and significantly so for all nine frogs at **63** dB and **83** dB, and for eight frogs at **73** dB ($p < 0.05$, Wilcoxon signed rank test). With a separation of 45" differences were significant for eight frogs at **63** dB, seven frogs at **73** dB, and eight frogs at **83** dB. The magnitude of the differences between neural responses to the contralateral and ipsilateral stimuli was greater with the speakers separated by 120" than by 45° for all intensities ($p < 0.05$, Wilcoxon signed rank test on average differences per frog). When calls from the ipsilateral and contralateral speakers were broadcast in overlapping fashion at **83** dB, degradation of the pulse structure of the stimulus calls was evident in the neural response (Fig. 5). However, the summed neural responses to each overlapping call were not significantly different to those obtained with alternating stimuli at a speaker separation of 120" (**83** dB). At a separation of 45°, the contralateral-ipsilateral response difference was smaller when the stimulus calls overlapped than when the stimulus calls alternated (p = **0.027,** Wilcoxon signed rank test on average differences per frog).

DISCUSSION

Male frogs in choruses often adjust the timing of their vocalizations in ways that reduce acoustic interference (Narins and Zelick, 1988; Klump and Gerhardt, 1992). For example, neighboring males of *H. microcephala* alternate notes when multinote calls overlap during pairwise natural interactions (Schwartz, 1993) and males of *H. versicolor* initiated more than 85% of advertisement calls during the interstimulus interval in a playback test (stimulus duration, 900 ms, stimulus period, 4 s) (Klump, unpublished data). However, when many males are calling, it becomes impossible for an adver-

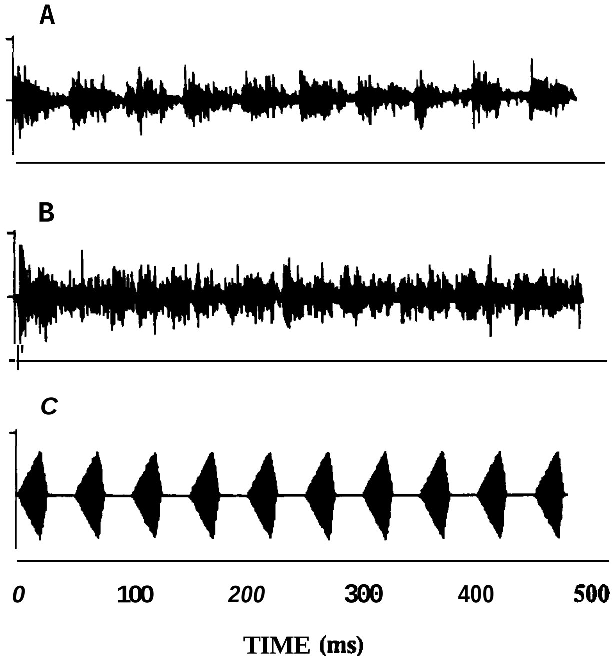

FIGURE 5 **Oscillogram of the multiunit neural response to the broadcast of a single advertisement call contralateral to the recording site in the torus semicircularis (A) and (B) two overlapping calls (speaker separation was 120" with the contralateral broadcast first). An** oscillogram of a single stimulus call is shown in C.

tising individual to avoid interference entirely. One solution to this problem is for a male to adjust call or note timing with respect to his closest neighbors. In this way overlap can be reduced with respect to those individuals whose loud calls are most likely to jeopardize seriously a male's chances of attracting a female (Brush and Narins, 1989; Schwartz, 1993). Female anurans also have traits that enhance their ability to acquire a mate in noisy environments. For example, a female frog's behavior and features of her auditory system (Capranica and Moffat, 1983; Narins and Zelick, 1988) may be well adapted for efficient extraction of biologically relevant signals from the background din of choruses. One reason efficiency may be important is because the time available to a female to choose a mate is limited (Sullivan, 1994). In particular, there may be temporal constraints on the physiological processes associated with oviposition and also a risk of predation (Jaeger, 1976; Ryan, 1985; Magnhagen, 1991; Heller, 1992; Schwartz and Tanner, in preparation), which increases with her time in the pond.

This study addressed the question of whether directional cues provided by a female frog's auditory system might assist her in the critical task just described. During tests of phonotaxis, directional cues were effective in *H. versicolor,* provided the angular separation between interfering call sources was sufficiently large (120"). Although the directionality of the auditory system may increase the signal-to-noise ratio at smaller separations, such an effect was not evident in the behavior of females in experiments using a separation of 45". Smaller directional contributions may augment small differences in sound intensities of the calls of interfering males at the female's position (of course, the extent to which directional contributions help in nature is unknown).

We used call timing relationships that remained static during female phonotaxis, although the relative call timing of males in a chorus would likely change as a female listened to potential mates. Data on the frequency of call overlap in multimale groups similar to those available for H. *microcephala* (Schwartz, 1993) would be valuable in estimating how often females are in situations similar to those we presented in the laboratory. The experimental data of Klump (unpublished) indicated that males overlapped portions of their calls with at least 30% of stimulus calls, suggesting that some overlap also occurs during natural pair-wise interactions. Further study is necessary also to learn why our results from female phonotaxis tests differ from those found using H. *microcephala* (Schwartz, 1993). Cues used by animals for the localization of a sound source are affected by interaural distance, and the task may pose a difficult problem for the auditory and nervous systems of species of very small size and so necessitate the use of mechanisms quite different from those used by larger animals (such as a pressure-gradient system or derivative thereof) (Michelsen, 1982). Although phonotaxis experiments have demonstrated that small frogs are as good as large frogs at sound localization (Rheinlaender and Klump, 1988), how size might influence a female's ability to exploit spatial separation of overlapping call sources for pattern recognition is unknown. The hypothesis that the size difference between *H. versicolor* and *H. microcephala* explains the different results obtained with these species could be evaluated by testing additional species that use ***Ah4*** calls (such as *Pseudacris triseriata* and *Hyla arenicolor*).

We attempted to estimate the strength of the contribution of directionality (at a 120" separation) by attenuating one of the adjacent speakers during the phonotaxis tests, E and F. Our results suggest that the contribution, as reflected in behavior, is quite weak and can be counteracted by a drop of only 3 dB SPL. This value is close to the estimate of 3 dB or less obtained by Schwartz and Gerhardt (1989) in phonotaxis experiments with H. *cinerea* when call sources were separated from noise sources by 90°. *H. cinerea* is similar in size *to H. versicolor.* The interaural vibration amplitude differences obtained using laser vibrometry and a frequency sweep as a stimulus were between 3 and 4 dB at an angle of sound incidence of ± 60" for H. *versicolor* at frequencies near the two spectral peaks of its advertisement call (see Fig. 3 in both Jorgensen, 1991, and Jorgensen and Gerhardt, 1991).

Our estimate of the difference between the neural response measured contralaterally and ipsilaterally averaged 9.6 dB SPL at 120" and 2.1 dB SPL at 45" during interfering broadcasts (83 dB SPL). During alternating broadcasts at 83, 73, and 63 dB SPL, averages at 120" were 9.2, 8.5, and 7.5 dB SPL, respectively. At 45" they were 6.6, 4.1, and 4.7 dB SPL, respectively. Thus, the effect of separation of sound sources measured neurophysiologically was of greater magnitude than our estimate obtained behaviorally (such as 3 dB or less at an intensity of 83 dB SPL and angular separation of 120"). It is important to remember, however, that despite the similarity of the stimulus presentations, experimental conditions were different, and we addressed different questions during the neurophysiology and behavior tests. For example, unlike the recording sessions, in the tests of behavior the females were free to move. Thus, although the speaker positions remained fixed, the orientation of the females relative to the sound sources changed as they engaged in head scanning and other movements during phonotaxis. Moreover, we asked the females to make a choice using all the sensory and neural "equipment" at their disposal. In the neurophysiology work, we asked what is the relative strength of contralateral and ipsilateral auditory input, using neural responses in hemispheres of the midbrain as an estimate. Aware of these differences, we offer some additional, not mutually exclusive, explanations for the discrepancy between the neural and behavioral estimates of the contribution of call source separation.

One possible explanation is that as the relative intensity of interfering calls was changed, the degradation of call temporal structure, from the female's perspective, was nonlinear (for example, steplike). Thus, the conspecific pulse rate from the closer (5") speakers was sufficiently discernable to elicit phonotaxis from females in test F when one of these adjacent speakers had been attenuated by 3 dB. To test whether this was the case, we conducted experiment 3 in which we repeated test F but positioned the speakers formerly separated by 120° adjacent to one another and attenuated calls from one of them by 9 dB (similar in magnitude to our neurophysiological measure of the directional contribution at 120°). Of the 19 females tested, those responding discriminated nearly unanimously (14:1) against the pair of speakers with one source at –3 dB (Binomial test, $p = 0.0018$). Therefore, this first possibility was rejected.

A second possibility is that the choices of females in test F were influenced by an inherent preference for callers close to one another relative to those widely separated. Such a preference in H. *versicolor* is supported by the results of test G, although the results of test G could also reflect a preference for males with high call rate if females perceived the alternating calls broadcast from the adjacent speakers as those of a single male. Perhaps a preference for males close to one another can act partially to counteract the degradative effects of call interference on selective phonotaxis, as long as the calls of one of the interfering callers is already somewhat attenuated at the position of the female. Thus, in test E it was not necessary to attenuate one of the adjacent speakers a full 9 dB before females failed to discriminate. In test B of experiment **2,** however, it was necessary to attenuate one of the speakers broadcasting interfering calls by 6 dB before discrimination occurred. In this test, each speaker pair was separated by 180° and so any preference for adjacent callers would not have influenced the responses of females.

A third possibility is that convergence of contralateral and ipsilateral input in the brain degraded the species-specific pulse rate. Thus, although angular separation improved somewhat the ability of females to extract or detect this critical call feature during phonotaxis, the improvement was not nearly as great as suggested by the results of the multiunit recordings from a single locus in the midbrain. Although the torus semicircularis receives both contralateral and ipsilateral input (Wilczynski and Capranica, 1984), sound presented from the contralateral side elicits the strongest response for the majority of neurons (Eggermont, 1988; Xu et *al.*, 1994). Despite this, signal convergence may occur in select areas within the torus or in higher centers of the central nervous system, which play a critical role in tasks similar to that which faced our test females. Elegant studies of grasshoppers have demonstrated how such convergence can influence pattern recogrution (von Helversen, 1984; von Helversen and von Helversen, 1990).

In our tests, call timing was adjusted so that pulses of the interefering calls interdigitated. As mentioned previously, when playbacks were made using this arrangement, it sounded to a human listener like a doubling of the pulse rate. However, the pulse shape was not affected, as it would be in natural choruses when interfering calls overlap in a range of phase relationships. Because pulse shape is a relevant call property (sensu Gerhardt, 1988) for females of *H. versicolor* (Gerhardt and Doherty, 1988; Diekamp and Gerhardt, in press), future work could explore the consequences of varying the phase during call overlap on female phonotaxis.

Finally, it would be especially interesting to examine the role of auditory system directionality and spatial filtering on selective phonotaxis during interference caused by heterospecifics (Schwartz and Gerhardt, 1989). This may be an especially important problem for frogs calling in multispecies assemblages found in the southern United States and the tropics (Gerhardt and Schwartz, 1995). Recent studies (Gooler et *al.,* 1993; Xu et *al.,* 1994) have demonstrated that there can be profound changes in frequency response properties of auditory neurons in the anuran midbrain concomitant with changes in sound direction. These directionality characteristics might be exploited by a female to enhance her ability to detect and localize a conspecific male when exposed to the calls of heterospecific males differing spectrally from the calls of her own species.

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