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The neuroethology of frequency preferences in the spring peeper

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ABSTRACT

We studied the relationship between auditory activity in the midbrain and selective phonotaxis in females of the treefrog, *Pseudacris crucifer*. Gravid females were tested in two-stimulus playback tests using synthetic advertisement calls of different frequencies (2600 versus 2875 Hz; 2800 versus 3500 Hz; 2600 versus 3500 Hz). Tests were conducted with and without a background of synthesized noise, which was filtered to resemble the spectrum of a chorus of spring peepers. There were no significant preferences for calls of any frequency in the absence of background noise. With background noise, females preferred calls of 3500 Hz to those of 2600 Hz. Multi-unit recordings of neural responses to synthetic sounds were made from the torus semicircularis of the same females following the tests of phonotaxis. We measured auditory threshold at 25 frequencies (1800–4200 Hz) as well as the magnitude of the neural response when stimulus amplitude was held constant and frequency was varied. This procedure yielded isointensity response contours, which we obtained at six amplitudes in the absence of noise and at the stimulus amplitude used during the phonotaxis tests with background noise. Individual differences in audiograms and isointensity responses were poorly correlated with behavioural data except for the test of 2600 Hz versus 3500 Hz calls in noise. The shape of the neural response contours changed with stimulus amplitude and in the presence of the simulated frog chorus. At 85 dB sound pressure level (SPL), the level at which females were tested, the contours of females were quite flat. The contours were more peaked at lower SPLs as well as during the broadcast of chorus noise and white noise at an equivalent spectrum level (45–46 dB/Hz). Peaks in the isointensity response plots of most females occurred at stimulus frequencies ranging from 3200 to 3400 Hz, frequencies close to the median best excitatory frequency (BEF) of 3357 Hz but higher than the mean of the mid-frequency of the male advertisement call (3011 Hz). Addition of background noise may cause a shift in the neural response-intensity level functions. Our results highlight the well-known nonlinearity of the auditory system and the danger inherent in focusing solely on threshold measures of auditory sensitivity when studying the proximate basis of female choice. The results also show an unexpected effect of the natural and noisy acoustic environment on behaviour and responses of the auditory system.

Why female animals prefer males with particular attributes has been of interest to evolutionary biologists since sexual selection was discussed by Darwin (1871). More recently, biologists have asked what features of the sensory systems of females are related to selection of a mate and hence potentially influence the evolution of male traits (Andersson 1994). Anuran amphibians are ideal subjects with which to explore these issues (Ryan 1994; Sullivan et al. 1995). Mate choice in many species is strongly affected by characteristics of the males' vocalizations or vocal behaviour. The vocal repertoires of frogs and toads are small, and each signal can be characterized in terms of some stereotyped spectral and temporal characteristics. The importance of specific call parameters for mate attraction can be assessed using synthetic calls, and behavioural data are available for many species of anurans (Gerhardt 1988, 1994a). The processing of sounds by the peripheral and central elements of the anuran auditory system has been studied extensively (Wilczynski 1992; Hall 1994). These kinds of data have been marshalled to explain patterns of intra- and interspecific discrimination by females as well as male vocal behaviour (Narins 1992; Ryan & Keddy-Hector 1992; Gerhardt 1994a; Ryan 1994; Diekamp & Gerhardt 1995).

There are a number of gaps in our understanding of both proximate- and ultimate-level explanations for female choice in anurans (Kirkpatrick & Ryan 1991; Gerhardt 1994a). At the proximate level, for example, the matched-filter concept (e.g. Capranica & Moffat 1983) used to describe the relationship between the auditory tuning of anurans and the species-specific features of

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their calls has been a useful heuristic. Nevertheless, at some levels of the auditory system the 'match' is at best relatively crude, and mistuning has been reported in some species (Ryan et al. 1992; Wilczynski et al. 1993). Although tuning to species-specific call features may improve signal-to-noise ratios and so assist females in detecting and locating a conspecific male, the degree to which intraspecific mate choice is influenced by the filtering properties of the female auditory system is not fully understood. The main difficulty is that neurophysiological estimates of frequency selectivity in frogs and toads are based on threshold measures, but these animals communicate at intensities well above threshold (Gerhardt et al. 1989), often in the presence of high ambient noise (Narins & Zelick 1988). Moreover, behavioural assessments of frequency selectivity have also been made at sound pressure levels (SPLs) well above threshold. Because the response of the auditory system is nonlinear (Moore 1989), we must be cautious when auditory tuning curves or audiograms (plots of the minimum sound level necessary to elicit a response from a neuron, group of neurons, or an animal as a function of stimulus frequency) are used to explain the behaviour of frogs in a chorus. For example, Capranica (1992) has stated that the focus on tuning curves in studies of sound communication may be unjustified, and the impact of nonlinearities in response to shifts in stimulus intensity and background noise on the psychophysics of hearing and neural coding of speech sounds is an area of active research in mammals (Pickles 1988). Another reason for caution is that we are ignorant about how individual variation in auditory response affects the behaviour of different males and females during communication. We know of no published attempts to correlate the behavioural and neural selectivity of particular individuals in anurans.

Our primary goal was to explore the relationship between auditory neurophysiology and behaviour in females of the spring peeper, *Pseudacris crucifer*. Males of this species advertise, often in dense choruses, during the spring across much of eastern North America. The tone-like advertisement call consists of a frequency-modulated 'peep' averaging about 3 kHz; the slight frequency modulation (about 15%) does not affect selective phonotaxis (Doherty & Gerhardt 1984). The range of variation in carrier frequency (based on power spectra averaging over the entire call) in our study populations was about 2670–3300 Hz ($\bar{X} \pm \mathrm{SD} = 3011 \pm 133$); the range from the initial frequency to the terminal frequency was 2600–3700 Hz (Diekamp & Gerhardt 1992). Females showed significant behavioural discrimination between a 'standard' call of 2875 Hz and those of two other frequencies at SPLs of 75 dB (Doherty & Gerhardt 1984). All females preferred the standard call to one of either 2600 Hz, which is at the bottom of the range of the frequency distribution, or 4000 Hz, which is well above this range. Females did not show significant preferences, however, when presented with the standard call and alternatives of 3300, 3500 or 3700 Hz. This lack of behavioural discrimination may have been due to the absence of a real preference by the females tested or differences in the preferences of individual females. Furthermore, this result does not mean that there was no perceptual discrimination. Because each subject did not receive multiple opportunities to choose, it is not possible to determine which of the first two possibilities is correct. Therefore, another goal of the present study was to check for consistency in the behaviour of females in two-choice tests of phonotaxis using stimuli of different frequency.

We also assessed the effect of noise on female discrimination. Noise levels often are very high in choruses of *P. crucifer* (see below). Nevertheless, during the course of each evening, as well as among nights, considerable variation in background noise can occur as the numbers of vocalizing males change.

Once we had tested for selective phonotaxis, we acquired neurophysiological data on the same females under similar stimulus conditions. We obtained multi-unit recordings from the torus semicircularis, the largest auditory centre in the midbrain of anurans and one that has been used to estimate auditory thresholds in different species of frogs (e.g. Ryan et al. 1990; Diekamp & Gerhardt 1992; Wilczynski et al. 1993). Neural audiograms and tuning curves of auditory nerve fibres of spring peepers have been published previously (Diekamp & Gerhardt 1992; Wilczynski et al. 1984). We acquired not only estimates of auditory threshold at different frequencies, but also a series of measurements of the relative magnitude of the multi-unit activity at the same frequencies. Because these latter measurements were obtained over a range of above-threshold intensities, they may more realistically portray the females' neural responses to the range of acoustic conditions encountered in nature.

METHODS

Noise Measurements

During three evenings in April 1994, measurements of background noise were made at a pond in the Baskett Wildlife area near Ashland, Missouri, U.S.A., with a Realistic sound-level meter (Radio Shack catalogue number 33-2050; C-weighting, fast RMS response). Chorus density was judged to be near average for this time of the breeding season. We made 28 measurements near the shore at heights ranging from a few inches above the water surface to approximately 1.5 m (the height of females perched in bushes). We were at least 3 m from the nearest calling male. We used these data to select a realistic intensity at which to broadcast noise during our behavioural and neurophysiological work (see also Brenowitz et al. 1984). However, the actual noise level any female will experience in nature depends on her particular position in or near the chorus and the number and locations of males calling at that time.

Behaviour

During the spring of 1994 and 1995, gravid adult female spring peepers were captured in amplexus from ponds in the Baskett Wildlife area. They were transported

on the same night to the University of Missouri and refrigerated at approximately 3°C. The following day, they were separated from their mates, placed in an incubator, and warmed to 20°C prior to testing in phonotaxis experiments. Each female was tested individually in an indoor semianechoic chamber (Gerhardt 1994b). The temperature (20°C) and humidity within the chamber were controlled externally, and noise-reducing silencers (Acoustic Systems, Austin, Texas) attenuated noise created by fans. An infrared light source was used to illuminate the testing area and the responses of the subjects monitored (Sanyo VDC-2524 CCTV Camera) on closed circuit television.

Before each trial, we placed a female in a small, centrally located, screen cage situated 1 m from each of two speakers (ADS-200) at opposite sides of the chamber. After exposing the frog to the test stimuli for 30 s, we lifted the cover of 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 min after showing phonotactic orientation movements (e.g. Rheinlander et al. 1979). Test stimuli were periodically switched between speakers to control for side biases. Choice by females was not significantly influenced by the source location of the stimuli (G<0.621 for each test).

Acoustic stimuli consisted of computer-synthesized tone bursts of different frequency (unpublished software by G. M. Klump) of 150-ms duration and 25-ms rise and fall times (sample rate=20 kHz, 8 bits/sample). Calls were output (1 call/s) using a Supersound FX (engineering version; Silicon Shack, San Jose, California) digital-to-analog interface board and an IBM compatible 386 computer. We used two Nagra DSM amplifiers and equalized the SPL at 85 dB (± 1 dB; SPL re 20 µPa) at the females' release point with a calibrated Gen Rad 1982 precision sound-level meter (Fast RMS response, C-weighting). We chose this level based on measurements made from calling males (Gerhardt 1975; see Discussion).

Presentation of the synthetic calls was often preceded (by 5 min) and accompanied by the broadcast of filtered noise (Fig. 1). The noise was broadcast from two centrally facing speakers (Realistic Minimus-2.5), each 1.5 m from the central release point and at right angles to the line bisecting the two speakers that broadcast the call stimuli. The intensity of the noise (RMS SPL=75 dB at the release point) was well below that at which masking of the choice stimuli (at 85 dB SPL) would be expected to occur based on estimates of critical ratios in anurans (e.g. Moss & Megala Simmons 1986; see below). The noise was synthesized using custom software on a Commodore Amiga computer (linear congruential method; see Chamberlin 1985) and digitally band-pass filtered (FIR, 60 coefficients, 20 kHz sample rate, bandwidth=1400 Hz at − 6 dB) to resemble a typical dense chorus of spring peepers. The centre frequency of the noise (3050 Hz or 3150 Hz) was chosen so that the relationships of the frequencies of two pairs of synthetic calls (2600 versus 3500 Hz or 2800 versus 3500 Hz) were symmetric with respect to the noise band. For tests with stimuli of 2600 versus 2875 Hz, we used the 3050-Hz synthesized chorus noise. The (100 kilobyte=5 s) noise stimulus was repeatedly played back from two channels of a Commodore Amiga 1000 computer using FutureSound software and a Realistic SA-10 stereo microamplifier. In the software, we set a slightly different starting point and duration of the stimulus for each channel. Therefore, the noise broadcasts from each speaker were effectively uncorrelated and progressively time-shifted as these stimuli in RAM were 'looped' during testing. A recording of an actual chorus was not used in case females would be more likely to orient towards and approach such a noise source than one lacking the calls of individual males.

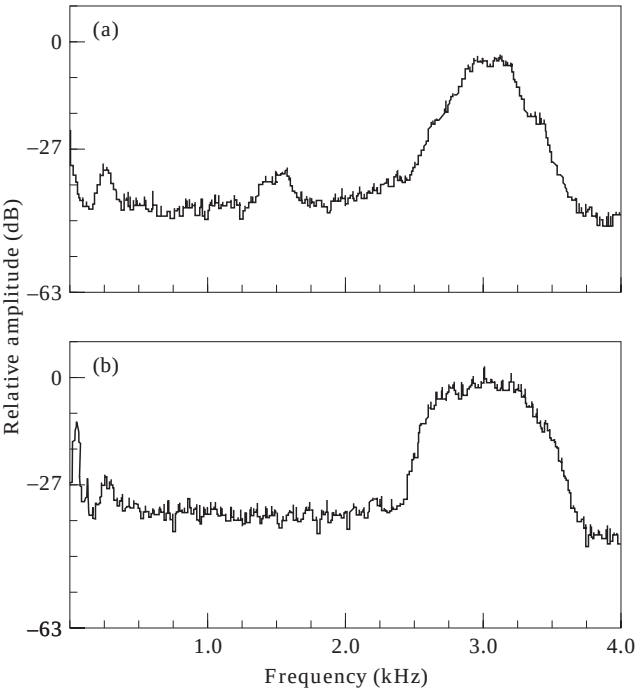

Figure 1. (a) Spectrum of a chorus of spring peepers recorded in Ashland, Missouri and (b) the synthesized chorus noise. The filtered noise was centred at 3050 Hz. Spectra were calculated over approximately 5 s using a 512 point FFT and a frequency resolution of 29.5 Hz.

Ninety-seven of the 117 females we tested responded in the phonotaxis experiments. Subsets of these frogs (Table 1) were presented with a choice between calls of 2800 and 3500 Hz (test 1), 2600 and 2875 Hz (test 2) and 2600 and 3500 Hz (test 3). We chose these frequencies because they fell within the natural frequency range of the calls of males and were identical or close to those used by Doherty & Gerhardt (1984). Behavioural and neurophysiological data also suggested that these frequencies differentially influenced female responses (for example, 3500 Hz elicited the strongest mean neural response with synthesized chorus noise in preliminary data). In each test, many females received an opportunity to choose synthetic calls both with and without broadcasts of the synthesized chorus noise. Although we were not able to obtain multiple phonotactic responses from all 97 animals (Table 1), 58 females were tested successfully more than once

Table 1. Numbers of *P. crucifer* females choosing calls of a particular frequency in two-choice phonotaxis experiments

Intensity (dB)		Frequency (Hz)		
Noise	Stimulus	Number choosing		*P**
Test 1		2800	3500	
0	85	27	28	NS
0	85	47	47 (19)	
75	85	14	17	NS
75	85	19	20 (8)	
Test 2		2600	2875	
0	85	14	12	NS
0	85	22	16 (12)	
75	85	10	8	NS
75	85	12	12 (6)	
Test 3		2600	3500	
0	65	10	18	0.19
0	85	16	20	NS
0	85	27	36 (27)	
75	85	6	17	0.03
75	85	11	30 (18)	

Experiments were performed with and without synthesized chorus noise. For each test condition, the upper row of results gives the first choices of all females tested, and the lower row gives the total of all choices (first choices+results of subsequent tests). The number of females tested more than once is given in parentheses.
*Two-tailed binomial probability for the first choice responses.

(usually twice, but up to five times) with particular pairs of choice stimuli to examine individual's consistency in choice.

Neurophysiology

Following behavioural tests, we refrigerated each female until we used her for neurophysiology, generally within 3 weeks. We obtained recordings from 66 females, 65 of which had been previously tested, but the complete data set from our neurophysiology protocol was not obtained for all of these animals. Subjects were anaesthetized in a buffered (sodium bicarbonate, pH 7.0) 0.2% solution of 3-aminobenzoic acid ethyl ester (MS-222; pH 7.2) for surgery. They were then blotted dry, weighed to the nearest 0.01 g, and measured (snout–vent length) to the nearest 0.5 mm. The midbrain was exposed using a dorsal approach through an aperture in the fronto-parietal cartilage and a small tear in the meninges. A small drop of mineral oil was placed on the exposed brain tissue and a local anaesthetic (lidocaine) applied to the region around the wound. These and additional procedures described below were approved by the Animal Care and Use Committee of the University of Missouri.

Following a recovery period of approximately 3 h, females were immobilized with an intramuscular injection of 0.007 mg/g of Pancuronium Bromide. The frogs were placed upright on a vibration isolation table (Kinetic Systems, Roslindale, Massachusetts) in an air conditioned (18–20°C), semianechoic chamber (Industrial Acoustics, Bronx, New York) lined with 10 cm thick acoustic foam (Illbruck, Inc., Minneapolis, Minnesota). Throughout each experiment, the animals were kept moist to aid cutaneous respiration by draping a wet piece of paper towel over their backs. Multi-unit activity was recorded with tungsten microelectrodes (0.5–1.0 MΩ; Micro Probe, Inc., Clarksburg, Maryland) lowered into the torus semicircularis using a Burleigh 6000 microdrive. Surface landmarks and depth had been established that reliably placed the electrode within this area of the brain using stained histological sections (Diekamp & Gerhardt 1992). 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 (sampling rate=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 (150-ms tones, 5-ms rise and fall times), synthesized on the Amiga computer (sampling rate=20 kHz), were low-pass filtered (9 kHz), amplified (Quad 303) and broadcast (every 2 s) from a speaker (Realistic Minimus-2.5) mounted on a pedestal (0.78 m tall) 0.75 m from the preparation. Stimulus intensity was software-controllable within a 36-dB range using the sound hardware of the Amiga, and a Leader (Model LAT-45) step attenuator was used for supplemental adjustments (1-dB increments). We also broadcast uncorrelated noise during recordings from many females (see protocol below) using a second Amiga 500 computer, a Realistic SA-10 microamplifier and two Realistic Minimus-2.5 speakers. Given the constraints imposed by the dimensions and location of our equipment within the semianechoic chamber, we placed these speakers to mimic as closely as possible their spatial arrangement in the female preference tests. The speakers were mounted on pedestals 60° to the right and left of the central speaker and 0.75 m from the preparation. Prior to each recording

session, sound levels at the location of the preparation were calibrated (1800–4200 Hz, 100-Hz steps) to the nearest 1 dB SPL using the Gen Rad 1982 precision sound-level meter. A calibration file was stored in computer memory and accessed by software during manual and computer-controlled adjustments of stimulus playback intensity.

We obtained measurements of multi-unit auditory thresholds (N=66 females) at 25 frequencies (1800–4200 Hz, 100-Hz steps). In 1994 we used two approaches with many of the females. In one approach, we used broadcasts of synthetic conspecific advertisement calls and an automated software routine. In a second approach, we manually determined thresholds using either computer-synthesized calls or a Clarke-Hess Model 748 function generator to present gated (150-ms) tone bursts and adjusted the Leader attenuator until neural responses were just audible relative to the background neural activity. These two methods yielded similar results, so we averaged thresholds to provide estimates for each female. In these and all other calculations, we converted threshold values in decibels to μbars before averaging and then converted them back to decibels. Because the manual determination of threshold was faster than using the computer software, in 1995 we performed only the former. Best excitatory frequency (BEF; equivalent to the characteristic frequency or CF) was usually estimated to within 50 Hz and threshold at BEF determined manually as described above.

We obtained isointensity neural responses over the 1800–4200 Hz range at stimulus intensities of 85, 75, 65, 55, 45 and 35 dB SPL (N=65 females). Nonlinearities in our playback system were compensated for in the software using the sound-level calibration files so that levels were flat ($\pm$1 dB) over the stimulus frequency range. For all frogs, we repeated the stimulus presentations twice. For subsets of females, we made nine additional presentations at call frequencies of 2600 (N=28), 2800 (N=7), 2900 (N=37), and 3500 Hz (N=39) to improve the reliability of our estimates of response strength at those call frequencies also used in the female choice tests. Neural responses were digitized in a 150-ms prestimulus period and during stimulus broadcast. These raw data were full-wave rectified and integrated during interstimulus periods and the results were stored on disk. We first calculated response strength as the difference between the integrated activity in the prestimulus and stimulus periods. We then normalized the responses for each female relative to the response of greatest magnitude elicited by the stimuli presented to the female. We averaged replicate measures of isointensity responses for each female at each frequency to provide estimates of response strength for each frog.

Following these procedures, we broadcast the filtered noise (3050 or 3150 Hz, 75 dB SPL at the location of the preparation) for 5 min (N=47 females). After 5 min, and while the noise broadcast continued, we presented the calls at 85 dB SPL. Three replicates were completed at each of the 25 frequencies plus an additional nine at 2600, 2800, 2900 and 3500 Hz (N=28, 6, 27, 28 females, respectively). Before making additional presentations of stimuli, we waited until the threshold at BEF had returned to its prenoise value (usually 10–15 min).

For some females, we also presented synthetic calls (85 dB SPL) during broadcasts of different kinds of background noise including a digitally looped segment of (1) a recording of a natural spring peeper chorus (75 dB SPL, N=10 females), and (2) white noise (75 dB SPL, N=5 females; 88 dB SPL, N=19 females). We used the natural spring peeper noise to obtain neural responses that we could compare with those acquired during broadcast of our synthetic spring peeper chorus and to verify whether they had similar effects on the females. White noise (Gen Rad 1390-B Random Noise Generator, 20 kHz bandwidth setting; Realistic SA-10 Stereo Amplifier, Minimus 2.5 speakers; flat $\pm$3 dB from 1 to 5 kHz) was used to test whether any changes in response associated with noise were dependent on the peculiar spectrum of the simulated chorus or could be reproduced by presenting white noise at the same intensity as the chorus (75 dB SPL) or at its estimated spectrum level of 45–46 dB/Hz. The latter level was obtained at an intensity of 88 dB SPL with our meter set for C-weighting and 80 dB SPL when the meter was set for flat weighting to measure in the 4-kHz octave band (frequency range: 2828–5656 Hz; spectrum level=45.5 dB/Hz). The masked threshold of a tone (in dB) relative to the spectrum level of broadband noise can be used to calculate an estimate of frequency selectivity at the frequency of the tone known as the critical-ratio (CR) bandwidth (Ehret & Gerhardt 1980; Moore 1989). Threshold measurements using advertisement calls at BEF during white noise broadcast (88 dB SPL) allowed us to estimate CR bands for 19 females.

We performed an additional set of broadcasts with five females to test for the possibility of an overall shift in the neural response as a function of stimulus intensity level during the broadcast of noise. Noise-induced shifts in rate-level functions occur in mammalian auditory nerve fibres (Costalupes 1985) and in auditory fibres of the eighth nerve in the frog *Eleutherodactylus coqui* (Narins 1987). We broadcast five repetitions each of the 2600-Hz call and 3500-Hz call at six intensities of 35–85 dB SPL (in 10-dB ascending increments), with and without a background of synthesized chorus noise. We used these data to construct plots of neural response versus stimulus intensity.

Following neurophysiology, females were given a recovery period of at least one week and were subsequently released at the site of capture.

RESULTS

Noise Measurements

Noise levels in the pond ranged from 75 to 86 dB SPL (median=82). To be conservative, we chose the low value of 75 dB SPL as the intensity to broadcast noise in our experiments.

Behaviour

Results (first choices) for the entire population of tested females did not reveal significant preferences for calls of

any frequency in the absence of background noise (Table 1). Differences in the proportions of females choosing particular frequencies did not change when the synthesized chorus noise was broadcast (chi-square test) in either test 1 or test 2. However, in test 3, there was a significant preference for the 3500-Hz call over the 2600-Hz call (P=0.034, two-tailed binomial test) during broadcast of the synthesized chorus noise.

Many of the females tested more than once with the same pairs of stimuli were inconsistent in their behaviour during successive presentations of the choice stimuli (test 1, without noise: 13 of 19 females switched their choice; test 1, with noise: five of eight switched; test 2, without noise: four of six switched; test 2, with noise: two of six switched; test 3, without noise, 13 of 27 switched). In test 3 with synthesized chorus noise, only three of 18 females tested twice were inconsistent. When these three females were removed from the analysis of first responses, there still was a significant preference for the 3500-Hz call in noise (15 of 20 females; P=0.042, two-tailed binomial test).

Neurophysiology

Females had a mean BEF of 3369 Hz (median=3357, range 3000–3570 Hz, N=60) and mean threshold at BEF of 45 dB (median=44 dB, range 37–60 dB). BEF was not significantly correlated with females' snout–vent length (Spearman's $r=-0.14$, P=0.27; mean snout–vent length=30.4 mm, range 26.5–34.5, N=60).

Plots of isointensity responses, with and without chorus noise (3050 and 3150 Hz), and an audiogram for all females are shown in Fig. 2. Average response plots were relatively flat at higher stimulus SPLs, but were more peaked at lower SPLs and during the broadcast of background noise. The frequency of maximum response of individual females ranged from 1800 to 4100 Hz (45 dB: $\bar{X}$=3066 Hz, median=3200 Hz; 55 dB: $\bar{X}$=3278 Hz, median=3400 Hz; 65 dB: $\bar{X}$=3154 Hz; median=3300 Hz; 75 dB: $\bar{X}$=3038 Hz, median=3300 Hz; 85 dB: $\bar{X}$=2766, median=2800 Hz; 85 dB+75 dB synthetic chorus noise: $\bar{X}$=3208, median=3300 Hz). When tested without noise at the stimulus intensity used during the choice tests (85 dB SPL), the multi-unit neural responses to calls of 2800 Hz did not differ significantly from 3500 Hz (median of female's mean normalized responses=0.839 and 0.829, respectively; Wilcoxon signed-ranks test: $Z=-0.22$, N=65, P=0.83). Responses to 2600 and 2900 Hz were also similar to one another (medians=0.794 and 0.809; $Z=-1.12$, P=0.24). Responses to 2600 Hz were slightly weaker than to 3500 Hz (medians=0.794 and 0.829; $Z=-3.25$, P=0.0011).

Broadcast of the synthesized filtered noise and the digitized natural peeper chorus had similar effects on the neural response of females (Wilcoxon two-sample test). Choice of noise centre frequency (3050 and 3150 Hz) did not produce significant differences in neural responses at any call frequency (Wilcoxon two-sample test), so data were pooled for the analyses below. When synthetic chorus noise was broadcast, responses were significantly stronger to 3500 than to 2800 Hz (medians=0.424 and 0.326; Wilcoxon signed-rank test: $Z=-4.69$, N=47, P=0.0001), to 2900 Hz than to 2600 Hz (medians=0.358 and 0.227; $Z=-5.66$, P=0.0001), and to 3500 Hz than to 2600 Hz (medians=0.424 and 0.227; $Z=-5.67$, P=0.0001).

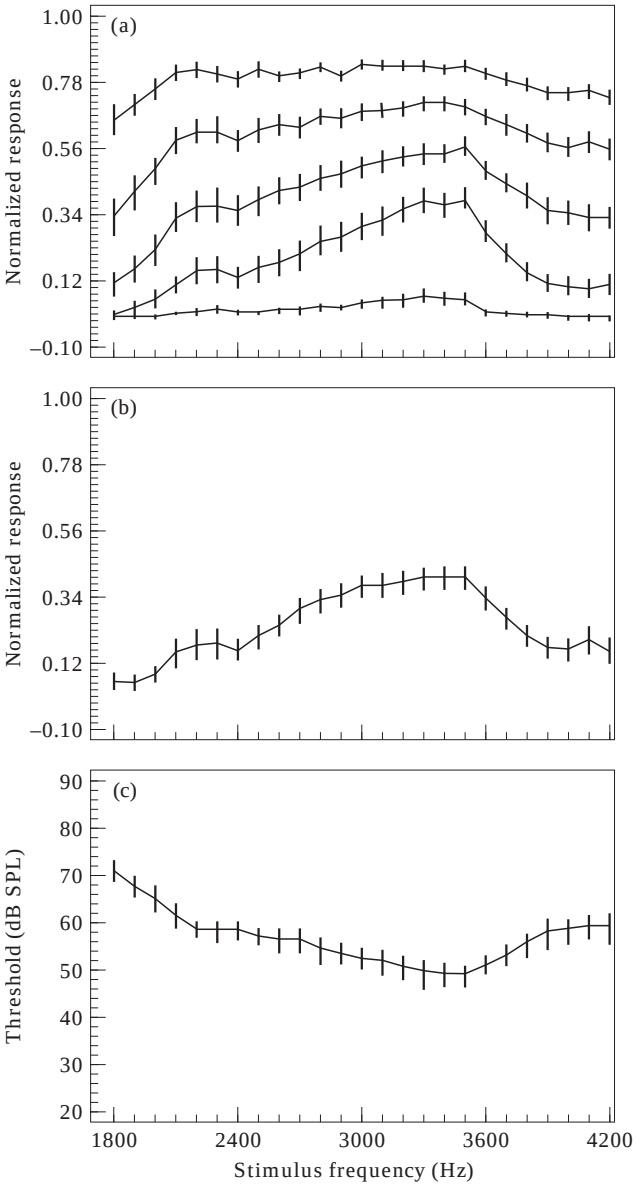

Figure 2. (a) Normalized isointensity response profiles obtained without broadcast of background noise at stimulus intensities (top to bottom) of 85, 75, 65, 55 and 45 dB SPL (N=65 females). (b) Responses with synthesized chorus noise (noise=75 dB SPL; stimulus=85 dB SPL; N=47 females). (c) Audiogram showing auditory thresholds at 25 call frequencies (N=66 females). If only data from the 47 females that were exposed to chorus noise are used, the resulting curves are nearly entirely coincident with those shown in (a) and (b). All data are presented as means (±2 SE).

Neural thresholds were significantly lower at 3500 than at 2800 Hz (median thresholds=47.1 and 52.0 dB, respectively; Wilcoxon signed-rank test: $Z=-6.14$, N=66, P=0.0001), at 3500 than at 2600 Hz (medians=47.1 and 54.1 dB, respectively; $Z=-6.68$, P=0.0001), and at 2900

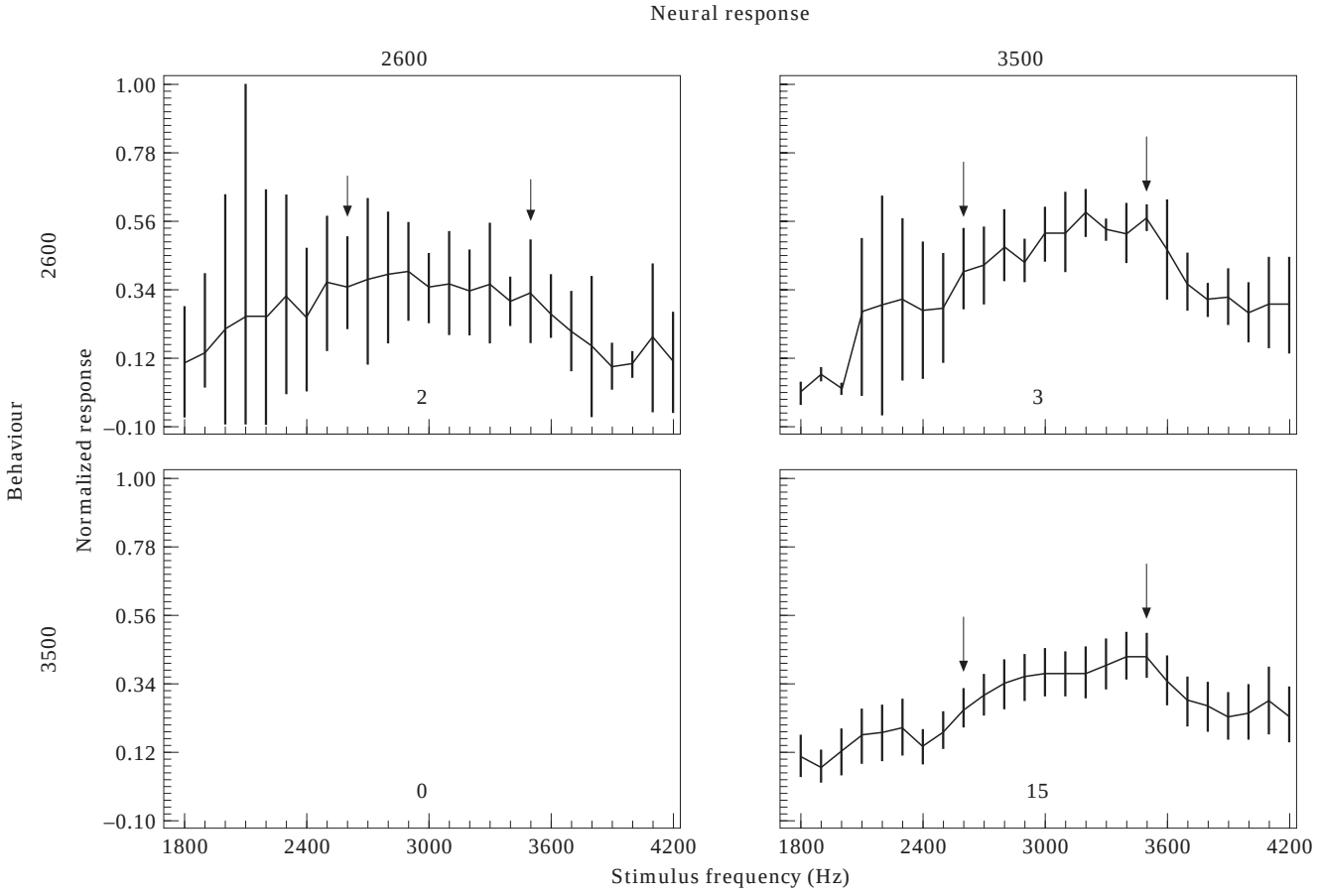

Figure 3. Normalized isointensity response profiles (mean±2 SE) of 20 females that chose either 2600 or 3500 Hz calls and also provided neurophysiological data during the broadcast of chorus noise. The plot in each quadrant is the average response profile for the females that had a stronger neural response at a particular frequency and had a particular preference in the choice test. The number of females contributing to each plot is indicated. Response strengths to 2600 and 3500 Hz are indicated by the arrows to facilitate their comparison. See the legend of Table 2 for additional explanation.

than at 2600 Hz (medians=50.8 and 54.1 dB, respectively; $Z=-5.57$; $P=0.0001$).

Association Between Neural Responses and Behaviour

We also tested whether there was an association between a female's neural response and her choice during phonotaxis. That is, we asked if those females that chose a particular call frequency during a phonotaxis test also had a stronger neural response to that frequency than to the alternative frequency.

Such association was not evident during either the no-noise or noise condition for the choices of 2600 and 2900 (2875) Hz or of 2800 and 3500 Hz. For the call stimuli of 2600 and 3500 Hz, females tended to choose the frequency to which their neural response was strongest, but this nonindependence was only evident when noise was broadcast (Fig. 3, Table 2; $P=0.025$, exact unconditional test of independence; Berger & Boos 1994; Berger 1996). If auditory thresholds at 2600, 2800, 2900 and 3500 Hz were used as the neural measure, the data failed to show a significant association with the results of the choice tests (Table 2) for each of all frequency pairs used in the choice tests.

Response to White Noise

The isointensity response during presentation of white noise at 75 dB SPL was depressed relative to the no-noise condition, and the shape of the isointensity response plot was somewhat flatter than that obtained during presentation of the synthesized chorus noise at the same intensity (Fig. 4b,c). When the noise intensity was raised to 88 dB SPL, the shape of the neural response versus frequency plot was more similar to the plot obtained with chorus noise, although not quite as peaked (±2 standard error lines did not overlap from 2600 to 3500 Hz; Fig. 4e,f).

The mean masked threshold of females during noise broadcast at 88 dB SPL (45.5 dB/Hz) was 69.3 dB, yielding a critical ratio estimate of 23.8 dB (+2 SE=25.5 dB, −2 SE=21.7 dB). This critical ratio estimate corresponds to a critical ratio-band estimate of 240 Hz (bandwidth=$10^{\text{ratio}/10}$; Scharf 1970).

Table 2. Tests of independence of the results of neurophysiology and the female choice tests

	Neural response					
Choice	Quiet		Noise		Neural threshold	
	2800	3500	2800	3500	2800	3500
2800	6	8	1	6	2	12
3500	3	12	1	10	0	16
	P=0.19		P=1.00		P=0.13	
	2600	2900	2600	2900	2600	2900
2600	5	7	0	7	0	12
2900	5	9	1	11	1	13
	P=1.00		P=1.00		P=1.00	
	2600	3500	2600	3500	2600	3500
2600	4	10	2	3	3	11
3500	5	11	0	15	0	15
	P=1.00		P=0.03		P=0.06	

In the individual tables, each quadrant indicates the number of females that had a stronger neural response (or lower threshold; tied values are removed) at a particular frequency and had a particular preference in the choice tests. For example, in the table at the lower left, 10 females chose 2600 Hz over 3500 Hz but had a stronger neural repsonse to the 3500 Hz call. For neural response, choice results are presented for conditions with (noise) and without (quiet) broadcast of the synthesized chorus noise. Threshold data were obtained without chorus noise. *P*-values (exact unconditional test of independence; two-sided hypotheses, multinomial model) are given below each table. Results for each pair of call frequencies were qualitatively similar, if only the subset of females for which all data were available in both quiet and noise were considered.

Shift in Response-intensity Level Functions

A shift in the neural response as a function of stimulus intensity during the broadcast of chorus noise was evident (Fig. 5) at both 2600 Hz (right shift of about 28 dB) and 3500 Hz (right shift of about 28–32 dB). Shifts were approximated by moving the plots along the intensity axis until the curves were superimposed. More precise quantification requires that shifts be measured at the 50% saturation response points on the functions. These points were not available for the response-level functions in noise, because the saturation intensity (i.e. the level at which the response ceases to increase with further increases in intensity) was not reached at a stimulus intensity of 85 dB SPL.

Auditory thresholds at BEF were raised by an average of 24 dB SPL (N=36 frogs) during the broadcasts of synthetic chorus noise. Immediately following the cessation of noise, the elevation in threshold was 9 dB SPL relative to the prenoise value (N=9 frogs).

DISCUSSION

Frequency Coding by the Anuran Auditory System

One theory for the coding of frequency assumes that the peripheral auditory system operates like a bank of band-pass filters (Scharf 1970). Thus, within its linear dynamic range, the rate of firing of an auditory fibre is proportional to the acoustic energy falling within its pass-band or tuning curve. Moreover, at sound intensities above threshold, yet below those that produce a saturation of firing rate, deviations from the neuron's best excitatory frequency will elicit a reduction in firing rate (Pickles 1988; Moore 1989). Differential stimulation of auditory fibres tuned to different frequencies may facilitate discrimination of frequencies or help encode the spectral structure of harmonically complex sounds. In mammals, the basilar membrane is tonotopically organized and so sounds of different frequency may stimulate different spatial patterns of neural activity along its length (Zwicker 1970). Such a mechanism of encoding differences in frequency is called a rate-place code.

The frequencies of our synthetic stimuli fell within the range of maximum sensitivity of primary auditory neurons innervating the basilar papilla, one of the two principal organs of hearing in the inner ear of anurans (Wilczynski et al. 1984). The basilar papilla is a simple resonant structure lacking tonotopic organization, and the tuning of its hair cells is purely mechanical (Zakon & Wilczynski 1988). Therefore, a rate-place mechanism of frequency coding is absent (Simmons & Buxbaum 1996) and, for a given individual, neurons innervating the basilar papilla have similar BEF (although neurons may have a range of thresholds; Wilczynski 1992). Thus intensity-independent discrimination of frequencies stimulating only the basilar papilla is unlikely; the same difference in neural response could result from presentation of tones of two frequencies at the same SPL, or tones of the same frequency at different SPLs. At frequencies above 1 kHz, tuning curves obtained from anuran peripheral fibres are typically broader than in mammals, and critical ratios tend to increase with BEF (e.g. Freedman et al. 1988). Using data from multi-unit recordings, we estimated an average critical ratio bandwidth of 240 Hz, a value consistent with estimates obtained at similar frequencies from another hylid (Ehret & Gerhardt 1980; Moss & Megala Simmons 1986;

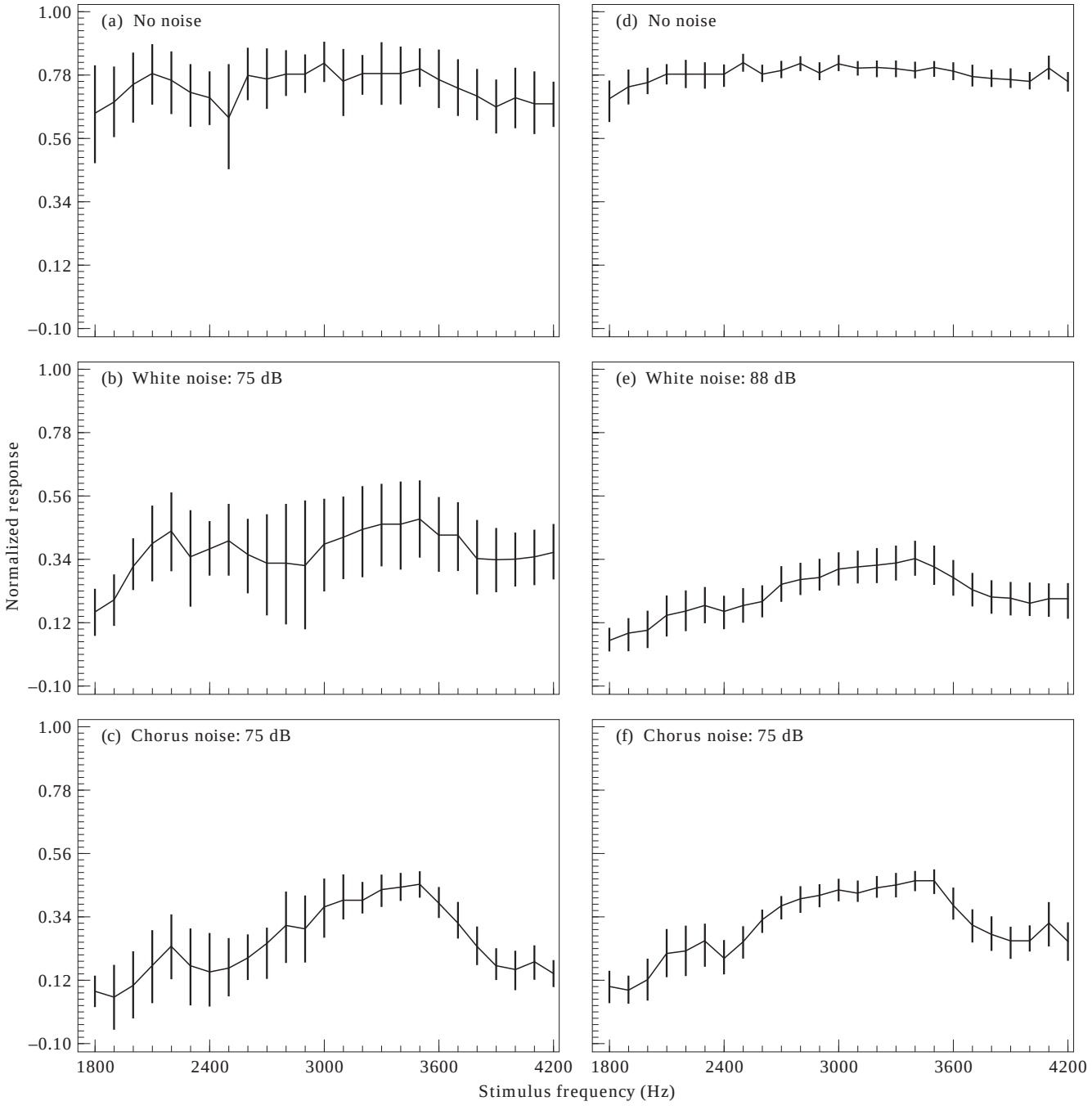

Figure 4. Normalized isointensity response profiles (mean±2 SE) of the same five females during presentation of stimulus calls at 85 dB without noise (a), with white noise at 75 dB (b) and chorus noise at 75 dB (c). Normalized isointensity response profiles of the same 19 females during presentation of stimulus calls at 85 dB without noise (d), with white noise at 88 dB (e) and chorus noise at 75 dB (f).

Gerhardt et al. 1990). These findings suggest that the ability to resolve differences among frequencies stimulating fibres innervating just the basilar papilla is poor (Zakon & Wilczynski 1988). Although the anatomy and physiology that could provide a place mechanism for frequency discrimination has been documented for the amphibian papilla (Zakon & Wilczynski 1988), the other principal inner ear organ of hearing found in anurans, such a mechanism would necessarily be confined to the much lower band of frequencies (100–1200 Hz in spring peepers; Wilczynski et al. 1984) to which this organ is maximally sensitive. Furthermore, because significant phase-locking by auditory nerve fibres is absent for frequencies above 900 Hz in anurans (e.g. Narins & Hillery 1983), frequency discrimination cannot be based on a purely temporal neural code. Accordingly, a female frog such as a spring peeper may prefer a call of one frequency to another because of a difference in the total firing rate of all peripheral auditory neurons stimulated by the two calls (Ryan & Keddy-Hector 1992).

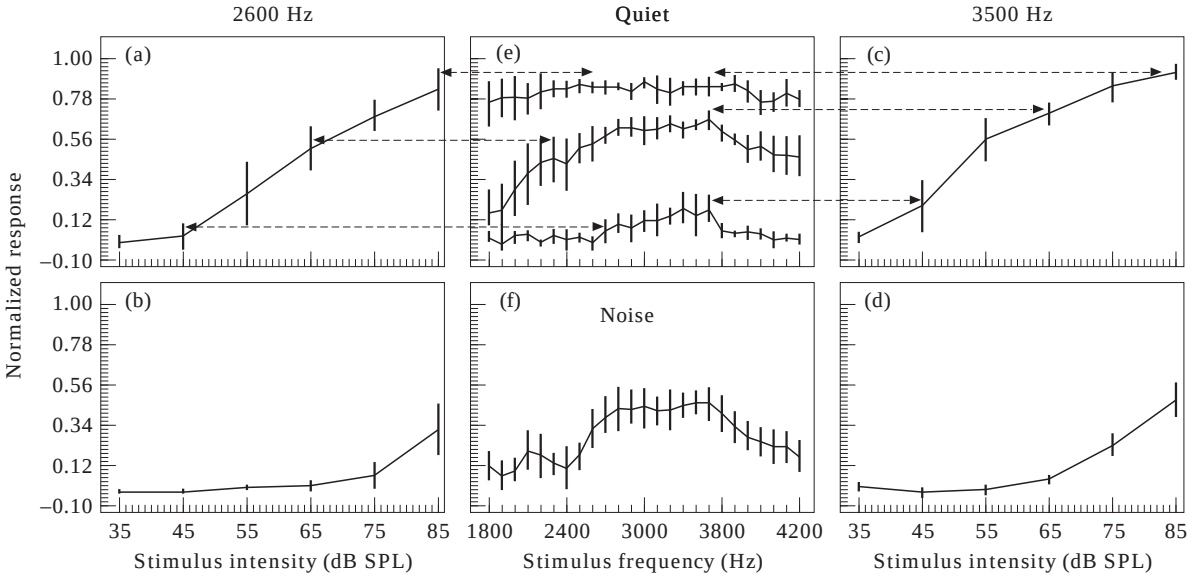

Figure 5. Plots of normalized response as a function of stimulus intensity level for calls of 2600 Hz and 3500 Hz with (b, d) and without (a, c) broadcast of chorus noise. Data show the average (±2 SE) responses for five females. Note the shift to the right of the response-intensity level functions in the noise relative to the quiet condition. Also shown (e, f) are mean isointensity response profiles for the five females obtained earlier over the 1800–4200 Hz frequency range for stimulus intensities: (e) for 85, 65 and 45 dB SPL in 'quiet' and (f) for 85 dB SPL in 'noise'. Note that the responses at the equivalent stimulus conditions match up well on the central and lateral plots (indicated by the arrows).

Information from the auditory periphery ascends the auditory pathway through a series of nuclei in the central nervous system that process this information based on the rate and timing of neural spikes (Wilczynski 1992; Hall 1994). Our measure of neural response strength in the torus semicircularis, therefore, reflects responses of neurons innervating the basilar papilla, a few neurons innervating the amphibian papilla (at the highest stimulus levels and lowest frequencies), and contributions from the intervening stations in the auditory pathway that project to the torus semicircularis. There are three morphologically well defined auditory nuclei within the torus semicircularis (Walkowiak & Luksch 1994), but our electrodes were designed to record activity from a large number of neurons. Thus, our recordings almost certainly reflect the activity of all types of spike-generating neurons in this structure.

Comparison with Previous Studies of Neurophysiology

Wilczynski et al. (1984) recorded the responses of single, primary auditory neurons, using a closed system of acoustic stimulation. They found that neurons in female peepers (N=11) innervating the basilar papilla were most sensitive to stimulus frequencies near the mean call frequency of their population in Ithaca, New York (mean BEF=2939 Hz; mean threshold=76.3 dB SPL; mean dominant frequency=2895 Hz). The multi-unit audiograms we obtained from females indicated a higher frequency of maximum sensitivity (median=3357 Hz) at a lower threshold (median=44 dB SPL) than the values reported in 1992 by Diekamp & Gerhardt (median frequency=2800 Hz; median threshold=63 dB SPL, N=6); the mean dominant frequency in the Missouri populations from which the females were collected was about 3000 Hz.

The explanation for these differences is unknown. Indeed, a major emphasis of this paper is that estimates of BEF and absolute sensitivity vary considerably from individual to individual. Thus, making strong predictions about sexual selection based on a comparison of mean values derived from audiograms and mean dominant frequency in the calls of males is risky, especially when sample sizes are small. Moreover, as emphasized in the next section, our present study shows that even such individual variability does not successfully predict preferences of individuals for frequency differences within the range of variation in the calls of males in the same population.

Behaviour and Relevant Neurobiology

Our results indicate that the conditions in which female *P. crucifer* use frequency differences to choose among conspecific males are likely to be limited. With low background noise and at a playback level of 85 dB, females did not show preferences between alternatives falling within the range of variation in the calls of males in the same population that differed by as much as 900 Hz. Whereas the audiogram based on all individuals might predict, on average, preferences for frequencies slightly above the average in the population over frequencies at the lower end of the range of variation, the isointensity function at 85 dB was relatively flat over the entire range of frequencies produced by conspecific males (Fig. 2).

Our interpretation of the results of other studies of anurans is that threshold measures were also poor

predictors of frequency preferences at the population level. In a study of three populations of the cricket frog, *Acris crepitans*, Ryan et al. (1992) found that the mean BEFs of the female's basilar papilla were lower than the mean dominant frequency of male's calls. When females were offered a choice between calls with their population's mean dominant frequency and calls of either lower or higher dominant frequency, significantly more females responded to the calls of lower frequency in only four of six choice tests. Moreover, in one of these four tests, there was a significant preference where none should have been expected (see data for Bastrop, Texas; Figure 8.2 in Ryan 1994). In the túngara frog, *Physalaemus pustulosus*, the population average tuning of the basilar papilla is close to 2100 Hz and the mean dominant frequency of the chuck note is 2500 Hz (Ryan 1994). When tested with complex calls using tone or chuck frequencies of 2100 and 3000 Hz at 82 dB (peak SPL), females failed to show a significant preference (Wilczynski et al. 1995), although there was a strong bias in favour of the lower frequency (63% of females chose calls with low-frequency tones and 70% chose calls with low-frequency chucks).

Frequency differences might become important when females compare males whose calls they hear at lower intensities, and Doherty & Gerhardt (1984) reported one significant preference within the natural frequency range of male calls at a playback level of 75 dB. Moreover, the neural response profiles were more peaked at lower call intensities. Results from experiments with the green treefrog, *H. cinerea*, also showed the intensity-dependence of frequency preferences. Although finer preferences were observed at low rather than high playback levels within the band of frequencies (0.6–1.2 kHz) to which the amphibian papilla was maximally sensitive, the opposite trend was observed in tests involving frequencies (2.1–4.2 kHz) to which the basilar papilla was maximally sensitive. We have no explanation for this discrepancy; the maximum absolute sensitivity in the low-frequency range was greater in *H. cinerea* than in *P. crucifer*, but the absolute sensitivity in the high-frequency range was similar (Lombard & Straughan 1974; this paper). In humans, frequency discrimination declines at higher sound intensities if the test frequencies are above those at which a periodicity rather than a rate-place code is believed to operate (i.e. above 3 kHz; Dye & Hafter 1980). The rate-based mechanism is more relevant to our results. Dye & Hafter argued that reduced discrimination in humans results from a broadening of the tuning curves of eighth nerve fibres as stimulus intensity increases. In our frogs, such broadening, coupled with the effect of rate saturation at high intensities near BEF, would flatten isointensity response plots.

Females are, however, probably only rarely exposed to choices of calls at the lower call intensities, which might permit better fine-scale, frequency discrimination in *P. crucifer* because this species typically calls within dense choruses. The mean SPL of the calls of a male at 50 cm is about 94 dB (Gerhardt 1975), and thus a playback level of 85 dB corresponds to the level a female would experience at a distance of about 1.4 m from a male, assuming attenuation due only to a spherical spread of sound. We do not know at what distance(s) from males the females make mate-choice decisions, but males are often much closer together than 1.4 m (Gerhardt et al. 1989); thus females in the midst of a chorus would frequently be exposed to calls at a higher intensity than 85 dB SPL. Moreover, when separated from potential mates by distances greater than 2 m, the calls of males would often be lower in SPL than the background noise of the chorus.

Females often changed their preference from one test to the next, thus indicating that there may be little variation in preference among individuals. On the one hand, these data suggest that population-level estimates of frequency preferences are probably not masked by variation in individual preferences, although additional multiple testing is needed to confirm this trend. On the other hand, the inconsistency within individuals compounds the problem of interpreting the lack of correlations between frequency preferences and neurophysiological estimates of frequency sensitivity in the same individuals. Additional studies in which both behavioural and neurophysiological estimates of frequency selectivity are derived from the same individuals are badly needed.

Effect of Chorus Noise on Frequency Discrimination

The problem imposed by limited dynamic range on the rate-coding of frequency is well recognized (Pickles 1988), and solutions such as temporally based coding, range fractionation and dynamic range shifts have been discussed for anurans (Capranica & Moffat 1983; Narins & Zelick 1988; Schwartz & Simmons 1990). Frequency discrimination in noise has been tested in psychophysical studies of humans (Harris 1966; Henning 1966; Dye & Hafter 1980) and cats (Hienz et al. 1993) although, to our knowledge, improved discrimination in the presence of noise has not been reported. In the neotropical treefrog *Hyla ebraccata*, noise can reduce discriminability (Wollerman 1995). In our study, population-level frequency preferences and a significant association between neural response strength and frequency preference at the individual level occurred only in the presence of synthesized chorus noise. Why might this have been so?

We infer from our multi-unit midbrain data that at high sound intensities, such as 85 dB SPL, the firing rate of many basilar papilla neurons might be at or close to saturation. This was reflected in the relatively flat profiles of the isointensity response plots (Fig. 2) and in the drop in slope of the response-intensity level functions (Fig. 5, upper half). Activation of some of the most sensitive amphibian papilla neurons could also have contributed to increases in firing on the low-frequency side of the range of stimulus frequencies we used. At 85 dB without chorus noise, females did not discriminate among call frequencies. At lower stimulus intensities, frequencies lower or higher than females' BEFs elicited a reduced aggregate firing rate than frequencies at BEF. Thus, our isointensity response plots became more peaked between 3200 and 3500 Hz. More peaked response functions were

also observed when chorus noise was used during playback of calls at 85 dB SPL; in this situation, the median, normalized multi-unit response was nearly twice as great at 3500 Hz as at 2600 Hz (Fig. 2). Our data indicate an elevation in noise of auditory thresholds and suggest a concomitant change in the linear operating range of peripheral auditory neurons (see below).

Another factor that may explain the results of the choice tests in noise is differential masking of calls of 3500 and 2600 Hz. These stimuli were presented at the same intensities and their frequencies were symmetric with respect to the centre frequency of the synthesized chorus noise (3050 Hz). Signal-to-noise ratios during stimulus broadcasts might, however, differ after filtering by the female's auditory system. A bell-shaped filter centred near 3500 Hz would, for example, pass more signal power at 3500 Hz than signal power at 2600 Hz.

The density of calling males in frog choruses can vary considerably from night to night, as well as during an evening, and the acoustic environment changes accordingly. Aggressive calling thresholds in males of the Pacific treefrog *H. regilla*, change as a function of the duration and intensity of acoustic stimulation by advertisement calls, so auditory system plasticity affects behaviour in anurans (Rose & Brenowitz 1991; Brenowitz & Rose 1994). One adaptive scenario is that the nervous system of female frogs adjusts to the acoustic environment to facilitate the perception of differences in call frequency among males. Thus, on quiet nights when the few calling males in the chorus are well separated, differences in a female's neural responses to call frequencies are manifest because call intensities are well below those that cause rate saturation in most auditory neurons. On nights when the density of calling males is higher, background noise shifts the linear operating range of auditory neurons, and so the ability of females to perceive differences in the call frequencies of potential mates is maintained. Forester & Czarnowsky (1985) failed to find a relationship between male size and mating success, and we observed a significant preference in noise for call frequencies only at the extremes of the species' distribution. Therefore, it is unlikely that such a dynamic process facilitates female choice for call frequency in *P. crucifer* in nature, although we do not rule out this possibility for other species of frogs at intermediate chorus densities. However, increased acoustic complexity (Gerhardt 1987; Telford et al. 1989) and masking of males' calls in choruses of very high density (Ehret & Gerhardt 1980; Schwartz & Wells 1983; Gerhardt & Klump 1988; Simmons et al. 1992) are two phenomena that may counteract any positive effect of noise by making discrimination by females more difficult.

Although improved discrimination of call frequencies with noise may have a limited impact on frequency-based intraspecific mate choice by female anurans, at moderate intensity levels, noise-induced shifts in the operating range of auditory neurons could help females to discriminate conspecific from heterospecific males in a mixed-species chorus environment. Such a process could be particularly important to female frogs attempting to breed in choruses where many species can call syntopically (Gerhardt & Schwartz 1995). Furthermore, low levels of broad-band noise have been shown to enhance phase-locking of auditory fibres to tones in mammals (Rhode et al. 1978; Lewis & Henry 1995). If the same phenomenon applies to anurans, then chorus noise could improve their resolution of temporal properties of advertisement calls under some circumstances. Low levels of background noise may even improve call detection by male frogs (Narins et al. 1997).

Bases for Changes in Neural Response in Noise

The change in isointensity response during chorus noise may have been largely due to a rightward shift in the response-intensity level functions that was also evident during this noise condition. This shift probably reflected an underlying shift in the rate-intensity level functions of basilar papilla neurons. The good match between the response-intensity level functions and the isointensity response at 2600 and 3500 Hz both with and without chorus noise (Fig. 5) supports this hypothesis. When using broadband noise at a spectrum level close to that used in our study, Narins (1987) observed rate-level shifts of up to 20 dB while recording from fibres of the eighth nerve of *E. coqui*. Unlike those of the Narins' study, our results reflect the combined responses of fibres in the auditory periphery as well as additional effects mediated by more centrally located neurons (see Phillips & Hall 1986 for a discussion of how noise-induced changes in responses of peripheral neurons might shape rate-level functions of cortical neurons of the cat).

A temporary threshold shift is an important element of the change in neural activity that we obtained with chorus noise. Such a shift is observable in the response-intensity level plots, and we measured a shift at BEF both with and immediately after noise broadcast. Adaptation of auditory system neurons during the prolonged noise broadcast, perhaps due to depletion of neurotransmitter at synapses in hair cells (Henry & Mulroy 1995), may account for much of the depression in response strength relative to the no-noise condition at a stimulus intensity of 85 dB SPL. Decrements in firing rate to advertisement calls in eighth nerve fibres of the bullfrog accompany increases in the intensity of broadband background noise (Simmons et al. 1992). Similar observations were reported by Narins (1987) at and below tone intensities eliciting rate saturation for eighth nerve fibres of *E. coqui*.

Does the change in neural responses we observed with chorus noise represent a response to noise in general or was it specific to the noise produced in a chorus of spring peepers? Our data support the former interpretation. The isointensity response curve with a white noise background at 75 dB SPL was elevated but flatter in shape relative to the curve obtained with chorus noise at the same intensity (Fig. 4b,c). The spectrum level of the white noise at 75 dB (32 dB/Hz) was lower than that of the chorus noise at 75 dB, so its reduced effect on the neural response is not surprising. When the intensity of white noise was raised to 88 dB SPL, so that its spectrum level was close to the spectrum level of the chorus noise at 75 dB SPL, the shape of the response curve was similar,

although not identical, to that obtained with chorus noise (Fig. 4e,f). We suggest that this small difference is related to the increased contribution of masking in shaping the auditory response to calls of different frequency using white noise at 88 dB. In our experiments, more energy from a white noise masker would have fallen within a critical band centred at BEF (about 3500 Hz in the audiogram in Fig. 2) than from the filtered chorus noise at an identical spectrum level (also see Figure 3.3 in Patterson & Moore 1986). Thus the partial masking of stimulus calls was more effective with white noise at 88 dB SPL than with chorus noise at 75 dB SPL.

Implications for Studies of the Neuroethology of Anuran Communication and Mate Choice

Our results have implications for other studies of anuran amphibians that attempt to link tuning of the auditory system to mate choice by females. Such an association may be valid when choices confronting a female are relatively large, as is often the case between conspecific and heterospecific males. The association may not be valid for fine-scale discrimination tasks between conspecifics.

Isointensity functions were superior to audiograms in explaining the behaviour of female spring peepers only when differences in neural response strength to alternative stimuli were profound (2600 versus 3500 Hz with background noise). Data from the barking treefrog *Hyla gratiosa* also indicate that isointensity functions may not be particularly reliable as a predictor of behavioural discrimination of call frequency by female frogs (J. Schwartz & K. Murphy, unpublished data). This result is not surprising, given that coding schemes based on neural firing-rate in the mammalian auditory periphery do a poor job of explaining the psychophysical performance of humans in tests of intensity and frequency discrimination at high SPLs (Pickles 1986). Clearly, an understanding of the neural events that mediate call preferences in female anurans requires more research. Measures of neural response acquired by summing multi-unit activity or estimating auditory thresholds from a diffuse region in the midbrain may be insufficient for this purpose, even for species with calls as simple as those as the spring peeper.

Male mating success in frogs may be more closely tied to attributes of the advertisement call other than frequency. Indeed, females nearly always prefer high values of call duration, call rate and time spent advertising in the chorus to low values (Ryan & Keddy-Hector 1992; Gerhardt 1994a; Sullivan et al. 1995). Moreover, an increasing number of field studies have shown positive correlations between the last two properties and male mating success (Gerhardt 1994a). Such temporal aspects of calling might be less vulnerable than spectral properties to the confounding effects of absolute and relative intensity on the putative neural mechanism used by females to evaluate the calls of potential mates (e.g. relative strength or frequency of stimulation of auditory neurons).

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