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A precedence effect underlies preferences for calls with leading pulses in the grey treefrog, *Hyla versicolor*

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Abstract

The temporal relationship between signals often has strong and repeatable influences on receiver behaviour. While several studies have shown that receivers prefer temporally leading signals, we show that the relative timing of signal elements within overlapping signals can also have repeatable influences on receiver responses. Female grey treefrogs, *Hyla versicolor*, preferred overlapping conspecific advertisement call alternatives in which pulses were in the leading position relative to pulses in an alternative. The preference was maintained even when the first pulse of the stimulus with leading pulses began after that of the call with following pulses. To rule out the possibility of masking interference of the pulse pattern, we used a split-pulse design in which the playback of two nonoverlapping pulse elements were synchronized from spatially separated speakers. Females were attracted to the source of the short (6 ms) leading pulse element, which did not attract females in isolation even though its amplitude was 24 dB lower than the long (24 ms) following element, which did attract females in isolation. Taken together, our results fall within a range of phenomena that have been classified as precedence effects. However, to our knowledge, showing localization based on successive leading pulses rather than the very first-arriving pulse is a novel discovery for nonhuman animals.

Keywords

acoustic communication; precedence effect; signal timing

The social environment and receiver psychology play synergistic roles in shaping communication systems. For organisms that communicate acoustically, individuals often face overlapping signals arising from different sources. Most studies of temporal overlap between acoustic signals have focused on the differential attraction of mates by two or more nearby rivals of the same species (Greenfield & Roizen 1993; Richardson et al. 2008). In most cases, receivers have been found to prefer leading signals. Two sensory processes, masking and precedence effects, have often been proposed to underlie such preferences (Grafe 2005; Greenfield 2005). Simultaneous masking occurs when a target signal is rendered less perceptible to a receiver by a temporally overlapping sound source (Moore 2004). Psychophysical and behavioural correlates of masking include increased thresholds for the detection of signals, and several studies of frogs have examined how masking from chorus background noise influences the detection of mating signals in chorusing species (e.g. Gerhardt & Klump 1988; Wollerman 1999). The precedence effect describes a phenomenon by which two sounds arriving from different directions are perceived as a single auditory event, whose

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spatial location is determined mainly by the location of the leading sound (in humans: Zurek 1987; Litovsky et al. 1999). Psychophysical and behavioural methods have also demonstrated the operation of the precedence effect in nonhuman mammals, in birds and in two species of insects (Cranford 1982; Wyttenbach & Hoy 1993; Dent & Dooling 2004; Lee et al. 2009).

While most studies have considered the timing relationships between entire signals (calls, songs, etc.), the signals of many species have additional levels of structure (i.e. calls or songs that are made up of pulses, syllables or notes). The relative timing of such elements within overlapping signals may also have important influences on receiver behaviour. The few studies that have examined timing on such scales, however, have primarily focused on how female recognition is affected by overlap and have employed designs in which females were offered choices between overlapping and nonoverlapping alternatives (Schwartz 1987, 2001; Schwartz & Marshall 2006).

In this study, we examined how the relative timing of pulses within a pair of overlapping calls influenced the phonotactic behaviour of female grey treefrogs, *H. versicolor*. This study was partly motivated by an earlier experiment examining the consequences of heterospecific overlap between two closely related species of treefrogs in which Marshall et al. (2006) found a surprising result in which a significant majority of females of the tetraploid species of grey treefrog (*H. versicolor*) approached a sound source broadcasting calls of a diploid sister taxon (*H. chrysoscelis*) when there was near-total overlap of these calls. Although the playback conditions of overlap in which the errors occurred was highly artificial in that, unlike the calls of two frogs vocally interacting in nature, the alternative stimuli always started at exactly the same time and completely overlapped during all presentations to females, the attraction of *H. versicolor* to heterospecific calls under these conditions provided clues to the perceptual correlates of receiver responses to overlapping signals.

In the few other studies considering acoustic overlap between the signals of two different species, the emphasis has been on masking of the signals of one species by the signals of the other species (Schwartz & Wells 1983; Greenfield 1993; Páez et al. 1993). Masking is an unlikely explanation for the positive responses towards the heterospecific calls in the study of Marshall et al. (2006). First, in the original experiments, females showed normal phonotactic orientation and movement to one of the sources of overlapped calls even though response times were longer than when there was no overlap. Moreover, females of *H. chrysoscelis* reliably approached the source of conspecific calls in similar conditions of overlap. Finally, females of *H. versicolor* did not show reliable phonotaxis towards the calls of *H. chrysoscelis* presented in isolation (see also Bush et al. 2001); thus, their responses to that stimulus in the overlapped condition were not attributable to any inherent attractiveness of the heterospecific calls.

An alternative hypothesis, that these results were caused by some form of precedence effect, was based on the fact that in the overlapping calls, more of the pulses of *H. chrysoscelis* stimuli were in a leading position relative to the pulses in *H. versicolor* stimuli because the *H. chrysoscelis* calls comprised more pulses repeated at a higher rate than the *H. versicolor* stimuli (Marshall et al. 2006). An argument against this hypothesis is that the classical precedence effect postulates that the arrival of the very first sound dictates the perception of the location of the composite signal; indeed, an alternative term for the effect is `the law of the first wavefront' (Blauert 1971). Yet in the experiments of Marshall et al. (2006), the very first pulse of alterative, overlapping signals started at the same time, which suggests that the perceived location of the signal was based on the timing relationship between signal elements following the very first pulse.

In this study, we tested female *H. versicolor* with synthetic calls to confirm that the perceived location of a pair of overlapping pulsed signals was determined by the phase relationships of

pulses within these signals and not by the first-arriving pulse. Using a playback design in which the individual pulses within an *H. versicolor* call were divided into two segments of differing attractiveness to females, we then showed that a pulse element that is ineffective in eliciting phonotaxis in isolation can determine the perceived location of a composite signal when it is placed in a leading position relative to a pulse element that does elicit accurate phonotaxis in isolation. In this paradigm we presented the alternative signals from spatially separated locations in a manner that did not interfere with the fine-scale temporal properties of the composite call, which are important for signal recognition in this species.

MATERIALS AND METHODS

General Methods

During 2004 and 2005, gravid females of *H. versicolor* were collected while in amplexus from breeding ponds in Boone County, Missouri (U.S.A.). These ponds contain exclusively *H. versicolor*, but are within 30 km of populations that are sympatric with *H. chrysoscelis*. Females were placed in individual containers within an ice-filled cooler to delay oviposition and maintain their receptivity to mating signals. Prior to testing, females were allowed to warm up to the testing temperature of 20 °C. All females were returned to the site of collection, usually 2–3 days after collecting took place.

Testing took place within a temperature-controlled room (2.7 ×4.4m) lined with echo-attenuating acoustic foam. A 2m diameter circular arena consisting of an acoustically transparent outer wall (hardware cloth lined with black fabric) was placed in the centre of the room. A pair of Analog-Digital Systems 200 loudspeakers was placed outside of the arena 10 cm from the arena wall with an angular separation of 90° relative to the female release site in the centre of the arena. The inside edge of the arena was marked along its circumference at 10° intervals to allow an observer to record where a female contacted the wall relative to the locations of the speakers broadcasting the alternative stimuli. Female phonotaxis was observed under infrared illumination with a low-light sensitive camera via a television monitor outside of the chamber. Our facilities, testing and animal care and release procedures were approved by the University of Missouri Animal Care and Use Committee (Protocol No. 1910).

Acoustic signals (44 kHz sampling rate and 16-bit resolution; Windows PCM format) were output from a Dell laptop computer (Dell Inc., Austin, TX, U.S.A.) using Adobe Audition v1.5 software (Adobe, San Jose, CA, U.S.A.) via an external digital-to-analogue device (WAMI Box, EGO Systems Cupertino, California, U.S.A.) and custom-built amplifiers. Stimuli consisted of synthetic *H. versicolor* advertisement calls that were created using custom software. These calls consisted of two phase-locked sinusoids (2.2 kHz and 1.1 kHz; 1.1 kHz @ −6 dB re: 2.2 kHz). Pulse durations were 24 ms with 24 ms intervals between pulses (18 pulse total duration), and pulse shape was given species-specific values. Alternative stimuli were copied into separate channels of a stereo sound file, and their relative timing was adjusted according to the experiment. During playback, stimulus calls were presented once every 4 s at 83 dB SPL (sound pressure level in decibels re. 20 µPa, C-weighted; adjusted using the `fast' root-mean-square setting of a Larsen Davis model 720 sound level meter, Larsen Davis, Depew, NY, U.S.A.) at the female release site. The signal amplitude of the *H. versicolor* advertisement calls at the female release site was 3 dB less than that in the previous study (Marshall et al. 2006) because the arena was larger than the previous study (which used a 1.5 m diameter arena), and the speakers were thus at a greater distance from the female release site than in the Marshall et al. (2006) study.

Experiment 1: Female Responses to Pulse Timing within Overlapping Advertisement Calls

In the first experiment, we measured phonotactic orientation of female *H. versicolor* to a pair of overlapping synthetic *H. versicolor* advertisement calls in which the 24 ms pulses of one call led those in an alternative call by a fixed delay (Fig. 1). The calls in the alternative channels were set to delays of 2, 6, 12 and 18 ms between the leading and lagging pulses. We also performed a test with a delay of 6 ms between the pulses in which the call with leading pulses was reduced in amplitude by 6 dB (to 77 dB SPL) relative to the call with the lagging pulses. Finally, females were also tested with the stimulus in which the call with pulses that led by 6 ms began 42 ms (equivalent to one pulse period) after the call with lagging pulses (Fig. 1b). This resulted in the call with lagging pulses leading the call with leading pulses (i.e. it was the first call to arrive at the female's location in each broadcast cycle). Separate groups of females were tested with each stimulus.

Experiment 2: Test of Localization Dominance by Leading Pulse Elements

In experiment 1, the alternative signals were synthetic signals made up of a series of pulses that were modelled after *H. versicolor* advertisement calls and were thus of equal intrinsic attractiveness to females. In experiment 2, the pulses of such a signal were split into two elements such that one part was unattractive to females in isolation and the other part was attractive in isolation. We then tested the hypothesis that a train of unattractive elements, each placed in a leading position relative to an attractive element, would nevertheless influence the localization of a composite signal that resulted when trains of each kind of pulse element were played back synchronously, each from a different location.

Each unattractive element consisted of the initial 6 ms of a pulse of the standard synthetic call of *H. versicolor* used in experiment 1, and each attractive element consisted of the last 18 ms of such a pulse (Fig. 2a). These values were chosen on the basis of the results of single-stimulus experiments with females of *H. versicolor* (Schul & Bush 2002), and we confirmed our expectations with single-speaker tests. Such tests also confirmed the attractiveness of the composite signal that resulted when series of the two different pulse elements were broadcast concurrently from spatially separated sources. Attractiveness was quantified by phonotaxis scores derived from female response times for each test stimulus relative to responses to a standard conspecific advertisement call stimulus (Bush et al. 2001;Schul & Bush 2002).

The choice to use sections of advertisement call pulses was motivated by previous research that demonstrated that female *H. versicolor* are sensitive to the temporal shape of pulses within advertisement calls (Diekamp & Gerhardt 1995; Gerhardt & Schul 1999; Bush et al. 2001). Disruption of the conspecific temporal pattern can reduce the attractiveness of stimuli to females of this species (Schwartz 1987; Schwartz & Marshall 2006), and we therefore used a stimulus that would be attractive when the alternative pulse segments were presented together.

To create the stimuli comprising the 6 ms pulse segments, a 24 ms synthetic *H. versicolor* advertisement call pulse modelled after a typical pulse of an advertisement call of *H. versicolor* was edited by removing the remainder of the pulse after the initial 6 ms. The 18 ms pulse segment was similarly created by editing out the first 6 ms of the pulse. The pulses were cut at the zero-crossing point of the waveform, and the cut edges were shaped with 0.5 ms linear ramps. The respective pulse sections were copied into alternative channels of a stereo sound file for 18 repetitions with a periodicity of 48 ms. The relative timing of the separate channels was adjusted to maintain the normal pulse structure when the two channels were broadcast simultaneously (i.e. the 6 ms portion led the 18 ms portion).

To set the amplitude of the stimuli consisting of pulse segments, we initially used an advertisement call stimulus with unedited pulses to set the amplitude of the speakers to 83 dB

SPL at the female release point. Because the files consisting of the pulse segments had the same energy as the equivalent sections within the standard stimulus, this ensured that relative stimulus energy of the different segments was maintained. We recorded the test stimuli at the female release site using a Marantz PMD-670 solid-state recorder (D&M Professional, Itasca, IL, U.S.A.) and a Sennheiser ME-88 microphone (Sennheiser Electronic Corporation, Old Lyme, CT, U.S.A.). The recordings were analysed using Raven (Cornell University, Ithaca, NY, U.S.A.) in order to verify that the broadcast stimulus timing and amplitudes were consistent with that in the stimulus files. We also measured the amplitudes of the stimuli in the testing chamber using the impulse settings on the sound level meter, and the SPL of the stimuli was 69 dB and 85 dB for the stimuli consisting of the 6 ms and 18 ms pulse segments, respectively.

Females were first presented with the same synthetic call of *H. versicolor* used in experiment 1; this was broadcast from a single speaker (Fig. 2b). Subsequently, females were tested in randomized order with the following: the stimulus comprising the 6 ms pulse segments broadcast from a single speaker; the stimulus comprising the 18 ms pulse segments from a single speaker; and both call segments presented in a synchronized fashion with the 6 ms segment leading the 18 ms pulse segment (Fig. 2c). In synchronized playback, each element was played back from a separate speaker, with a 90° angular separation between speakers. Finally, females were again tested with the standard synthetic call played back from a single speaker. Females were given at least a 5 min break between tests, and the location of the speaker (s) was moved between each test.

Two aspects of female phonotaxis were considered. First, we measured the phonotactic orientation of females by recording where they contacted the arena wall relative to the locations of the speaker(s) broadcasting the stimulus or stimuli. Second, we quantified the strength of the phonotactic response by measuring the speed with which females approached the stimuli. We timed each response, beginning when the female was free to move from the release point and ending when she contacted the wall in front of a speaker (±5° relative to centre of the speaker face) broadcasting a stimulus. Phonotaxis scores were computed as in Bush et al. (2001). That is, we divided the mean time that a female took to respond in the two tests with the standard call stimuli by the time that she took to respond to a particular test stimulus (6 ms pulse segments, 18 ms segments, or both segments together). A phonotaxis score of 1 indicates that a test stimulus and the standard advertisement call are equally attractive; lower scores indicate that alternatives are less attractive than the standard call. A score of 0 was assigned if the female failed to contact the 10° segment of the arena wall in front of a stimulus source in under 5 min. To ensure that such a failure to respond reflected the unattractiveness of a stimulus rather than a lack of phonotactic receptivity, we only included scores in the analysis from females that met the response criterion in both tests with the standard stimuli. In addition, we tested a separate group of females with the pulse segment order reversed (the 18 ms segments in a leading position relative to the 6 ms segments; Fig. 2d). In this latter test, we only measured female orientation relative to the locations of the source of the pulse segments.

RESULTS

Experiment 1: Phonotactic Responses to Pulse Timing within Overlapping Advertisement Calls

When presented with overlapping calls in which one alternative led the other by a fixed delay, female *H. versicolor* demonstrated behaviours typical of phonotaxis to mating signals, including head scans (movement of the head while the body remains stationary during a sound's broadcast) and zigzag movements that lateralize the speakers location during sequential hops in response to the playbacks. In contrast, nonresponsive females will either show a complete lack of movement, or walk/jump randomly with respect to the sound broadcast. As measured

by where females contacted the edge of the arena relative to the stimulus sources, the orientation of females was nonrandom and clustered near the location of the sound sources (Table 1, Fig. 3). For each delay between the leading and lagging pulses within the completely overlapping calls, the direction of female phonotaxis was displaced towards the location of the speaker broadcasting the advertisement call with leading pulses (Table 1). Figure 1a shows the phonotactic orientation of females in the test in which there was a 6 ms delay between the pulses in the overlapping calls. Even when the call with leading pulses was reduced in intensity by 6 dB relative to the call with lagging pulses, females approached the source of the stimulus with leading pulses (Table 1). When the call with leading pulses (6 ms delay) was delayed by one pulse period relative to the call with lagging pulses, such that it began after the call with lagging pulses, females still oriented towards the call with leading pulses (Table 1, Fig. 3b).

Experiment 2: Test of Localization Dominance by Leading Pulse Elements

Of the 19 females tested in experiment 2, two failed to respond to the second standard stimulus, and the analysis presented here is for the 17 females that completed the entire series of tests. Females tested with only the stimulus consisting of the pulse segments shortened to 6 ms were not reliably attracted, as indicated by the low phonotaxis scores (Fig. 4). Although 17 females were tested with the 6 ms pulse segments, only 13 reached the wall of the arena during the 5 min test period, and these females did not consistently arrive near the sound source (Rayleigh test: $r = 0.37$, $N = 13$, $P = 0.18$; Fig. 5a). By contrast, when a stimulus consisting of the long (18 ms) pulse segments was played back from one speaker, females were reliably attracted to the sound source (Fig. 4) and accurately located it ($r = 0.71$, $N = 17$, $P < 0.001$; Fig. 5b). When the two signals were played back simultaneously from separate speakers and synchronized so that the two parts were emitted in the normal order, females reliably responded as they did to the longer pulse segment alone (Fig. 4), but they oriented and moved to the sound source of the short, leading pulse segment ($r = 0.97$, $N = 17$, $p < 0.0001$; Fig. 5c). We tested 11 females with the pulse segments in reverse order. Female orientation was nonrandom (Rayleigh test: $r = 0.72$, $N = 11$, $P = 0.02$), but in this test, movements were towards the source of the 18 ms segments (mean + SD direction of phonotaxis = $93.5 + 46.3°$, $N = 11$); locations of speakers broadcasting 18 ms and 6 ms segments referenced at $90°$ and $0°$, respectively).

DISCUSSION

Female Preferences for Calls with Leading Pulses and Perceptual Correlates

Female *H. versicolor* oriented towards and approached the source of advertisement calls with leading pulses over a range of delays between pulses in alternative stimuli. Females still oriented towards the call with leading pulses even when it was delayed relative to the call with lagging pulses. This result suggests that signal-timing preferences are operating in this system at the level of pulses within overlapping calls and not at the level of entire calls (the first pulse to arrive at their position) and is consistent with the lack of preferences based on the timing of entire calls reported previously in *H. versicolor* (Klump & Gerhardt 1992).

While both masking and precedence effects have been proposed to underlie preferences for leading signals in chorusing organisms, it is difficult to disentangle potential perceptual processes that underlie signal-timing preferences using conventional stimuli and playback designs. In the second experiment, we used a paradigm that took advantage of well-established preferences for pulse duration in *H. versicolor* to show that preferences for leading pulses probably arise because they strongly influence the perceived location of the source of the advertisement calls. Our demonstration that an unattractive stimulus (very short sections of pulses) influenced the direction of female phonotaxis in a leading timing position strongly supports this interpretation.

Our results are highly consistent with characteristics of the precedence effect described in humans. The precedence effect actually comprises several related perceptual phenomena regarding how listeners perceive leading and lagging stimuli (Litovsky et al. 1999; Litovsky & Shinn-Cunningham 2001). The most striking phenomenon is when there are short delays between leading and lagging sounds, leading sounds dominate the perceived location of the sound sources. Indeed, localization dominance by leading sounds is most strongly expressed in humans over latencies on the order of several milliseconds between sounds, which are of the scale of the timing differences among the alternative pulse and pulse elements studied here. A second important correlate of the precedence effect is that listeners often perceive sounds arising from different locations as a single auditory event under many conditions, a phenomenon termed perceptual fusion (Litovsky et al. 1999). In experiment 2, female *H. versicolor* must have clearly perceived the 18 ms pulse segments as part of a composite signal because the 6 ms pulse segments did not elicit phonotactic responses on their own. Thus, it is likely that the two segments were perceptually fused into a single auditory image that was localized at the source of the leading pulse segments. This hypothesis is supported by the results of preliminary tests in which the paths of females of *H. versicolor* were deflected towards a point between the loudspeakers when the amplitude of the lagging pulses was increased relative to that of the leading pulses, suggesting that the females perceived a phantom source at that location. Perceptual grouping of widely separated sounds under different conditions has been found in another study with *H. versicolor* (Bee & Riemersma 2008) as well as in the túngara frog, *Physalameus pustulosus* (Farris et al. 2002, 2005).

Implications for Communication

The frog auditory system comprises features that are unique, such as the internally coupled tympana and two kinds of auditory organs, as well as those that are shared with other terrestrial vertebrates (Mason 2007; Wilczynski & Endepols 2007). The finding of a precedence-like effect of leading signals suggests another commonality of frog auditory processing with that of birds and mammals. The precedence effect is widely considered to be a mechanism that serves to minimize contradictory sound source location cues arising from echoes by using small differences in the arrival times of sounds (Zurek 1987). While the importance of reverberation in frog communication has been largely unexplored (Feng & Schul 2007), many species communicate in habitats that are cluttered with vegetation and other potential sources of echoes. Further experiments in which the timing relationships, relative intensities of the leading and lagging pulses or pulse sections and the angular separation of the speakers are varied could explore similarities and differences among species in perceptual phenomena, such as summing localization and the generation of a phantom sound source.

The importance of signal element order within overlapping calls for receiver behaviour and the operation of precedence effects have broad implications for animal communication. Many chorusing species communicate within noisy aggregations of closely spaced signallers, and in such environments, receivers often face overlapping signals arising from multiple individuals. For species with signals that are temporally structured into pulses, syllables or other discrete elements, the relative timing of signal elements within overlapping calls probably has important implications for communication and mate choice. Our results suggest that reduced localizability of signals may be a cost of signal interference in such species and a selective factor for the timing adjustments that minimize signal overlap in grey treefrogs and many other chorusing animals. Indeed, some species actively adjust the timing of signal elements within overlapping calls (Schwartz 1993; Martínez-Rivera & Gerhardt 2008).

Sources of signal overlap often also include members of other species, and the results of this experiment suggest an explanation of the phonotactic errors made by female *H. versicolor* when presented with conspecific advertisement calls that were overlapped by those of the

closely related *H. chrysoscelis* (Marshall et al. 2006). It is likely that the phonotaxis towards the source of heterospecific calls arose because these calls interfered with the ability of females to localize the conspecific signals when the calls overlapped. The calls of *H. chrysoscelis* have a greater number of pulses and a nearly two-fold pulse rate relative to those of *H. versicolor*. Thus, during signal overlap, these short pulses may be more likely to be in a leading position and thereby have a disproportionate influence on the perceived location of calls. In mixed-species choruses, male *H. versicolor* adjust the timing of advertisement calls in response to calls of conspecific males and calls of *H. chrysoscelis* to avoid overlap, and these behaviours may serve to ensure that conspecific signals are localizable.

We suggest that multiple mechanisms probably underlie the widespread preferences of receivers for leading signals. Preferences for leading calls in frogs occur over extremely short delays on the scale of those used in this experiment (Grafe 1996, 1999), and preferences based on the timing of signal elements probably operate in other species with temporally structured calls. At these timescales, precedence effects may often be a dominant influence on receiver behaviour. Preferences for leading signals in species in which preferences are expressed over much longer delays and in species with signals that that lack fine-scale temporal structure may be more likely to result from masking and other phenomena (e.g. Römer et al. 2002). Further studies with a broad range of species are needed to elucidate the role of various perceptual processes that determine selective phonotaxis to overlapping signals.

Acknowledgments

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(a)

(b)

10 ms

50 ms

(c)

1 m
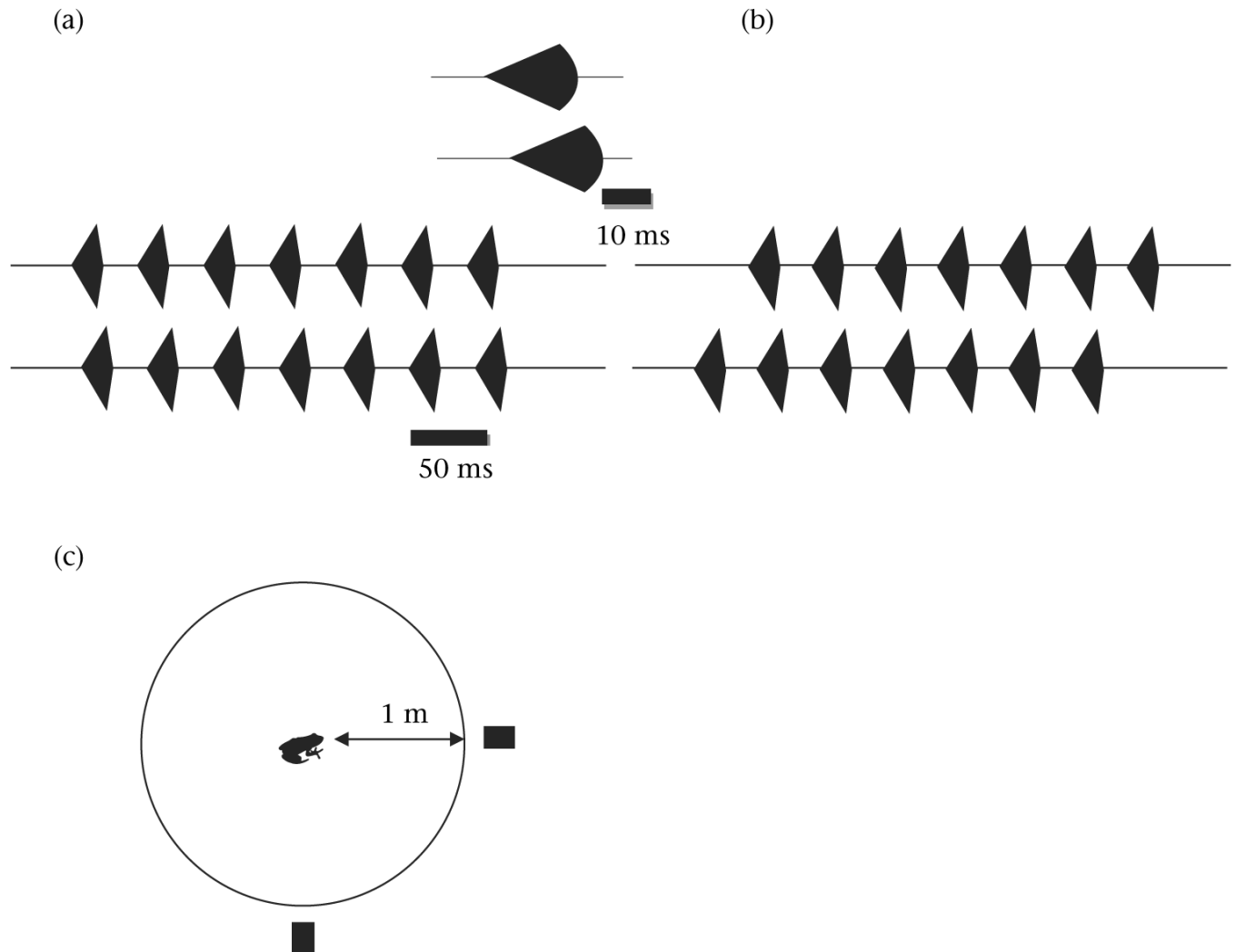
Figure 1.
Playback stimuli used in experiment 1. (a) Overlapping synthetic advertisement calls in which the pulses of one alternative led those of the other by a fixed delay. For clarity, the depicted stimuli contain fewer pulses than the actual stimuli, which were 18 pulses long. The inset shows the timing of the pulses within the overlapping calls, which was a delay of 6 ms in this case. (b) Overlapping stimuli in which the call with leading pulses was delayed to start after the call with lagging pulses. In this test, the first pulse of the call with lagging pulses and the last pulse of the call with leading pulses did not overlap with pulses in the alternative stimulus. (c) Configuration of the playback arena showing the locations of the speakers broadcasting the alternative stimuli.
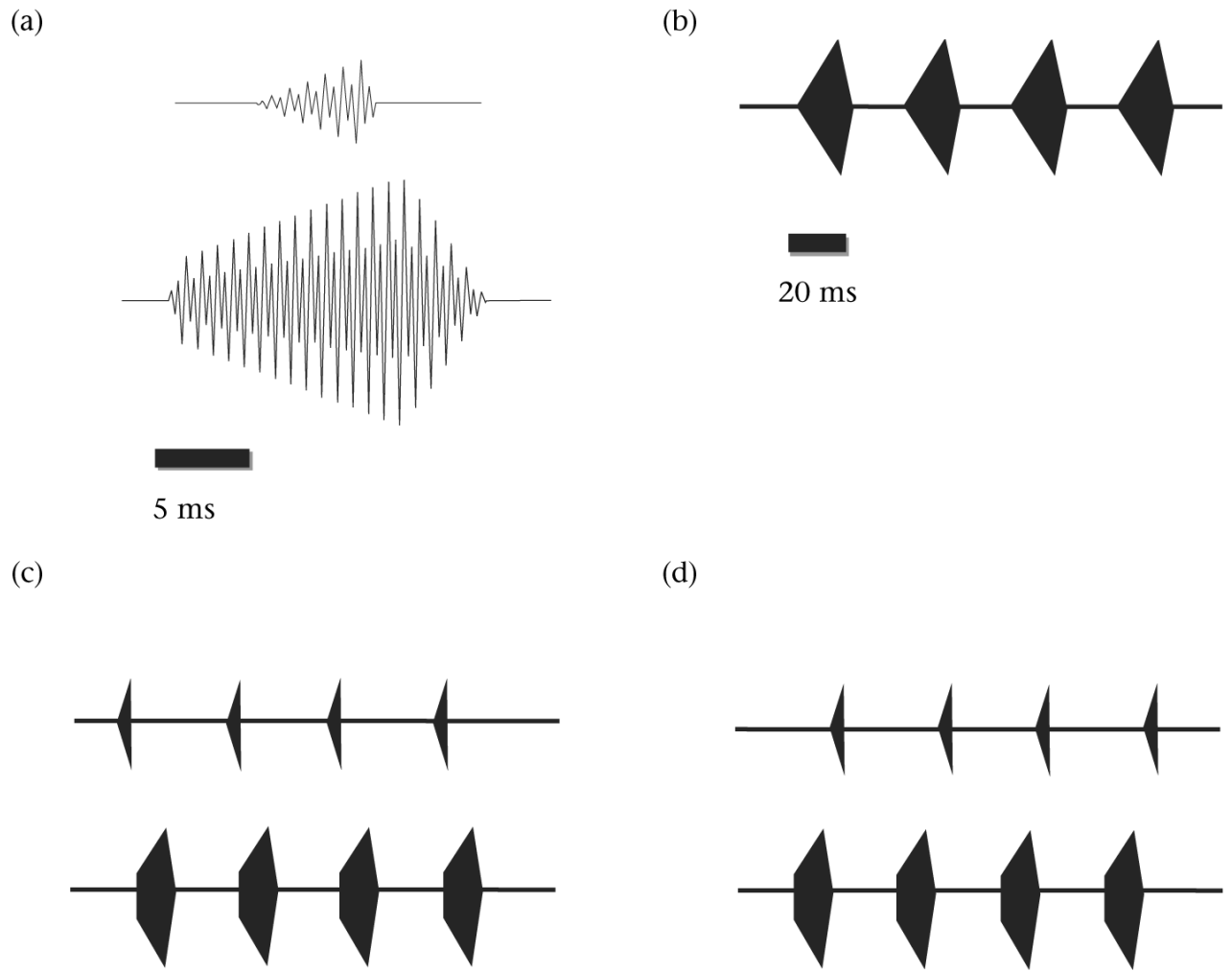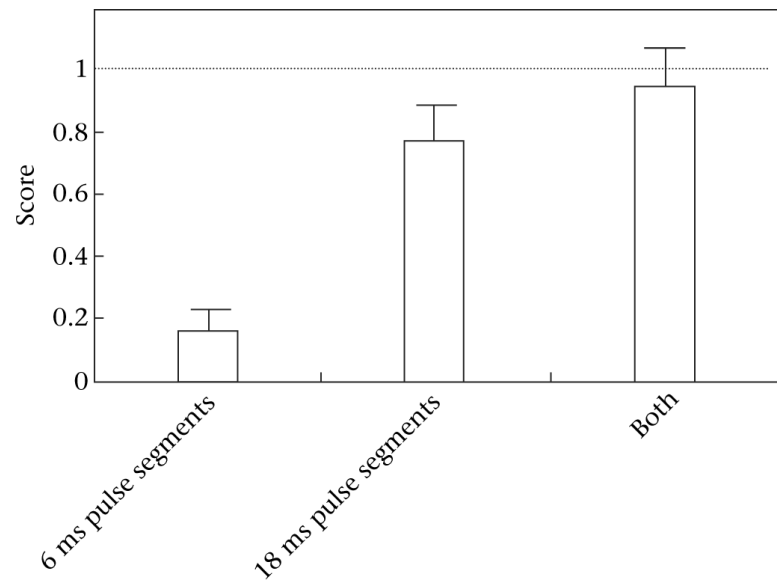
Figure 2.
Stimuli used in experiment 2. (a) Oscillograms of individual segments of the short (top trace) and long (bottom trace) pulse segments. (b) Unmodified advertisement call stimulus. (c) Synchronized playback of the alternative segments with the short pulse segments leading the longer segments. (d) Synchronized playbacks of the segments in reverse order.
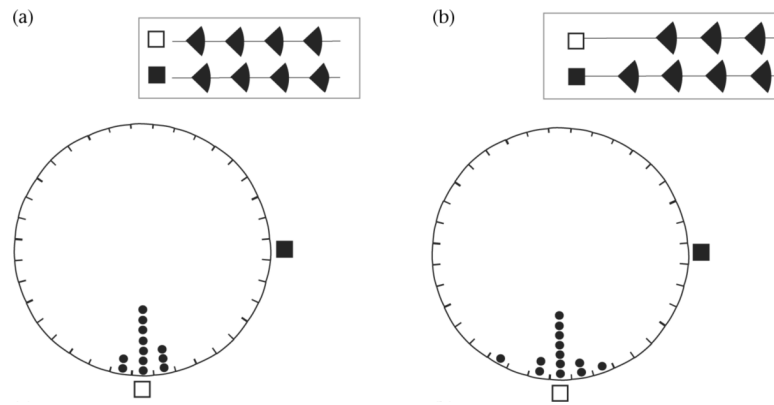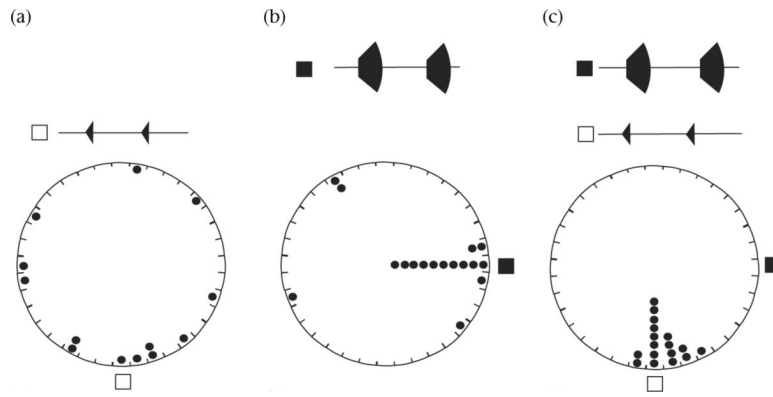
Figure 3.
Phonotactic orientation of female H. versicolor in the playback arena in response to overlapping advertisement call stimuli in experiment 1. (a) Pulses were timed such that those in one call (□) led those of the other call (■) by a delay of 6 ms. (b) Same pulse timing relationship as in (a) except the call with leading pulses was itself delayed by one pulse period such that it began after the stimulus with lagging pulses. Each point represents the contact point along the edge of the arena by a single female.

Figure 4.
Phonotaxis scores showing the strength of female phonotactic response ($\bar{X}$ + se) for the various stimuli used in experiment 2. Higher scores indicate stimuli that were more attractive to females. We used a cutoff score of 0.4 to differentiate between stimuli that were attractive and unattractive to females.

Figure 5.
Phonotactic orientation of female *H. versicolor* relative to the location of the speaker broadcasting the advertisement call stimulus. (a) Responses to stimuli consisting of only the first 6 ms of conspecific pulses. Four of the 17 females tested did not reach the wall of the arena during the 5 min test period. (b) Responses to the stimuli consisting of 18 ms segments of each pulse. (c) Responses to both segments broadcast together (with the 6 ms segments preceding the 18 ms segments), but originating from separate speakers.

Table 1

Phonotactic orientation of female *Hyla versicolor* relative to the locations of speakers broadcasting overlapping advertisement calls with different delays between leading and lagging pulses

Delay between pulses	N	Mean angle of phonotaxis (°)[*]	SD	95% CI	$r^{\dagger}$	$P^{\ddagger}$
2 ms	13	358	9.7	(352–4)	0.99	<0.001
6 ms	12	1	6.4	(357–5)	0.99	<0.001
12 ms	13	357	4.6	(354–0)	1.0	<0.001
18 ms	13	8	21.9	(354–21)	0.93	<0.001
6 ms, lead pulse call @ −6 dB	11	2	14.0	(352–11)	0.97	<0.001
6 ms, lead pulse call delayed	13	359	11.3	(352–6)	0.98	<0.001

[*] Speakers broadcasting the calls with leading pulses and following pulses are referenced at 0° and 90°, respectively.

[†] Concentration of mean angle.

[‡] Rayleigh test of significance of mean angle (Zar 1999).