Restoration of call attractiveness by novel acoustic appendages in grey treefrogs

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Ethologists have often reported preferences for novel signals, especially if they are more extravagant than normal signals. Such preferences presumably reflect sensory biases that may promote the evolution of both novel and complex signals. We tested behavioural responses of female grey treefrogs, *Hyla versicolor*, to novel complex calls in relation to the response properties of previously described temporally selective neurons in the auditory midbrain. As predicted by the selectivity of interval-counting neurons, females discriminated against synthetic advertisement calls containing a gap, a missed pulse or a pulse of abnormally short duration. The addition of a novel tonal appendage to such defective calls often resulted in partial restoration of the attractiveness of the signal. The restorative effect occurred only when an appendage with a higher amplitude followed (rather than led) the defective call. Our results show how the consideration of proximate mechanisms can provide insights about the evolution of complex signals; the behavioural results, in turn, suggest new ways of assessing the response properties of the auditory system.

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Acoustic communication plays a prominent role in mate choice in many kinds of animals, and acoustic signals may convey information about the species, physical condition and heritable fitness of a prospective mate. However, not all preferences are based on such properties, and strong responses to exaggerated, supernormal stimuli have been observed since the early days of ethology (Tinbergen 1951; Andersson 1994). Formal explanations based on pre-existing sensory biases (Ryan & Rand 1993) or hidden preferences (Arak & Enquist 1993) have received much attention and some empirical support. Such biases may contribute to the evolution of complex signals.

Whereas most species of frogs and toads produce calls consisting of a single acoustic element that is repeated, some exceptional species, scattered throughout a wide range of taxonomic groups, produce calls with multiple acoustic elements (Gerhardt & Huber 2002). In two groups of anurans, experiments have shown that adding elements of the calls of another species to the single-element species-typical call can result in a novel complex call that is preferred to the simple normal call (Ryan & Rand 1993; Gerhardt et al. 2007). Although these experimental results support the idea that pre-existing biases may underlie the evolution of complex calls, several questions arise about the specific sensory mechanisms. Are complex calls more attractive simply by virtue of the greater duration of sensory stimulation? If not, then what are the characteristics of novel signals that make them more attractive than conspecific signals? Are these characteristics common to a wide variety of taxa or specific to a few exceptional species? To the extent that a widespread set of criteria exists, are there also common, underlying sensory mechanisms or constraints?

Studies of grey treefrogs (*Hyla versicolor* and *Hyla chrysoscelis*), in which males produce simple trills, showed that adding a novel element to the trill usually enhanced the attractiveness of the resulting complex call relative to the trill alone, provided that the novel element followed the trill in time (Gerhardt et al. 2007). Subsequent research has corroborated this temporal order effect in *H. versicolor* over a wide range of values of duration of the trill and appendages (novel acoustic elements) and magnitudes of the silent gap between elements (H. C. Gerhardt & S. C. Humfeld, unpublished data). By contrast, the order effect was weaker in *H. chrysoscelis*, and given short silent gaps, complex calls with leading appendages often had enhanced attractiveness (see also Seeba et al. 2010). Despite their close relationship, such differences are not entirely surprising because females of the two species differ significantly in the fine-scale temporal criteria by which they recognize conspecific trills (Schul & Bush 2002).

Our goal was to relate the behavioural effectiveness of different variants of novel complex calls to the response properties of a class

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of temporally selective auditory neurons found in the midbrain and characterized in several species of frogs. Our data are derived from experiments with *H. versicolor*, the grey treefrog species in which the duration, shape and silent interpulse interval all play a role in call recognition (Schul & Bush 2002). Our experiments were framed in part by descriptions of a class of temporally selective neurons (interval-counting neurons), identified in the torus semicircularis of several anuran species (Adler & Rose 1998; Edwards et al. 2002). The torus semicircularis, a homologue of the inferior colliculus of higher vertebrates, is a key point in the central auditory system, integrating forebrain inputs, acting as a transition between the brainstem and forebrain, and interfacing with the motor system (Wilczynski & Endepols 2006). Thus, the response properties of neurons in this part of the brain are almost certainly important for selective phonotaxis (orientation and movement towards sound). These neurons have the property of pulse period selectivity, with the proviso that they fire only after a threshold number of correct pulse periods (reciprocal of pulse rate). An individual pulse elicits small excitation followed by inhibition. A series of pulses presented with optimal pulse periods result in enhanced excitation that overcomes inhibition and results in spikes. Pulse periods that are too short or too long in duration reset the counting process. If the interval between pulses is increased, continued inhibition and a decrease in excitation result in neuronal resetting and the absence of spikes (Edwards et al. 2007). In *H. versicolor*, behavioural experiments show that pulse duration must also exceed some minimum value and that interpulse silent periods (and hence pulse duty cycle) can then vary over a wider range than found in natural calls (Schul & Bush 2002). Thus, in addition to testing signals with an abnormally long pulse period, we assessed the effects of shortening the duration of only one or three pulses in a trill.

Our main goal was to test the hypothesis that following appendages in grey treefrogs are effective because once interval-counting neurons integrate and fire in response to a threshold number of pulses with species-typical duration and period, the majority of these cells will continue to respond tonically throughout the duration of the stimulus (Adler & Rose 2000). This property might ensure that almost any acoustic element with the appropriate spectral composition and amplitude could be effective in maintaining stimulation of these cells, thus increasing the attractiveness of the novel complex call relative to the simple call. Here we addressed the specific question of whether an appendage could restore the attractiveness of a signal with inappropriate intervals (Schwartz et al. 2010) or pulses of suboptimal duration (Schul & Bush 2002). These altered signals alone were less attractive than standard calls with species-typical pulse periods and durations. We will show that in *H. versicolor* the answer is positive for following but not for leading appendages over a wide range of trill and appendage durations.

METHODS

Animal Collection

In 2010–2012, gravid *H. versicolor* females were collected from local breeding ponds in Boone County, Missouri, U.S.A. Females were placed in individual containers and stored in an ice-filled cooler to delay oviposition. All females were returned to their collection site after experimentation, usually 2–3 days after collection.

Acoustic Stimuli

Synthetic advertisement calls were created (16 bits per sample, 20 kHz sampling rate) using custom software (J. J. Schwartz,

unpublished software). These stimuli were modelled after natural advertisement calls and have been found to be as attractive as prerecorded natural calls (Gerhardt 2005b). Each stimulus had a spectrum consisting of two spectral peaks with frequencies (1.1 and 2.2 kHz, relative amplitude of the 1.1 kHz peak was −6 dB) close to the mean values in calls of male *H. versicolor* from the populations from which we collected females (Gerhardt 2005a). The pulse shape in synthetic calls was also similar to that of typical natural calls.

We generated three standard calls that corresponded to short, average and long calls of males in local populations (10, 18, 24 pulses). The call period was 4 s at 20 °C with a 50% pulse duty cycle (25 ms pulses and 25 ms silent interval between pulses). To prevent call overlap in tests using long duration appendages, the call period was increased to 5 s.

For tests using incorrect pulse periods, 'gap' calls were created by removing one pulse from a standard call, resulting in one abnormal silent interval of 75 ms. Gaps were created by dropping pulses from one of three possible locations in the call: early, midway, or late. For the short call stimulus, gaps were created by dropping the third, fifth or ninth pulse of the call; for the average stimulus, dropped pulses were either the fifth, ninth, or 14th (Fig. 1); and the long call stimulus had either the fifth, 12th, or 20th pulse dropped.

For tests using pulses of suboptimal duration, affected pulses were half the normal pulse duration (12.5 ms) with rise/fall time ratios (80% of pulse is rise time, 20% of the pulse is fall time) comparable to natural pulses. The pulse period was not modified to maintain a 50% pulse duty cycle; therefore, after a suboptimal pulse, the silent interpulse interval was 37.5 ms instead of 25 ms. Suboptimal pulses (one or three pulses) were placed starting at the third pulse of the call in a standard call containing either 10, 18, or 24 pulses.

All appendages were created by the tone generator function in Adobe Audition v2.0 (Adobe Systems Inc., San Jose, CA, U.S.A.) and had the same spectrum as the standard and altered advertisement calls. Appendages were separated from the advertisement call by a silent interval of 50 ms (the equivalent of one pulse period). The amplitude–time envelopes of the appendages and the silent interval with respect to the advertisement call part of the complex calls are shown in Figs 3–8. We also used a sound level meter (described in Experimental Procedure) to adjust the amplitude of appendages independently of the trilled part of the complex call. The usual 'fast' RMS value of the standard (220 ms) appendage was
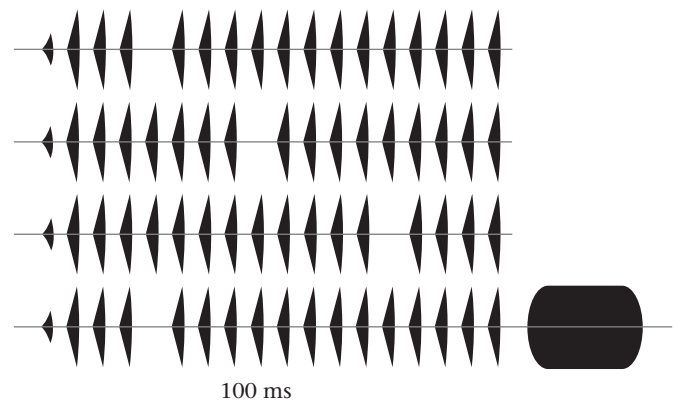

Figure 1. Examples of *H. versicolor* calls of standard duration (18 pulses) with missing pulses ('gaps') and a tonal appendage. Gaps were the equivalent of one dropped pulse, resulting in a 75 ms interval. The tonal appendage (bottom oscillogram) is 220 ms in duration and 91 dB SPL fast RMS.