A complex mechanism of call recognition in the katydid *Neoconocephalus affinis* (Orthoptera: Tettigoniidae)

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SUMMARY

Acoustic pattern recognition is important for bringing together males and females in many insect species. We used phonotaxis experiments on a walking compensator to study call recognition in the katydid *Neoconocephalus affinis*, a species with a double-pulsed call and an atypically slow pulse rate for the genus. Call recognition in this species is unusual because females require the presence of two alternating pulse amplitudes in the signal. A Fourier analysis of the stimulus-envelopes revealed that females respond only when both the first and second harmonics of the AM spectrum are of similar amplitude. The second harmonic is generated by the amplitude difference between the two pulses making up a pulse-pair. Females respond to double pulses that have been merged into a single pulse only if this amplitude modulation is preserved. Further experiments suggest that females use a resonance mechanism to recognize the pulse rate of the call, supporting a neural model of rate recognition in which periodic oscillations in membrane potential are used to filter the pulse rate of the signal. Our results illustrate how a reduction in pulse rate extends the opportunities for females to evaluate fine-scale temporal properties of calls, and provide further evidence for the importance of oscillatory membrane properties in temporal processing. The results are discussed with regard to evolutionary changes in call recognition mechanisms within the genus.

Key words: Fourier analysis, acoustic, communication, call recognition, pattern recognition, resonance.

INTRODUCTION

Temporal patterns of acoustic signals are important for mate recognition in many frogs and insects. Repetitive amplitude modulations generate a variety of parameters that females can use to identify conspecific males, including pulse rate, pulse rise time, the durations of pulses and silent intervals, and the rates or durations of verses composed of individual pulses. Females of closely related species often assess different temporal parameters to recognize conspecific calls (Schul and Bush, 2002). For example, the katydid *Tettigonia cantans* uses pulse rate to recognize the conspecific signal, whereas *T. viridissima* measures pulse and interval durations (Schul, 1998). Similar patterns exist for the crickets *Teleogryllus oceanicus* and *T. commodus* (Hennig, 2003; Hennig et al., 2004) and the frogs *Hyla versicolor* and *H. chrysoscelis* (Schul and Bush, 2002).

The katydid genus *Neoconocephalus* has been used as a study system to address behavioral, evolutionary and neurophysiological aspects of acoustic communication (e.g. Greenfield and Roizin, 1993; Greenfield, 1994; Schul and Sheridan, 2006; Beckers and Schul, 2008). The calls of most *Neoconocephalus* species have exceptionally fast pulse rates in the range of 150–220 Hz at 25°C (Greenfield, 1990), imposing constraints on the ability of females to use fine-scale measurements of temporal properties for call recognition; the capacity of the sensory system to encode parameters such as pulse rise time or pulse duration decreases at such fast rates (Franz and Ronacher, 2002). Accordingly, females of species that call at high rates rely on relatively crude temporal properties (e.g. the absence of gaps) for call recognition (Deily and Schul, 2004; Deily and Schul, 2009; Beckers and Schul, 2008).

Calls with a fast, uniform pulse rate represent the ancestral state in the genus *Neoconocephalus* (Snyder, 2008) and the majority of

species have maintained this simple pattern. Several species (five out of 25 with described calls) (Greenfield, 1990) produce calls with a derived pulse pattern: pulses are grouped into pairs or double pulses, i.e. the calls comprise alternating pulse periods. The rate at which these double pulses are repeated is much slower than the original single pulse rate, and should be easily resolved by the sensory system. In most species with such double pulses, females respond to the two pulses merged together into a single long pulse and evaluate the rate of the merged pulses. Other temporal parameters (e.g. interpulse intervals, pulse durations) are not evaluated in these species (Deily and Schul, 2004; Beckers and Schul, 2008).

A second evolutionary modification of the pulse pattern is a reduction in the pulse rate. Five of the 25 *Neoconocephalus* species with described calls (Greenfield, 1990) have dramatically reduced their pulse rates to under 50 Hz; intermediate rates between 50 and 100 Hz do not occur in this genus (Greenfield, 1990). These slow pulse rates would allow, at least in principle, more sophisticated temporal processing than is observed in the fast calling *Neoconocephalus* species.

The tropical species *N. affinis* is the only known *Neoconocephalus* species to adopt both of the modifications to the standard pulse pattern, producing double-pulsed calls with a pulse rate of approximately 26 Hz (single pulse rate, or 13 Hz double pulse rate) (Greenfield, 1990). Here, we characterize the call recognition mechanism in *N. affinis* using a behavioral paradigm. We identify which temporal parameters of the calls are evaluated by females to determine whether *N. affinis* females make use of the fine-scale temporal properties available to species with slow pulse rates, and to identify how the double pulse pattern contributes to call recognition in this species. We found that although the double pulse

rate is a critical parameter, other temporal parameters also play significant roles.

Recognition of pulse rates could be accomplished by a variety of different neural mechanisms. Behavioral responses to synthetic stimuli are used to distinguish among the various models that have been proposed (see Bush and Schul, 2006). Resonant neural properties probably underlie rate recognition in several orthopteran species (Bush and Schul, 2006; Gerhardt and Huber, 2002; Webb et al., 2007). We tested this hypothesis in *N. affinis* and found evidence that this species uses a resonance mechanism to recognize the rate of double pulses.

MATERIALS AND METHODS

Eggs of *Neoconocephalus affinis* Beauvois 1805 were obtained from adults collected near the towns of Luquillo and Florida in Puerto Rico. After hatching, the insects were maintained in the laboratory on a diet of wheat seedlings, apples and cat food. Following the final molt, females were given 2 weeks to attain reproductive condition before use in experiments.

Phonotaxis

We conducted behavioral tests on a walking compensator (Kramer treadmill, M.P.I., Seewiesen, Germany) (Weber et al., 1981) in an anechoic chamber at 25±1°C. In short, the insects were placed on top of a sphere, free to walk but kept in place by compensatory sphere rotations, while acoustic signals were presented from loudspeakers located in the insect's horizontal plane. The intended direction and speed of the animal were read out from the control circuitry [see Schul et al. (Schul et al., 1998) for a sample walking path]. The experiments were performed in the dark except for an infrared light used to monitor the movements of the animal on the sphere. The infrared light was positioned directly above the animal, eliminating the light as a directional cue (for details, see Weber et al., 1981; Schul, 1998).

Call recordings

Calls of eight males were recorded in an anechoic chamber at an ambient temperature of 25±1°C. The specimens were placed in small screen cages 15 cm in diameter. The microphone was placed 20 cm dorsal of the calling male. Calls were recorded with a 1/4 in (0.63 cm) free field microphone (GRAS 40 BF; Holte, Denmark), amplified (GRAS 26 AC and 12 AA), high-pass filtered (1000 Hz, Krohn Hite 3202; Brokton, MA, USA), and digitized using a custom-made A/D-converter system (16 bit resolution, 250 kHz sampling rate). This setup provided a flat (±1 dB) frequency response in the range from 2 kHz to 70 kHz.

Amplitude spectra were calculated using BatSound (Ver. 1.0, Pettersson, Uppsala, Sweden) by fast Fourier transformation (Hamming window, frame length 1024) and averaged over a 1 s section of each call. The spectra of the calls of all species had a narrow-band low frequency component and broad components of lower amplitude in the ultrasound range.

Stimulation

Synthetic stimuli were generated using a custom-developed DA-converter/amplifier system (16 bit resolution, 250 kHz sampling rate). Signal amplitude was adjusted using a computer-controlled attenuator and delivered *via* one of two loudspeakers (Motorola KSN1218C; Schaumburg, IL, USA) mounted at a distance of 150 cm in the horizontal plane of the insect and separated by an angle of 115 deg. We measured signal amplitude using a 1/4 in (0.63 cm) condenser microphone (GRAS 40BF) positioned 1 cm above the top of the sphere, and a Bruel and Kjaer (Naerum, Denmark) sound level meter (B&K 2231). All stimuli were presented at 80 dB peak SPL (re. 20 μPa); this amplitude represents a distance of 2–5 m from a calling male (Schul and Patterson, 2003).

Stimuli were generated based on our analysis of male calls (see Results). To generate our stimuli, we added two sine waves of 12.5 kHz and 25 kHz (at –6 dB relative to 12.5 kHz) to mimic the natural spectrum of *N. affinis* calls. We used the resulting sinusoid as carrier signal, to which we subsequently applied amplitude modulations. In preliminary experiments, we identified an artificial stimulus that was as attractive as high quality recordings of natural calls, i.e. females responded with similar response strength to synthetic and natural calls. The synthetic stimulus had the simplified spectrum described above and consisted of two abutting pulses that alternate between 75 and 100% amplitude. The lower-amplitude pulse was 37 ms in duration (30 ms rise, 5 ms plateau, 2 ms fall) and the higher amplitude pulse was 41 ms in duration (29 ms rise, 10 ms plateau, 2 ms fall). This stimulus was used as the control stimulus for all experiments described below and is shown in Fig. 2. Note that the pulse durations of the control stimulus differ from those of the natural calls, as they included both the opening and closing semi-pulses of the natural calls.

Experimental protocol

The experimental protocol is described fully in Schul (Schul, 1998) and Bush et al. (Bush et al., 2002). Briefly, each stimulus is presented for approximately 1.5 min from each of the two loudspeaker positions; data from the two positions are combined to eliminate any directional biases in individual animals. Each insect is initially presented with the control stimulus, followed by two test stimuli, then the control etc., until all stimuli in the series have been presented. We imposed a 1-min period of silence between each stimulus presentation. Individual females were typically presented with four to seven test stimuli and three to four controls per series, and were given a break of at least 24 h between series. Test stimuli were presented in a pseudo-random sequence within each experiment, and the sequence was varied among individual females. We could not detect any effect of stimulus sequence on female responses. Data were collected from 98 females over a period of 5 months. Only females responding to the standard situation were included in the tests. For ease of reading, descriptions of our test stimuli are given in the corresponding Results section.

Data analysis

We quantified female responses to the test stimuli relative to their responses to the control stimulus as a 'phonotaxis score' (PS), which included measures for three criteria that positive phonotaxis should meet: (1) the relative walking speed, describing the locomotion activity elicited; (2) the vector length, describing the accuracy of orientation; (3) the orientation relative to the orientation during the control stimulus. Phonotaxis scores range from approximately +1 (perfect positive phonotaxis) to –1 (perfect negative phonotaxis). Phonotaxis scores close to 0 indicate either no response or random orientation. [For details of the data analysis and calculation of the phonotaxis score see Schul (Schul, 1998).] We tested eight to 11 different females for each stimulus and present phonotaxis scores as means ± standard error of the mean (s.e.m.).

Female responses were considered significant if the average response was at least 50% of the response to the standard call model. Note that the application of significance criteria merely emphasizes the relative attractiveness of stimuli and is not meant to classify

stimuli as 'recognized' or 'not recognized' [for a detailed discussion see Bush et al. (Bush et al., 2002)].

Modulation spectra of the stimuli

Following the reasoning of Schmidt et al. (Schmidt et al., 2008), we re-analyzed the phonotaxis data by calculating the Fourier spectrum of the amplitude modulation ('envelope') of each stimulus, and correlating properties of these spectra to the phonotaxis scores. This analysis should reveal which Fourier components of the modulation spectrum influence attractiveness, if any. We extracted the amplitude modulation (amplitudes from 0 to 1) of 77 synthetic stimuli from the experiments described below with a temporal resolution of 1 ms (=1 kHz sampling rate). We then calculated the spectrum of the amplitude modulation through fast Fourier transformation (FFT; window length 1024; Hamming window) using BatSound software. We compared the locations and amplitudes of the peaks in the spectra relative to the phonotaxis score for the stimulus.

RESULTS
Call analysis

In katydids, sound is produced during opening and closing movements of the forewings (Walker, 1975; Heller, 1988). The sound produced during opening movements (opening pulses) is of much lower amplitude and shorter duration than that produced during closing movements (closing pulses; Fig. 1A). We refer to the sound produced during one wing-cycle as one pulse (i.e. it includes both opening and closing pulses). The temporal structure of *N. affinis* calls consists of alternating pulses that differ in both amplitude and duration (Fig. 1). The first closing pulse of a pair (c1) is of lower amplitude and shorter duration than the second closing pulse (c2; Fig. 1; peak amplitude of c1:c2=0.75±0.05; c1 duration: 22.2±1.0 ms, with 15 and 2 ms rise and fall times, respectively; c2 duration: 32.0±3.4 ms, with 20 and 2 ms rise and fall times, respectively). The durations of the low amplitude opening pulses are 15 ms (o1; Fig. 1) and 9 ms (c2), generating a double pulse rate of 12.8±0.6 per second. All values are given as means ± s.d. (*N*=8).
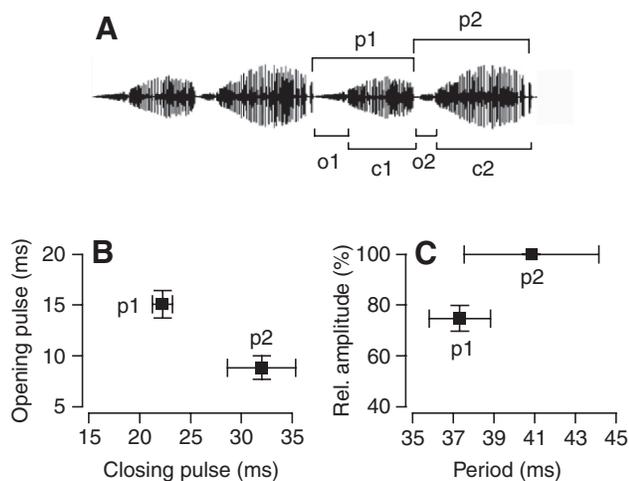

Fig. 1. Male calls of *Neoconocephalus affinis*. (A) Typical oscillogram clarifying the double pulse structure of the call. Brackets indicate periods 1 and 2 (p1, p2) as well as opening and closing pulses (o1,o2, c1,c2). (B) Durations of the opening pulses and of the closing pulses (means±s.d., *N*=8). Values are given separately for period 1 and 2. (C) Amplitude of the closing pulse (relative to that of c2) as a function of pulse period. Owing to the alternating pulse periods, two value combinations are given in B and C.

Experiment 1

First we tested the importance of the low-amplitude opening pulses for the attractiveness of male calls. A digital recording of a natural call (stim. 1 in Fig. 2) was as attractive as our synthetic control stimulus (PS=0.93±0.02, *N*=8). Replacing the opening pulses of the natural call with silence significantly reduced the attractiveness of the recorded call (stim. 2, PS=0.77±0.07, *N*=8; Wilcoxon paired-sample test, T^+=36, T^-=0, *P*<0.01). We generated a synthetic version of the stimulus that lacked opening pulses by shortening the rise times in the control stimulus by 15 ms and inserting silent intervals of 15 ms between the pulses (stim. 3). This stimulus elicited responses comparable to those elicited by stimulus 2, the modified recording that lacked opening pulses (PS=0.68±0.06, *N*=11). These results indicate that silent intervals between the pulses reduce the attractiveness significantly, and that the opening pulses fill these intervals in natural calls.

Females did not respond to the final stimulus in this sequence, an unmodulated sinusoid (stim. 4, PS=−0.05±0.04, *N*=8), indicating that amplitude modulation is required to elicit positive phonotaxis of female *N. affinis*.

The next set of stimuli (Fig. 2; stim. 5–8) tested the importance of the double pulse pattern for female phonotaxis. Stimuli 5 and 6 contained only the rate of the double pulses, either by dropping pulse 1 of the control stimulus, or by merging the double pulse into one long pulse. Both stimuli elicited only weak, if any responses (PS=−0.04±0.04, *N*=8 and PS=0.31±0.11, *N*=8, respectively). Stimulus 7 contained the same number of pulses as the control, however, all pulses were equal in duration and amplitude, generating a single pulse rate of 25.6 Hz. This stimulus elicited only weak responses (PS=0.31±0.08, *N*=8).

Stimulus 8 was generated by reducing the amplitude of every other pulse to 75%. It differed from the control stimulus in that all
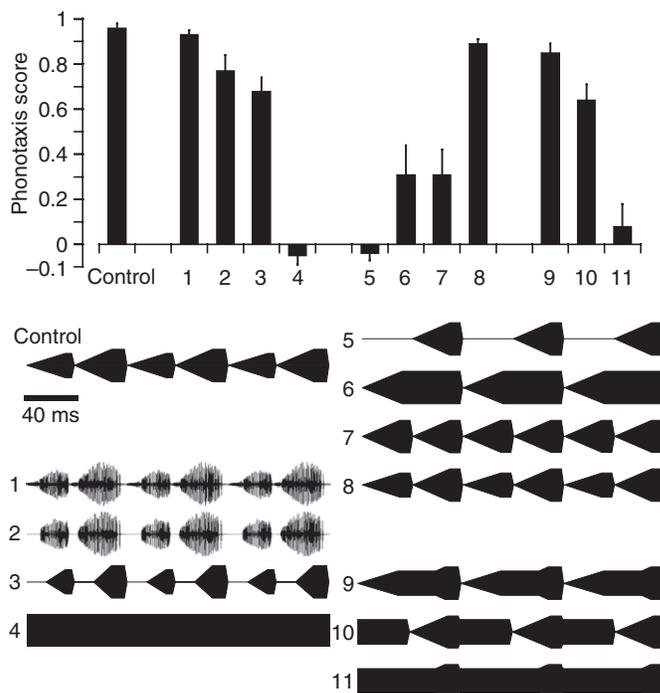

Fig. 2. (Top) Phonotaxis scores (means±s.e.m., *N*=8–11) of female *N. affinis* in response to various stimuli. (Bottom) Oscillograms of the stimuli used. This experiment tested the importance of opening pulses (1–4) and the double pulse structure (5–11). For further explanations see text.

pulses had the same duration, whereas in the control stimulus pulse 1 (the lower amplitude pulse) was 4 ms shorter than pulse 2. This stimulus was highly attractive, with phonotaxis scores (PS=0.89±0.02, N=8) comparable to that of the control. This set of stimuli indicates that the important feature of the double pulse is the difference in amplitude between pulse 1 and 2, but not the difference in pulse duration (or period).

Stimuli 9–11 were generated by merging pulses 1 and 2 of stimulus 8, but they differed in how the merging was accomplished. In stimulus 9, the fall of pulse 1 and the rise of pulse 2 were replaced by a plateau at 75% amplitude, resulting in one long pulse with two plateaus. This stimulus elicited strong responses (PS=0.85±0.04, N=11) comparable to that of stimulus 8 and the control. For stimulus 10, the pulses were merged by replacing the fall of pulse 2 and the rise of pulse 1 with a 75% plateau. Here, the higher plateau was leading the lower one. This stimulus was significantly less attractive than the previous stimulus (PS=0.64±0.07; Mann–Whitney U-test, U=26, N_1=N_2=11, P<0.01). Finally, all rise and fall ramps were replaced by a 75% plateau in stimulus 11, resulting in a continuous sinusoid with louder plateaus emerging at a rate of 12.8 Hz (= double pulse rate). This stimulus was not attractive to females (PS=0.08±0.10, N=11).

In summary, the results of experiment 1 indicate that silent intervals within the call render it unattractive, and that females only respond to merged double pulses if the relative amplitude difference between the two pulses is retained.

Experiment 2
The purpose of this experiment was to characterize the responses to the merged pulse (stimulus 9 above) with regard to both the amplitude difference between the first and second plateau, and the relative durations of the two plateaus. In both series of stimuli, the duration of the pulse was 78 ms. In the first series (Fig. 3A), the amplitude of the first plateau relative to the second plateau was varied from 20 to 100%. Stimuli in which plateau 1 had 50% or 75% amplitude were highly attractive; responses dropped when the first plateau was under 50% or greater than 75% of the amplitude of plateau 2.

In the second series (Fig. 3B), the amplitude of plateau 1 was held at 75%, but the durations of the two plateaus were varied while holding total pulse duration constant at 78 ms. Responses dropped when the duration of the second plateau was increased beyond 18.5 ms (with a corresponding decrease in the first plateau below 20 ms). Note that responses were high to the stimulus with a 0 ms second plateau. This stimulus included the second rise from 75% to 100% amplitude, but amplitude fell immediately from 100% to 0, creating a peak in place of plateau 2.

Experiment 3
We next tested which parameter of the merged double pulse was evaluated by females.

Pulse duration and consequently pulse rate were varied by independently varying the durations of the two plateaus. Other temporal parameters of the pulse were held constant (rise 1: 30 ms, rise 2: 7.5 ms, fall: 2 ms; plateau 1 at 75% relative amplitude). Note that when plateau 1 was 0 ms duration, the resulting pulse had a single 37.5 ms rise and a single plateau at 100% amplitude. When plateau 2 was 0, the rise from plateau 1 to plateau 2 was followed by an immediate fall to 0% amplitude. Between eight and 11 females were tested for each of the 37 points in the field. Responses occurred only if plateau 1 was longer than 20 ms. Significant responses were limited to the area along a diagonal from upper left to lower right
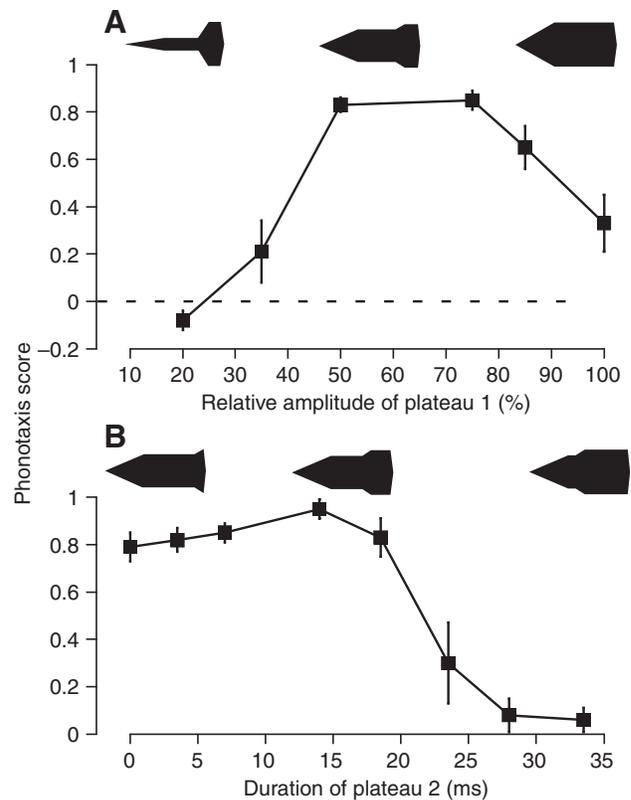

Fig. 3. Phonotaxis scores in response to stimuli that vary in (A) amplitude of plateau 1 relative to plateau 2 and (B) duration of plateau 2. Pulse duration was 78 ms in all stimuli. N=9–11 females per stimulus.

in Fig. 4, with the sum of both plateau durations of 38.5 ms. This diagonal corresponds to stimuli with a period of 78 ms (plateaus plus rise and fall times) equivalent to a pulse rate of approximately 12.8 Hz.

Experiment 4
This experiment compared the responses to stimuli with the same pulse rates but that differed in the scaling of the temporal components of the pulses (Fig. 5). Plateau 1 was at 75% amplitude relative to plateau 2 in both series of stimuli. In series 1, the durations of plateau 1 and plateau 2 were varied, but other temporal properties were held constant (rise 1=30 ms, rise 2=7.5 ms, fall=2 ms) to generate stimuli varying in pulse rate from 8.5 to 16.0 Hz. In series 2, all temporal properties of the pulse, including rise and fall times, were scaled proportionately to generate stimuli with the same pulse rates as in series 1. In both series, female responses were highest to stimuli with pulse rates of 12.8 Hz. The similarity in the shapes of the curves indicates that rise times have little influence on rate recognition in this species.

Spectral analysis of stimulus amplitude modulation
Figs 4 and 5 demonstrated that *N. affinis* females are attending to the pulse rate of the call. However, Figs 3 and 4 include stimuli that are unattractive despite having the preferred pulse rate. We calculated Fourier spectra of the amplitude modulation of the stimuli (Fig. 6A), and correlated the position and relative amplitudes of peaks in these AM spectra with the phonotaxis scores elicited by each stimulus when it was presented in the experiments. All stimuli described in the results section were included in this analysis except
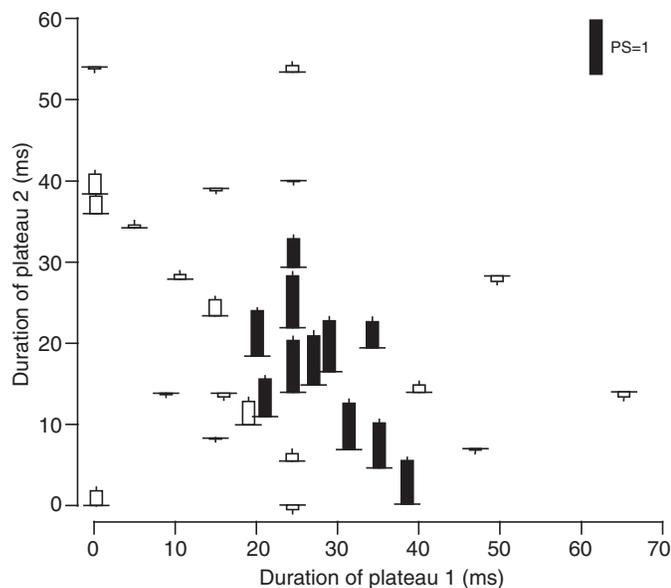
Fig. 4. Importance of the durations of plateaus 1 and 2 for phonotactic responses of *N. affinis*. The bars indicate the phonotaxis score (means±s.e.m.; *N*=8–11) for the respective parameter combination (see inset for the scale of the phonotaxis score; PS). The baseline of each bar is positioned on the duration of plateau 2. Black bars indicate significant responses, and white bars indicate non-significant responses. Rise and fall times were constant for all stimuli.

Fig. 5. Response function for stimuli that vary in pulse rate. Pulse duration (and therefore rate) was varied by altering proportionally the durations of the two plateaus (series 1) or of all temporal components of the pulse (series 2). *N*=8 females per stimulus.

those that contained silent intervals between pulses (Fig. 2, stim. 1–3, 5), yielding a sample size of 78 stimuli. Fig. 6B shows the phonotaxis score as a function of the first harmonic (=fundamental frequency) of the AM spectra; only stimuli with a first harmonic close to 12.8 Hz elicited strong responses. There were, however, many stimuli that possessed the 12.8 Hz peak and yet were associated with low phonotaxis scores. To explain this variation, we plotted the phonotaxis score as a function of the amplitude of the second harmonic relative to the first in the AM spectrum for all 39 stimuli with fundamental frequencies between 11.7 Hz and 13.7 Hz (i.e. the fundamentals which resulted in significant responses). Phonotaxis score decreased as the relative amplitude of the second harmonic decreased; no stimulus elicited significant phonotaxis if the amplitude of the second harmonic was 5 dB or more below that of the first harmonic. The regression between phonotaxis score and relative amplitude of second harmonic was significant (F=42.7, N=39, P<0.001; Fig. 6C). Note that the outlier at –28.4 dB was excluded from the statistical analysis. Thus, females respond with high phonotaxis scores only if the first harmonic in the AM spectrum is close to 12.8 Hz, and the second harmonic has high relative amplitude.

Experiment 5

The purpose of this experiment was to test whether rate recognition in this species is accomplished through neural resonance. Stimuli consisted of merged pulses in which every other pulse of the standard stimulus was elongated by 39, 78 or 117 ms. Of the three resulting stimuli, only the second stimulus is rhythmic with respect to the standard stimulus, such that the onset of every pulse corresponds to the onset of a pulse in the standard stimulus (Fig. 7, top). A strong response to the rhythmic stimulus coupled with reduced responses to both arrhythmic stimuli would provide strong evidence for the use of resonance in rate recognition [for justification, see Bush and
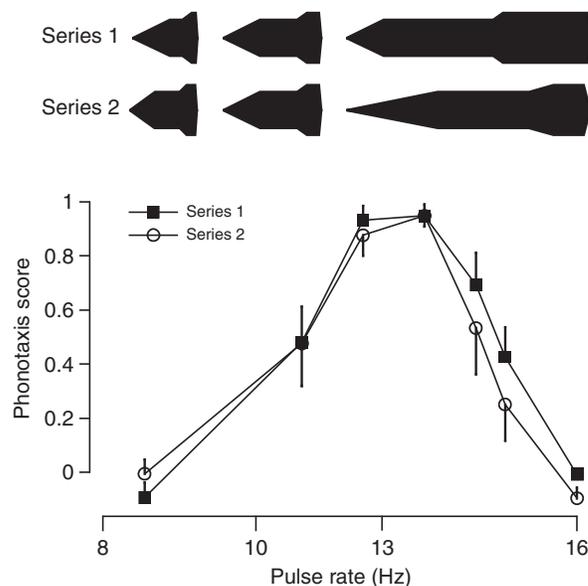
Schul (Bush and Schul, 2006)]. Three series of stimuli were tested. In series 1 and 2, every other pulse was elongated by increasing the duration of the first or second plateau, respectively. In series 3, both plateaus were increased proportionately to obtain the desired pulse durations. For all three series, the response to the rhythmic stimulus was highly significant and the responses to the arrhythmic stimuli were weak.

An additional series of rhythmic and arrhythmic stimuli were created by randomly generating 41 different pulses that varied in the durations of both plateaus. Pulse durations ranged from 50.5 to 131.5 ms. These pulses were shuffled into two different stimuli. In the rhythmic stimulus, the pulses were arranged such that the sum of adjacent pulse durations equaled an integer multiple of the standard period of 78 ms. That is, combinations of up to three pulses were arranged such that the sum of the pulse durations equaled 78, 156, or 234 ms. The arrhythmic stimulus consisted of the same 41 pulses shuffled into a random sequence with respect to the period of the standard stimulus. Females responded significantly more strongly to the rhythmic shuffle (PS=0.81±0.05) than to the arrhythmic shuffle [PS=0.05±0.11; Wilcoxon paired-sample test, N=8, T^+=36, T^-=0, P<0.01; Fig. 7, stimuli RH, AR (rhythmic and arrhythmic shuffle, respectively)].

DISCUSSION

Call recognition in *N. affinis* relies on multiple temporal components of the signal. Foremost, females rely on the presence of a 12.8 Hz component in the amplitude modulation, equivalent to the double pulse rate of the male call. Of additional importance is the presence of a strong second harmonic in the pulse rate, which is generated by the amplitude modulation between the first and second pulse within a pulse pair. Finally, *N. affinis* females are selective against the presence of silent gaps in the call. Other temporal features, such as the durations of rise and fall times, have little importance.
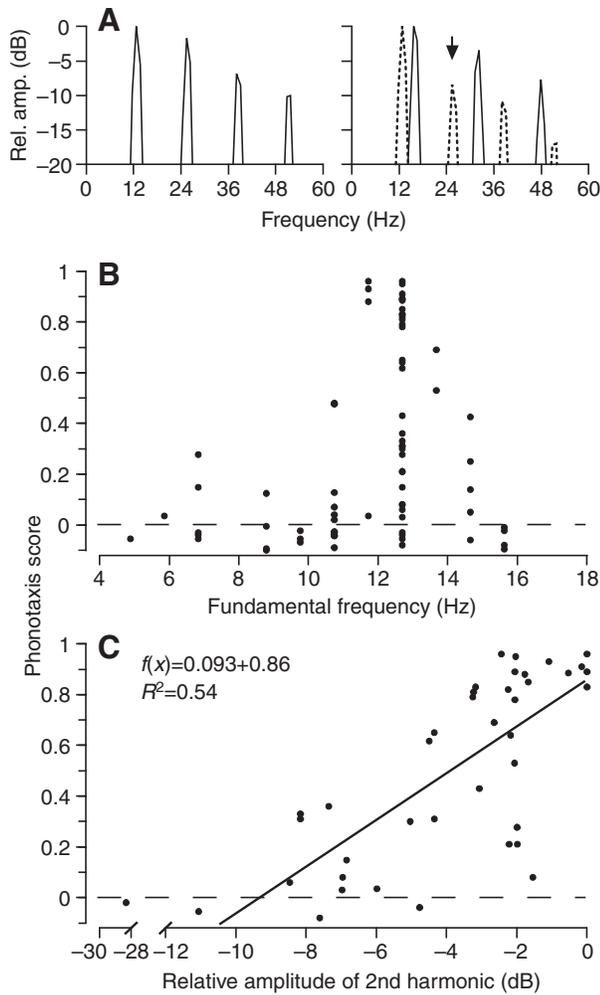
Fig. 6. Results of the spectral analysis. (A) Examples of AM spectra for three stimuli. Left: spectrum of an attractive stimulus [phonotaxis scores (PS)=0.85]; right: spectra of two different unattractive stimuli (dotted line: PS=0.06; solid line PS=−0.01). The arrow indicates the position of the second harmonic; note its attenuation relative to the first harmonic. (B) Phonotaxis scores as a function of the first harmonic in the AM spectrum of the stimulus. (C) The phonotaxis scores as a function of the amplitude difference between the spectral peak closest to 12.7 Hz and its second harmonic at or near 25.4 Hz. All data points in the frequency bins between 11.7 and 13.7 Hz are included.

These rules explain almost all of the temporal selectivity demonstrated in our experiments. Fig. 3, for example, illustrates that the 12.8 Hz rate alone is insufficient to stimulate phonotaxis: although all stimuli in this figure have the attractive rate, the phonotaxis score is dependent upon the relative amplitudes (Fig. 3A) and durations (Fig. 3B) of the two plateaus within the pulse. The spectral analysis provides an explanation for the shapes of these functions. The amplitude of the second harmonic in the FFT spectrum must be close to that of the fundamental frequency to elicit a strong response. In Fig. 8, we combine data showing the amplitude difference between the 12.8 Hz and 25.6 Hz FFT spectral peaks with the data from Fig. 3B, illustrating that a decreasing duration of plateau 1 results in a decreased amplitude of the second harmonic in the AM spectrum. Thus the importance of the relative duration of plateau 1 can be explained through the AM spectra of the stimuli.
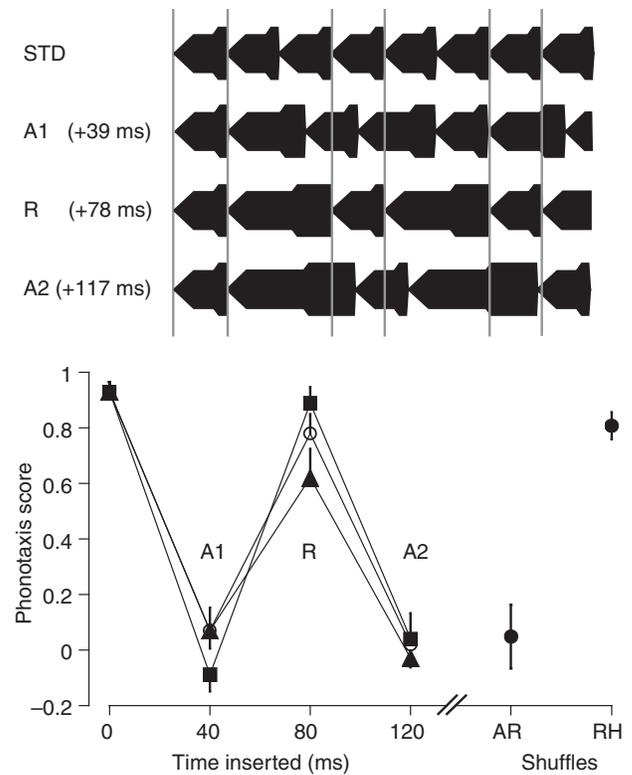

Fig. 7. (Top) Oscillograms illustrating how every other pulse was elongated to produce either arrhythmic stimuli (A1 and A2) or rhythmic stimuli (R) in which the subsequent pulse aligned with the correct timing of the standard (STD) pattern. (Bottom) Phonotaxis scores for the standard, arrhythmic and rhythmic stimuli (N=9 females per stimulus). The three series differed in whether pulses were elongated by lengthening the first plateau (squares), the second plateau (circles), or both plateaus (triangles). AR and RH represent phonotaxis scores to the arrhythmic shuffle and rhythmic shuffle, respectively, in which 41 pulses differing in duration were arranged in a sequence that was either arrhythmic or rhythmic with respect to the standard pattern (N=8).

Some variation in phonotaxis scores remains unexplained by the spectral analysis. Fig. 6C includes several stimuli with phonotaxis scores that are lower than predicted by the regression line. The FFT spectra of these stimuli contain additional peaks that are nonharmonic with 12.8 Hz (e.g. at 8.7 Hz), which may interfere with a neural resonance mechanism involved in filtering the pulse rate (see below).

Species of *Neoconocephalus* with single-pulsed calls recognize the calls by the absence of gaps, which represents the ancestral call recognition mechanism in this genus (Snyder, 2008). Silent gaps longer than a few milliseconds render calls unattractive in *N. robustus* and *N. nebrascensis*, which have retained the ancestral mechanism. Both species respond well to continuous sinusoids (Deily and Schul, 2004). *Neoconocephalus* species that produce double pulses, including *N. bivocatus* and *N. triops*, have a derived recognition system that attends to pulse rates rather than to the absence of gaps (Deily and Schul, 2004; Beckers and Schul, 2008), and thus require amplitude modulation. *N. affinis* uses both elements: while the primary cue is the pulse rate of the signal, the ancestral recognition mechanism is retained in the aversion to silent gaps. Although the intervals between closing pulses are much longer in *N. affinis* than in *N. robustus*, both species have solved this problem
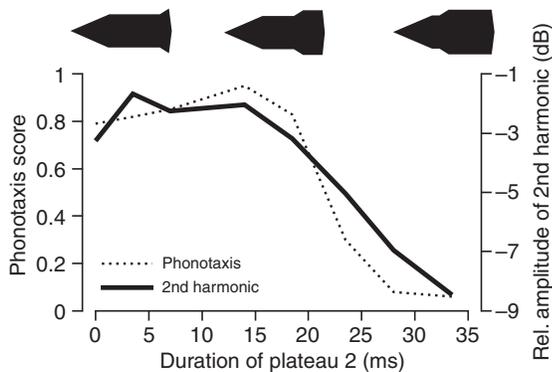
Fig. 8. Data from Fig. 3B plotted with the corresponding amplitude difference between the 12.7 and 25.4 Hz FFT spectral peaks.

by using opening pulses, which eliminate large gaps by effectively extending the rise time of the closing pulses (Figs 1, 2).

Experiment 5 provides compelling evidence that *N. affinis* uses a resonance mechanism to recognize the 12.8 Hz pulse rate. In all three stimulus series of experiment 5, females responded strongly to the rhythmic stimulus and failed to respond to the arrhythmic stimuli, even though the first arrhythmic stimulus in each series contained more periods of the correct duration per unit time. The ongoing rhythm of the call is therefore more important than the number of correct pulse periods. Moreover, a shuffle of 41 pulses of variable duration elicited a strong response when the pulses were arranged to correspond with the rhythm of the standard call, but elicited no response when the same pulses were arranged in a random sequence.

It is noteworthy that both *Tettigonia cantans* and *N. affinis* appear to use a resonance mechanism for rate recognition despite their phylogenetic distance (i.e. different subfamilies). This conservation or convergence of mechanism lends support to a model of rate recognition in which resonance occurs at the level of individual neurons, rather than emerging from more complex neural networks. Neurons with subthreshold oscillations in membrane potential can be excited by inputs with a periodicity that matches that of the oscillations (Izhikevich, 2001; Izhikevich et al., 2003). Such neural resonances occur in diverse hearing systems [e.g. bats (Covey et al., 1996; Galazyuk and Feng, 2001); crickets (Hennig et al., 2004)]. The intrinsic resonant properties of neurons are sensitive to changes in the time constants and relative abundance of voltage-gated ion channels (Hutcheon and Yarom, 2000). It is probable that the transition between resonant and non-resonant neurons happens easily and frequently in response to mutations in genes influencing these membrane proteins. Evolutionary changes in time constants or abundance of ion channels may be responsible for the differences among closely related species in the mechanisms of call recognition as described in the Introduction.

Spectral or temporal processing?

According to Fourier's Theorem, pattern recognition of amplitude modulations can be accomplished in the time domain or in the frequency domain (Schmidt et al., 2008). Which domain is used by an auditory system for the analysis of AM pattern can be determined based on the importance of the phase relationships of the different components of the AM spectrum: if the phase relationship is not important for pattern recognition, then processing in the frequency domain is likely, since the temporal pattern of the AM cannot be

unequivocally reconstructed (Hartmann, 1997). For example, a signal played backwards has an identical amplitude spectrum but different phase spectrum than the original. If the analysis takes place in the frequency domain, both signals should be equally attractive. Although we did not formally test this question here, our results provide clues to the situation in *N. affinis*.

The importance of the amplitude spectrum of the AM is demonstrated by Fig. 6. In addition, comparisons of responses to several stimuli suggest that phase information is unimportant for call recognition. For example, stimuli 9 and 10 in Fig. 2 have almost an inverse time structure and yet both elicited significant phonotaxis scores, and Fig. 5 demonstrates that the rise and fall times (which correlate with differences in the phase spectrum) (Schmidt et al., 2008) have no role in call recognition in *N. affinis*. Schmidt et al. (Schmidt et al., 2008) argue that rate recognition based on neural resonances takes place in the frequency domain. Taken together, these arguments strongly suggest that pattern recognition is based on frequency analysis in *N. affinis*. However, future work should test this hypothesis following the approach of von Helversen and von Helversen (von Helversen and von Helversen, 1998) and Schmidt et al. (Schmidt et al., 2008).

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