THE INFLUENCE OF SOUND SPECTRUM ON RECOGNITION OF TEMPORAL PATTERN OF CRICKET (*Teleogryllus oceanicus*) SONG

by

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Abstract

The phonotactic steering behavior of tethered flying crickets (<u>Teleogryllus</u> <u>oceanicus</u>) was examined as a measure of the insect's attraction to temporal patterns of calling song at different frequencies and intensities. A stimulus with a 5 kHz carrier becomes less attractive the further its pulse repetition rate deviates from 16 pulses/s. Increasing the intensity increases selectivity for temporal pattern. At sufficiently high intensity level crickets cease to respond to stimuli with altered temporal patterns.

High frequency neurons were suspected to be behind cessation of responsiveness to stimuli with altered temporal features. This hypothesis predicts that the effect on selectivity of increasing the intensity of the 5 kHz stimulus might be mimicked by adding a high frequency to the stimulus. My results contradict this hypothesis.

The response to a 30 kHz carrier demonstrates a dependency on the duration and pulse repetition rate of the stimulus. Crickets initially respond to 30 kHz with a movement away from the sound source at all repetition rates. However, if given enough time, their tendency for negative phonotactic steering decreases with increased pulse repetition rate. At high repetition rates negative responses are replaced by positive phonotactic steering. These findings are compared to similar observations previously reported in the literature and their behavioral significance is also discussed.

Résumé

Le comportement d'orientation du grillon (Teleogryllus oceanicus) en vol restreint, a eté mesuré comme indication de l'attraction au chant d'appel du male à différentes frequences d'émissions et d'intensités. Un son avec une frequence de 5 kHz devient de moins en moins attirant que le rythme de ses syllabes dévie de 16 tons/sec. Une hausse d'intensité augmente la séléction envers le rythme de répétition. A un niveau d'intensité suffisamment élevé les grillons cessent de répondre aux rythmes altérés.

Des neurones sensibles aux hautes fréquences ont été soupçonnés d'être derrière l'arrêt de répondre aux sons dont les composantes ont étés altérées. Cette hypothèse prédicte que les effets observés avec la hausse d'intensité pourraient être reproduits en ajoutant une haute fréquence au stimulus. Mes résultats contredisent cette hypothèse.

La réponse à une fréquence de 30 kHz se montra dépendante de la durée et du rythme de répétition du son. Au début, les grillons s'éloignent de la source du son. Cependant, si un temps suffisant est accordé, cette tendance se réduit de plus en plus que le rythme de répétition augmente. A des rythmes elevés, les grillons sont plutôt attirés et non plus repulsés. Ces résultats sont comparés à des observations semblables déjà décrites dans la littérature et la signification de ce comportement est discutée.

Acknowledgements

I am indebted to my supervisor, Gerry Pollack, for providing an invaluable guidance throughout my graduate training. I am greatful to him for the amount of learning I acquired and above all for setting an example of excellence and dedication. I would like also to thank those whom I shared lab space with: Silvia Beilin-Weitzner for all the help and support she provided, and Stan Banks for his friendship (and Tennis games). Thanks go to Don Kramer and Mark Chandler for helpful discussions in statistics. Finally, I would also like to thank the members of my committee Ron Chase , Bob Lemon, Valerie Pasztor, and Gerry Pollack.

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INTRODUCTION

The elaborate behavior of insects, and especially their acoustic communication has always drawn a lot of attention from biologists. Most crickets, for example, are primarily active nocturnally (Ulgaraj 1975) and must therefore rely on acoustic information in both their sexual behavior (Alexander 1960; Ulgaraj and Walker 1973; Popov and Shuvalov 1977; Loher and Rence 1978; Pollack and Hoy 1979, 81) and predator (mainly bat) avoidance (Shuvalov and Popov 1971; Moiseff et al. 1978; Nolen and Hoy 1986a), two crucial behaviors for survival.

Acoustic communication in insects has been thoroughly studied in many orders and particularly Orthoptera of which the crickets have gained most attention. For reviews on acoustic behavior in Gryllidae (crickets) and other groups such as Acrididae (grasshoppers and locusts) and Tettigoniidae (bush crickets) see (Alexander 1967; Michelsen and Nocke 1974; Otte 1977; Elsner and Popov 1978; Huber 1978; Gwynne and Morris 1983; Lewis 1983).

Only male crickets sing, a behavior associated with reproduction. By scraping its forewings together, the male produces, with each closing stroke, a relatively pure tone which varies between 3 and 8 kHz depending on the species (Hoy et al. 1982). The opening stroke is silent. The timing of opening and closing produces a rhythmic, species-specific, stereotyped song. Males produce three types of songs: calling song, courtship song, and aggression (rivalry) song. These three songs differ in their carrier frequency and/or temporal pattern. The primary function of calling song is female attraction. Both males and females hear by means of tympanal organs located on their forelegs. The morphology of the tympanal organs is well documented (see Young and Ball 1974; Eibl 1978). Males and females are attracted by the calling song (CS) (Ulgaraj and Walker 1973) and sex identification of the approaching cricket must therefore follow. This is achieved through tactile, mainly antennal, contacts (Loher and Rence 1978). Depending on the sex identified the calling male then produces either an aggressive or a courtship song.

Regen (1913) broadcast a male calling song through a telephone and observed female response. The female approached the telephone proving that attraction can be based on auditory cues alone. Since this pioneering work, phonotaxis in crickets has been extensively investigated both during walking (Weber et al. 1981; Thorson et al. 1982; Doherty 1985; Stabel et al. 1989) and in flight (Ulgaraj and Walker 1973; Moiseff et al. 1978; Pollack and Hoy 1979; 1981; Nolen and Hoy 1986a).

Phonotactic responses of flying crickets exhibit several kinematic and aerodynamic changes including wingbeat frequency, wing depression, elevation, and tilt, antennal swing, pitch and roll,etc. and abdominal flexion which is implicated in turning during flight (see May et al. 1988). In many studies of phonotaxis in tethered flying crickets the size and/or direction of abdominal flexions were monitored as indicators of steering (Moiseff et al. 1978; Pollack and Hoy 1979,81; Pollack et al. 1984).

Many investigations have demonstrated that female crickets are attracted to males (i.e. induced to perform positive phonotaxis) by the stereotypical species-specific calling song (Alexander 1967; Elsner and Popov 1978; Huber 1978; Moiseff et al. 1978; Oldfield 1980; Pollack and Hoy 1979). Furthermore, females preferentially orient toward the song of their own species (Walker 1957; Hill et al. 1972; Zaretsky 1972; Popov and Shuvalov 1977; Polack and Hoy 1979). Ulgaraj and Walker (1973), for example, showed that when the calling songs of Scapteriscus acletus and S. vicinus were simultaneously broadcast from two separate speakers, flying crickets were preferentially attracted to their conspecific calling song. It was also demonstrated that when T. oceanicus and T. commodus calling songs were presented simultaneously, female T. oceanicus exclusively steered toward the T. oceanicus song (Moiseff at al. 1978) and 95% of T. commodus females moved to their conspecific song (Hill 1974).

The natural calling songs of different species of crickets vary in carrier frequency and/or temporal pattern (Alexander 1967). In the Australian field cricket Teleogryllus oceanicus, the main subject of this thesis, the calling song has a carrier frequency of 4.5-5.5 kHz (Leroy 1964; Hill et al. 1972). The structure of this song is one of the most complex, consisting of repeated chirp and trill sections. Each section is characterised by a stereotyped sequence of pulses and interpulse intervals. The chirp consists, on average, of 5 pulses at 66.8 ms intervals (intrachirp). The trill consists of a sequence of 9 doublets of pulses with 122.8 ms interval between doublets (intertrill) and 41 ms between pulses of a pair (intratrill) (Bentley and Hoy 1972).

The effects on attraction of alterations of the spectral or temporal parameters of the calling song vary among different species. In Scapsipedus marginatus the species-specific pulse interval is the only feature necessary for freely walking females to locate the sound source. Severe alterations of chirp interval, fundamental frequency, and number of pulses per chirp do not affect the phonotactic response (Zaretsky 1972). Similarly Thorson et al. (1982) determined that in Gryllus campestris L. females tested on a Kramer treadmill the 30 Hz syllable rate of the 5 kHz carrier is the chief, and in some cases necessary and sufficient, parameter for recognition and tracking. On the other hand, Stout et al. (1983) found that in Acheta domesticus all features of the song (frequency, syllable period, syllable duration, chirp rate, number of syllables, chirp duration, and intensity) are important for recognition, although some are more important than others.

In T. oceanicus, frequency, temporal structure, and intensity all influence phonotactic responses (for effects of different features see Moiseff et al. 1978; Pollack and Hoy 1981; Pollack 1982; Pollack et al. 1984; Doolan and Pollack 1985).

Song discrimination and direction of the response depend on the frequency of the stimulus. The importance of frequency in song discrimination was demonstrated by Hill (1974) and Popov et al. (1975). They showed that crickets are attracted more by the conspecific calling song when it is played with the normal carrier frequency than with other carrier frequencies. Oldfield (1980) also found that female T. oceanicus presented with synthetic calling songs with carriers between 2.5 kHz and 12 kHz showed the highest proportion of turns to 3.5 kHz and 4.5 kHz, less attraction to frequencies between 5.5 and 9 kHz and indifference to 2.5 and 12 kHz carriers. Stout et al. (1983) also studied the effects of frequency on song discrimination by determining thresholds for positive response to a model of the calling song in Acheta domesticus. A lower response threshold indicates a more attractive stimulus. They found that at 4 kHz, threshold is close to 45 dB and is probably a little lower at 5 kHz. When the frequency is changed to 3 kHz, the threshold is increased by 40 dB.

In addition to threshold, the direction of the response also varies with carrier frequency. Moiseff et al. (1978) studied tuning of behavioral thresholds to a model of the calling song in T. oceanicus with carriers between 3 and 100 kHz. They found that in the range 3 to 12 kHz crickets are highly sensitive to a narrow frequency band. The lowest threshold for a positive response is between 4.5 and 5.5 kHz which corresponds to the calling song carrier frequency. A secondary area of high sensitivity is more broadly tuned to frequencies between 20 and 100 kHz and corresponds to negative phonotaxis. Pollack et al. (1984) reported that crickets both in tethered flight and walking on a spherical treadmill, attempted to turn toward a song model when played with a 5 kHz carrier, and away from it when played with a 33 kHz carrier. Nolen and Hoy (1986a) also showed that positive phonotactic steering in T. oceanicus is best tuned to 5 kHz and restricted to frequencies below 9 kHz. On the other hand, ultrasound in the range between 20 and 100 kHz elicits only negative phonotactic steering.

While carrier frequency is important, the temporal pattern is required for, and is probably the basis of, species-specific recognition (Walker 1957; Zaretsky 1972; Popov and Shuvalov 1977; Pollack and Hoy 1979, 1981). Pollack and Hoy (1979) demonstrated that crickets can recognize conspecific calling song from its temporal pattern. Flying T. oceanicus females were allowed to choose between two synthetic songs: their species calling song and that of T. commodus. Although the natural songs of these two species differ both in carrier frequency and temporal pattern the synthetic songs were presented at identical carrier frequencies. All T. oceanicus females preferred their conspecific song. Weber et al. (1981) showed that Gryllus campestris females, both sequentially and in two choice situations, could discriminate between the species calling song and wrong songs which have no syllable structure (burst), or have a wrong syllable repetition rate.

Pollack and Hoy (1981) demonstrated that T. oceanicus females respond to many other patterns besides the natural song. Particularly, the chirp section of the song was found to be the most attractive. A 'continuous chirp' song in which the trill section was eliminated and the chirp section is extended indefinitely was favoured by female crickets over the natural calling song. The chirp component was also found more attractive than the trill component. Also, the natural calling song was preferred to a song model in which the chirp section was eliminated and the trill repeated. Species specificity was also emphasized by the fact that either of the two components (chirp or trill) was favoured over a T. commodus song.

Weber et al. (1981) further demonstrated that in G. campestris a modified temporal pattern was favoured over the natural calling song. This species has a calling song with a 5 kHz carrier consisting of 'chirps' each having 4 syllables repeated at 30 ms intervals (Weber et al. 1981). They showed that attractiveness is increased with a simulated song having identical carrier and syllable intervals to the natural call but with increased number of syllables per chirp in the range 4-8. In addition to frequency and temporal pattern, phonotactic steering is also sensitive to sound intensity. The effects of intensity vary depending on the spectral and temporal pattern of the stimulus. With the model calling song above threshold levels, the direction of phonotactic steering is not affected by intensity. Stout et al. (1983) showed that with Acheta domesticus (walking in an orientation arena) model calling songs with carriers between 4 and 5 kHz elicited positive responses over the full intensity range studied of 45 to 85 dB, with 45 dB being very close to threshold. Similarly, tracking of the calling song in G. campestris does not depend on intensity in the region 60 to 90 dB (Weber et al. 1981). Doolan and Pollack (1985) also showed that in T. oceanicus increasing the intensity of a song with unaltered temporal pattern does not reduce its attraction. With the model calling song the only influence of intensity is that on the size of abdominal flexions associated with flight steering. Pollack and Plourde (1982) found that in T. oceanicus abdominal flexions increase in size with intensity up to 70 dB, reach a plateau from 70 to 90 dB and slightly decrease at higher intensities.

The impact of the stimulus intensity becomes critical as the temporal pattern deviates from that of the normal calling song. Doolan and Pollack (1985) showed that when intensity is sufficiently increased, female T.

oceanicus cease to respond to songs in which the pulse rate and/or pulse duration are altered. Selectivity of crickets for temporal parameters is therefore intensity-dependent. In some cases at high intensity, crickets were repelled rather than attracted by the stimulus. The aversive nature of such high intensity low frequency sounds was confirmed by Nolen and Hoy (1986a). They studied phonotactic steering movements of T. oceanicus, T. commodus, and G. bimaculatus in response to single tone pulses at frequencies (3 to 6 kHz) near their respective calling songs. Differences among the three species were found both in thresholds and the number of crickets responding. However, and more importantly, sufficiently high intensities caused most (80-100%) of the crickets that responded to steer away from the sound source.

In addition to its role in mediating sexual behaviors, acoustic communication in crickets is involved in bat avoidance. Insectivorous bats locate their prey by producing high frequency (10 to 100 kHz) sound pulses which are reflected by the body and wings of flying insects (Griffin et al. 1960). Bats monitor these returning echoes by which they obtain information about the location, speed, and type of the flying insect (Schnitzler et al. 1983; Suga 1984). Roeder (1967b) showed that moths sense the ultrasonic biosonar sounds of foraging bats by means of their simple ears. Subsequently, they perform negative phonotaxis in response to distant bats (Roeder 1967b) and evasive maneuveours (multidirectional unpredictable flight) in response to loud ultrasounds of a closely approaching bat (Miller 1983). Green lacewings have also been observed to perform ultrasound-induced evasive flight maneuvers (Miller and Olesen 1979). The response of flying crickets to low and high intensity ultrasounds is very similar to that of moths, and in addition their frequency sensitivity is remarkably similar to that described by Roeder (1967b) in moths (Nolen and Hoy 1986a). Negative phonotaxis in crickets in response to high frequencies was therefore interpreted as a bat avoidance behavior (Popov and Shuvalov 1977; Moiseff et al. 1978; Nolen and Hoy 1984, 1986a, 1986b).

Negative phonotaxis is a short latency behavior, is readily elicited by frequencies between 20 and 100 kHz, and does not require a particular temporal pattern (Nolen and Hoy 1986a). Pollack et al. (1984) demonstrated that in _T. oceanicus_ a sound with a 33 kHz carrier would elicit negative phonotactic responses whether played with a non-calling song pattern (2 pulses/s) or as a calling song model.

In this thesis, I further investigate the findings of Doolan and Pollack (1985) concerning the effect of stimulus intensity on temporal pattern selectivity. Doolan and Pollack (1985) suggested that the increase in temporal pattern selectivity with increased intensity might be due to stimulation of neurons tuned to high frequencies. I explain and test this hypothesis.

Increasing stimulus intensity increases selectivity for temporal parameters. A similar effect of intensity in reducing the range of effective temporal parameters was previously described for certain auditory neurons in the ventral nerve cord in tettigoniids (Rheinlaender 1975) and for neurons in the auditory cortex in the bat (Suga 1965, 1977). For these neurons the effective range of frequencies narrows as stimulus intensity increases beyond some value. Inhibitory interactions were reported to be behind the nature of such response (Suga 1965; Rheinlaender 1975).

In crickets, negative phonotaxis is mediated by neurons tuned to high frequencies (Moiseff and Hoy 1983; Nolen and Hoy 1984), which can also be excited by intense 5 kHz sound (Nolen and Hoy 1987). Furthermore negative responses to high intensity low frequency (3-6 kHz) single tone pulses were similar in form and latency to negative phonotactic steering elicited by low intensity ultrasound (Nolen and Hoy 1986a).

Doolan and Pollack (1985) suggested that the increase in selectivity for temporal pattern with increasing intensity might be due to stimulation, at these high intensities, of the high frequency-tuned neural pathway responsible for negative phonotaxis. Activation of this pathway might in turn suppress positive phonotaxis at certain temporal patterns reflecting therefore an increase in selectivity. Based on this suggestion I test whether the effect on selectivity of increasing the intensity of the 5 kHz stimulus might be mimicked by adding ultrasound to the stimulus.

MATERIALS AND METHODS

Adult virgin females of Teleogryllus oceanicus were used two to four weeks after their imaginal molt. Crickets were raised in a 14:10 light/dark cycle at temperatures between 24 and 28 °C, with a constant supply of water and Purina Cat Chow.

Sound stimuli. The 'continuous chirp' stimulus described by Pollack and Hoy (1979) (see Introduction) consisted of 30 msec sound pulses, with rise and fall times of 5 msec, repeated at a rate of 15/sec (based on the 67 ms average intrachirp interval from Bentley and Hoy 1972). I used similarly shaped pulses, at repetition rates of 8-32 per second, including a 16 pps stimulus similar to the 'continuous chirp' and identical to the control call used by Doolan and Pollack (1985). The stimulus was generated electronically using a Grason Staddler Co. Timer Model 1216A. The 5 kHz carrier was produced by a Tektronix FG 501 sine wave generator and the 30 kHz carrier was generated by a Tektronix AF 501 Bandpass filter used in its sine wave generation mode. Frequencies were calibrated with a digital frequency counter (Hewlett Packard 3734A). Each signal was then attenuated independently by a Hewlett Packard (350 D) attenuator. The two signals were electronically added and their sum was gated by an electronic switch (Grason Staddler Co. Model 1287B) and then attenuated by a Hewlett Packard (350B) attenuator. The resulting signal was finally amplified by an Amcron

D150A amplifier and broadcast through two small loudspeakers. The speakers were in the same horizontal plane as the cricket, at a distance of 50 cm to the left and right of the midline of the cricket, orthogonal to the cricket's longitudinal axis. Stimuli were monitored on a Tektronix oscilloscope. Sound calibration was done with a Bruel and Kjaer measuring amplifier (Type 2610) using a Bruel and Kjaer (Type 4135) microphone. The microphone was calibrated with a Bruel and Kjaer calibrator (Type 4230). I used intensities between 60 and 90 dB RMS at the peak of the sound pulse envelope.

Behavioral measurement. Both hind legs were removed and the hindwings were shortened to prevent interference with the measuring device. The cricket was attached with wax at the pronotum to a wooden stick and placed ventral side up in a windstream to induce flight. Tethered flying crickets or crickets assuming a flight posture but without beating their wings (prothoracic and mesothoracic legs held close to the body, antennae straight, abdomen vibrating) were used, since in previous work no significant difference was found in their phonotactic behaviour (Doolan and Pollack 1985).

The response of a cricket to the sound stimulus was determined as a change in its abdominal position. May et al. (1989) showed that abdominal flexion in response to sound is associated with the attempt to turn.

Abdominal flexion was detected using an angle detector system based on that described by Koch (1980). In this method angles are detected as changes in the current induced in a coil by an alternating magnetic field. The cricket was placed in a high frequency magnetic field with a miniature sensing coil waxed to its dorsal abdominal surface parallel to its transverse axis. A sine wave generator (10 V peak-to-peak, 28 kHz) produced the magnetic field between two windings (diameter 15 cm, placed at 10 cm). Abdominal movements caused changes in the angle between the sensing coil and the two windings. These changes evoked variations in the induced AC current which were converted, by a custom-built circuit, to a DC signal (for more details see Doolan and Pollack 1985). The sign and magnitude of this signal were related to the direction and amplitude of the abdominal flexions. Abdominal movements were traced with a chart recorder (Harvard Apparatus Model 350) and a chart mover (Harvard Apparatus Model 480).

Behavioral tests. The order in which repetition rates were varied was random except for the control stimulus which was presented at the beginning and end of each series, as will be described in the results section. When more than one intensity level was tested they were presented in an ascending order.

The stimulus was switched between the two loudspeakers at 10 s intervals, 11 times. Response scoring was identical to that described by Doolan and Pollack (1985). For each switch, the position of the cricket's abdomen when the switch occured was compared with that after a 10 sec interval (i.e. just prior to the next switch). If after such an interval the abdomen was flexed further towards the sound source, the response to that switch was assigned a value of +1. If the abdomen flexed away from the sound source, a value of -1 was assigned. A value zero was assigned if the abdomen's position was unchanged. The resolution of the angle measuring system was approximately 1 degree. To allow the cricket to adjust to the new stimulus the response during the first 10 s was ignored. The individual scores to the sequence of the other 10 switches were averaged resulting in a Mean Response Score (M.R.S.). The M.R.S. could therefore vary from +1 for a perfect positive response to -1 for a perfect negative response.

All experiments were performed in an anechoic chamber, in the dark, at a temperature between 24 and 26 °C.

Statistical analyses. Statistical tests were taken from Sokal and Rohlf (1969) and Winer (1971). Paired t-tests were used to compare means when the same group of crickets was concerned, and independent t-tests when two different groups were considered. Before statistical tests were performed all data were arcsine transformed in order to homogenize variances.

RESULTS

Effects of increasing stimulus intensity on selectivity for temporal pattern

Doolan and Pollack (1985) found that alterations of the two major temporal parameters (pulse rate and pulse duration) of a 5 kHz 'continuous chirp' stimulus do not affect behavioral thresholds. However, they reduce the probability of positive responses occuring at suprathreshold intensity levels. The results in this section confirm and extend this finding.

I studied temporal pattern tuning to 5 kHz with three different groups of crickets: the first group was tested with 60 dB at 10 to 28 pps, the second with 70 and 90 dB at 8-16 pps, and the third with 70 and 90 dB at 18-32 pps. The stimulus is highly attractive at 16 pps (see materials and methods) and was therefore included, as a control, at the beginning and end of each series of tests to check if phonotaxis is maintained throughout. For the last group, which was not tesed with 16 pps, 18 pps was used instead as a control; at 18 pps the stimulus proved very attractive at 60 dB eliciting a M.R.S. of .94 ± .14 (± s.d.) which is not significantly different from that at 16 pps ($P>0.05$, paired t-test).

Comparison of the mean response scores to the control stimuli showed no significant difference between the beginning and the end of the experiments at any intensity (P>0.05, Table 1, paired t-tests). The behavior of the crickets was therefore stable over the duration of the experiment.

The responses of the crickets to different repetition rates are summarized in figure 1. At each of the three intensity levels the M.R.S. were found to depend on the repetition rate of the 5 kHz stimulus (see fig.1). All three tuning curves show a peak for positive phonotactic steering at 16-18 pps. At 70 and 90 dB, there is some indication of a secondary peak at about 24 pps.

At the two most attractive repetition rates (16 and 18 pps) increasing the intensity of the stimulus has no detectable effect on the M.R.S. (P>0.05, t-tests). In contrast, at other repetition rates M.R.S. decreased with increases in intensity. At 60 dB positive phonotactic steering (i.e. M.R.S. different from 0) was evoked at all the repetition rates studied in the range between 10 and 28 pps. As intensity was increased, some previously effective temporal patterns became ineffective. At 90 dB the range of repetition rates evoking positive responses narrowed to between 14 and 18 pps. Positive steering was also maintained at the secondary peak i.e. at 22 and 24 pps. This confirms the conclusion of Doolan and Pollack (1985) that selectivity for temporal patterns increases with stimulus intensity.

Table 1. Comparison of the response of crickets to control stimuli at the beginning and at the end of each series of tests.

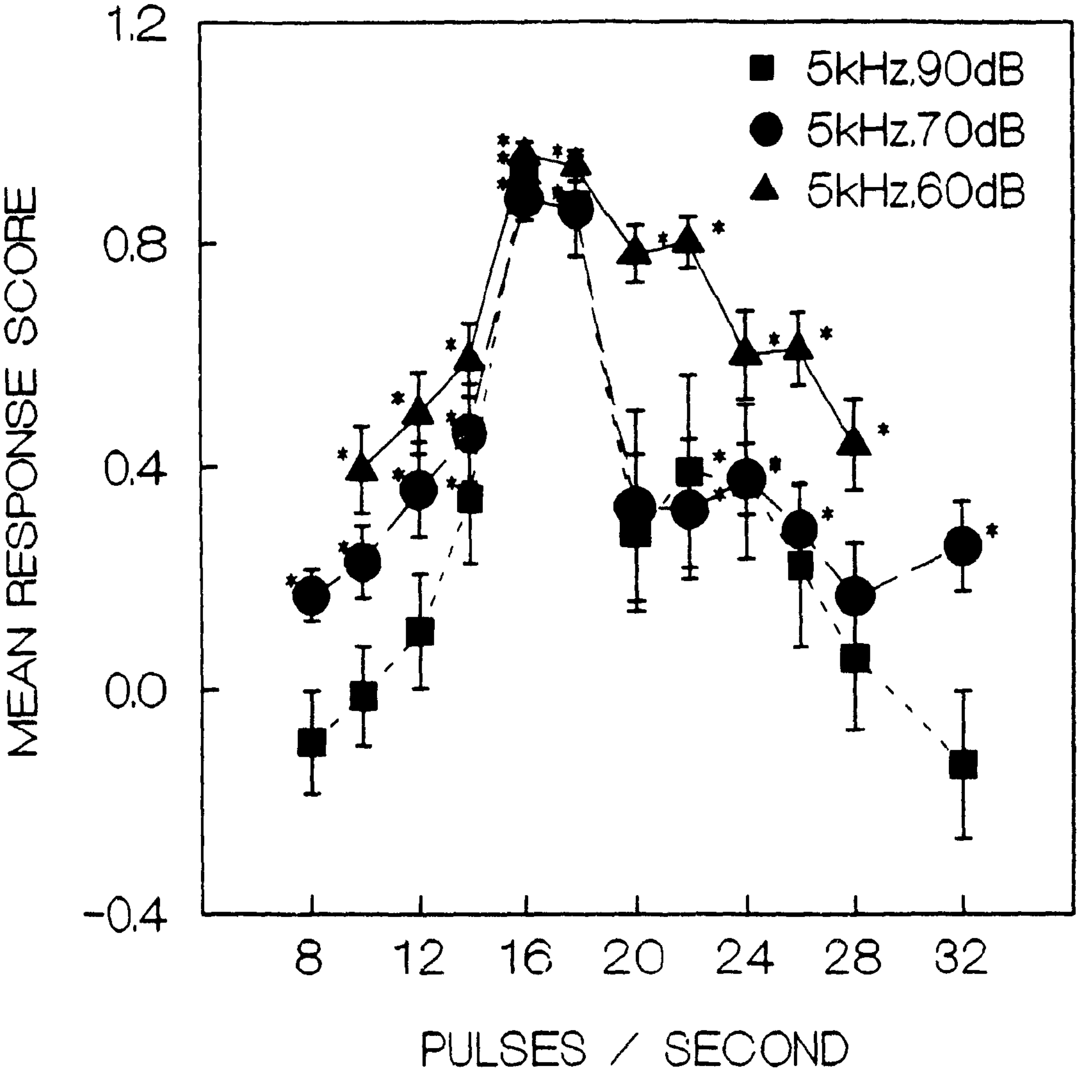
Intensity		M.R.S. beginning	± s.dev.	M.R.S. end	± s.dev.	P
60 dB	*	0.96	± 0.11	0.96	± 0.07	.72
70 dB	**	0.88	± 0.17	0.83	± 0.20	.17
	***	0.86	± 0.29	0.83	± 0.26	.28
90 dB	**	0.93	± 0.10	0.93	± 0.13	.83
	***	0.87	± 0.31	0.88	± 0.27	.92

* For a group of 23 crickets tested with 16 pps

** For a different group of 18 crickets tested with 16 pps

*** For a different group of 11 crickets tested with 18 pps

Figure 1. Increeasing the intensity of 5 kHz sharpens tuning. Phonotactic steering movements are expressed as response scores (mean ± standard error) at pulse repetition rate between 8 and 32 pps. The tuning curves become sharper as intensity increases reflecting an increase in temporal pattern selectivity. Asterisks indicate scores which are significantly different from 0. Three different samples were used: 23 crickets at 60 dB, 18 crickets at 70 and 90 dB for repetition rates 8-16 pps, and 12 crickets for 18-32 pps. Some of the 23 crickets tested at 60 dB were not tested at all the repetition rates. The sample size associated with each repetion rate is: 18 at 24 pps, 19 at 28 pps, 20 at 10 pps, 21 at 22 and 26 pps, 22 at 12, 14 and 20 pps, 23 at 16 and 18 pps.


■ 5kHz,90dB
● 5kHz,70dB
▲ 5kHz,60dB
MEAN RESPONSE SCORE
1.2
0.8
0.4
0.0
-0.4
8
12
16
20
24
28
32
PULSES / SECOND

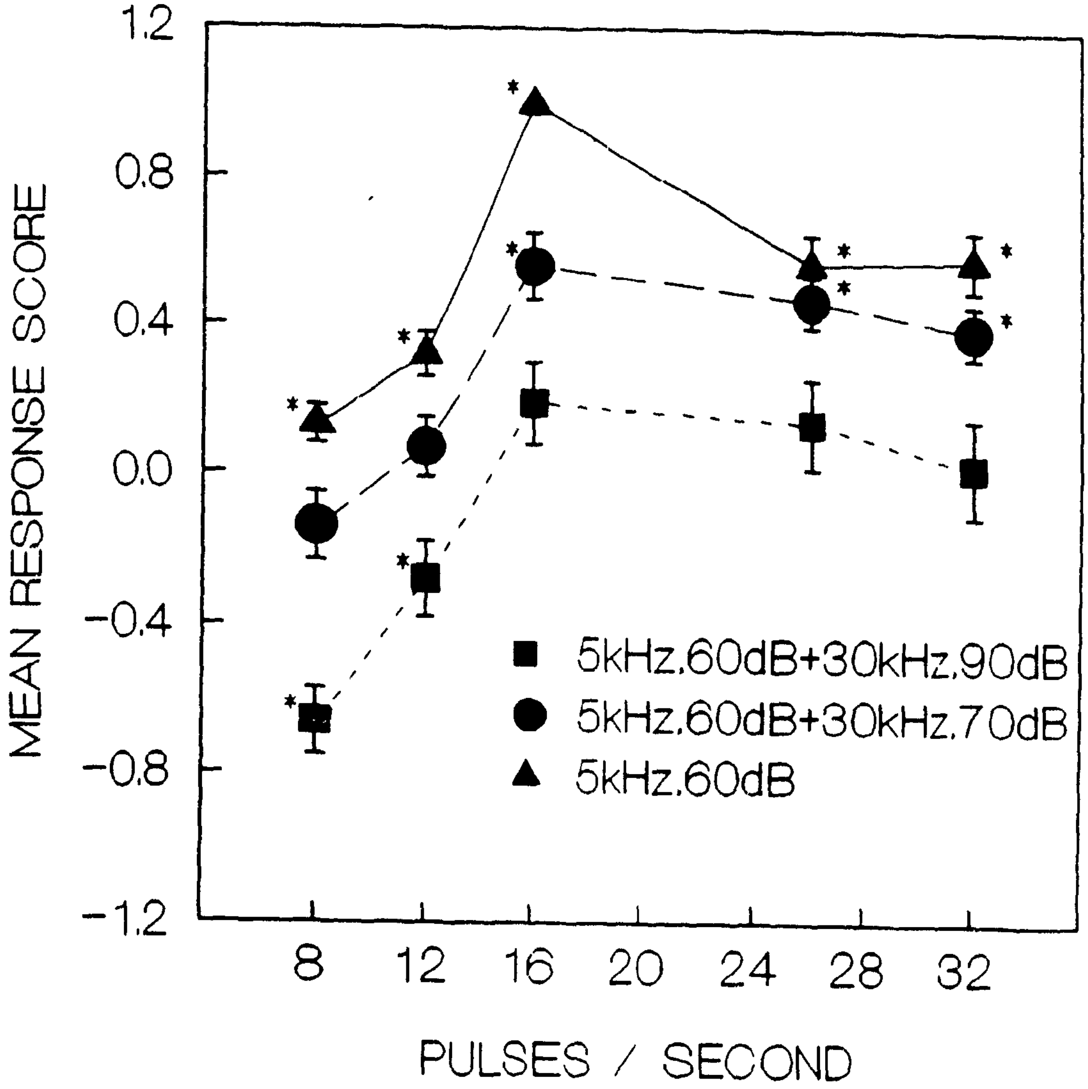
Phonotactic steering in response to simultaneous presentation of 5 kHz and 30 kHz

To test whether the increase in temporal pattern selectivity is due to activation of high frequency-tuned neurons I tested the selectivity with ultrasound added. The 5 kHz component of the stimulus was held at 60 dB and 30 kHz, when added, was presented at 70 dB and 90 dB. The responses to 5 kHz and those to the mixed stimuli (Fig. 2) were all dependent on the repetition rate of the stimulus (one-way Anova with repeated measures, $F_{4,116}=55.63$, $F_{4,116}=17.65$, and $F_{4,116}=20.03$ for 5/60, 5/60+30/70, and 5/60+30/90 respectively, $P<.001$).

Adding 30 kHz at 70 dB to the 5 kHz stimulus resulted in significantly lower M.R.S. at all repetition rates ($P<0.01$, paired t-test) except at 26 pps ($P>0.05$, paired t-test). Increasing the intensity of 30 kHz to 90 dB resulted in a further significant decrease in M.R.S. at all repetition rates ($P<0.01$, paired t-test).

Inspection of the tuning curves in Figure 2 suggests that adding ultrasound to the stimulus does not markedly affect selectivity. Rather the curves are shifted downward approximately uniformly as ultrasound intensity increases and, if anything, are less sharp than when 5 kHz is presented alone. To compare quantitatively the effect of adding ultrasound with that of increasing the intensity of a 5 kHz stimulus I compared the differences

Figure 2. Adding 30 kHz to 5 kHz depresses phonotactic scores. Response scores (mean ± standard error) are shown for 5 kHz and two mixed stimuli in which 30 kHz is added at 70 and 90 dB respectively. Asterisks indicate scores which are significantly different from 0. Sample size=30.


1.2
0.8
0.4
0.0
−0.4
−0.8
−1.2
MEAN RESPONSE SCORE
8
12
16
20
24
28
32
PULSES / SECOND
■ 5kHz,60dB+30kHz,90dB
● 5kHz,60dB+30kHz,70dB
▲ 5kHz,60dB
*

in M.R.S. between 16 pps and both 12 and 26 pps. Larger differences reflect greater selectivity for temporal pattern. As the intensity of the 5 kHz stimulus increases so do the differences in M.R.S. (Fig. 3A). This increase is significant between 60 and 90 dB (t-test for 12 vs 16 pps, P<.01; t-test for 16 vs 26 pps, P<.02). This confirms the increase in temporal pattern selectivity with increased intensity.

In contrast, when 30 kHz is added to the stimulus, differences in M.R.S. (Fig. 3B) reflect a trend toward a decrease in selectivity rather than an increase. The difference in scores was significant between 16 and 26 pps (t-test for 5/60 vs 5/60+30/70, P<.01; t-test for 5/60 vs 5/60+30/90, P<.01). Selectivity does not seem to differ between the two mixed stimuli. Thus the addition of 30 kHz decreases temporal pattern selectivity, while increasing the intensity of 5 kHz increases selectivity.

Another difference between the two effects is the appearance of a transient negative response when ultrasound is added. This negative component of the response is quickly replaced by positive phonotaxis. It was also dependent on the repetition rate since it only occurred at 16 pps. An example is shown in figure 4. Frequency of occurence of the transient negative response at 16 pps was about 1 in 7 (it happened 43 times with the 30 crickets tested i.e. out of 300 stimulus presentations).

Figure 3. Comparison of the effect of increasing the 5 kHz stimulus intensity with that of adding 30 kHz. A) Effects of increasing the intensity of 5 kHz on the differences in M.R.S. B) Effects of adding 30 kHz on the differnces in M.R.S.

Differences in M.R.S. increase with increased intensity of 5 kHz but decrease with the addition of 30 kHz. Scores compared here are the same ones reported in figures 1 and 2. Bars indicate standard errors.

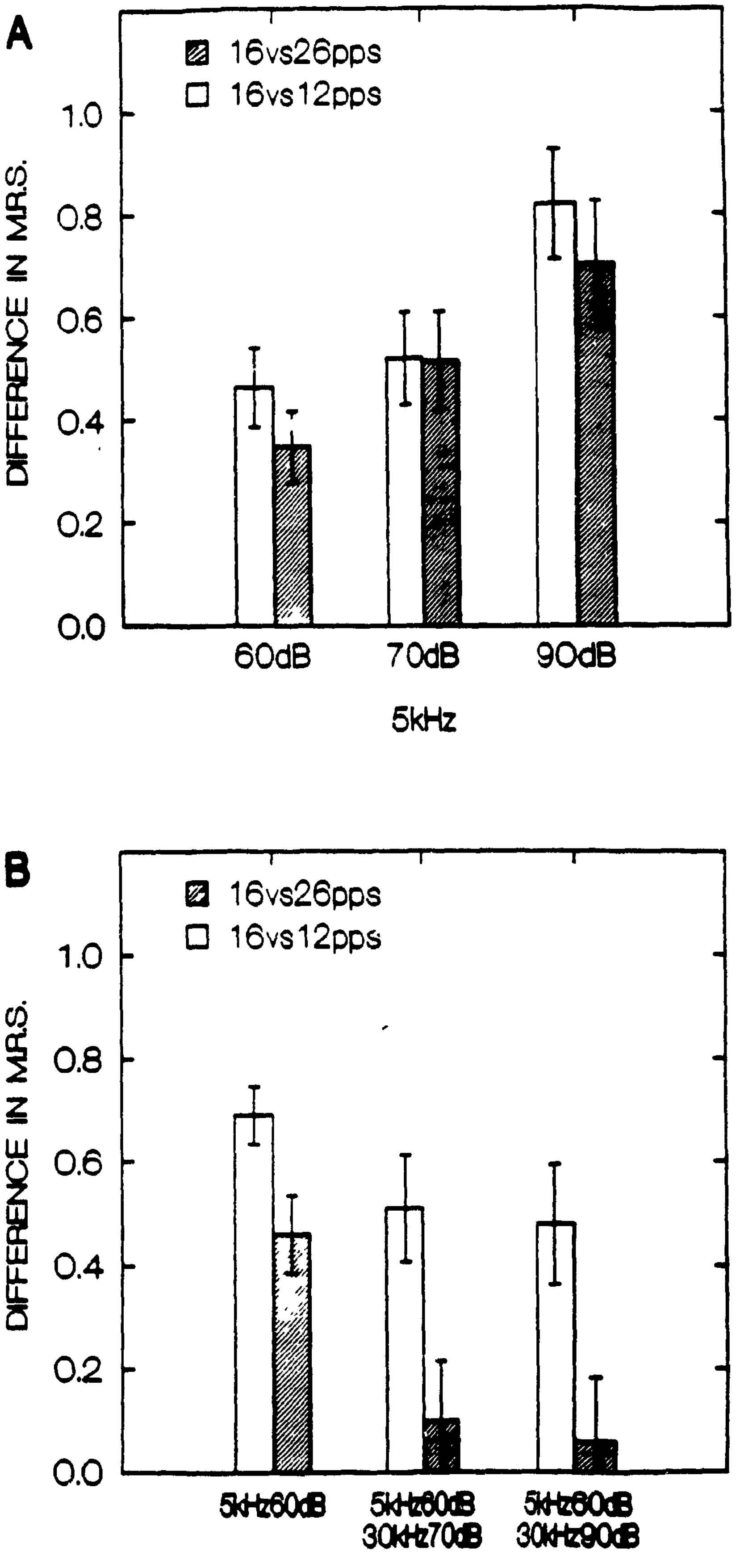
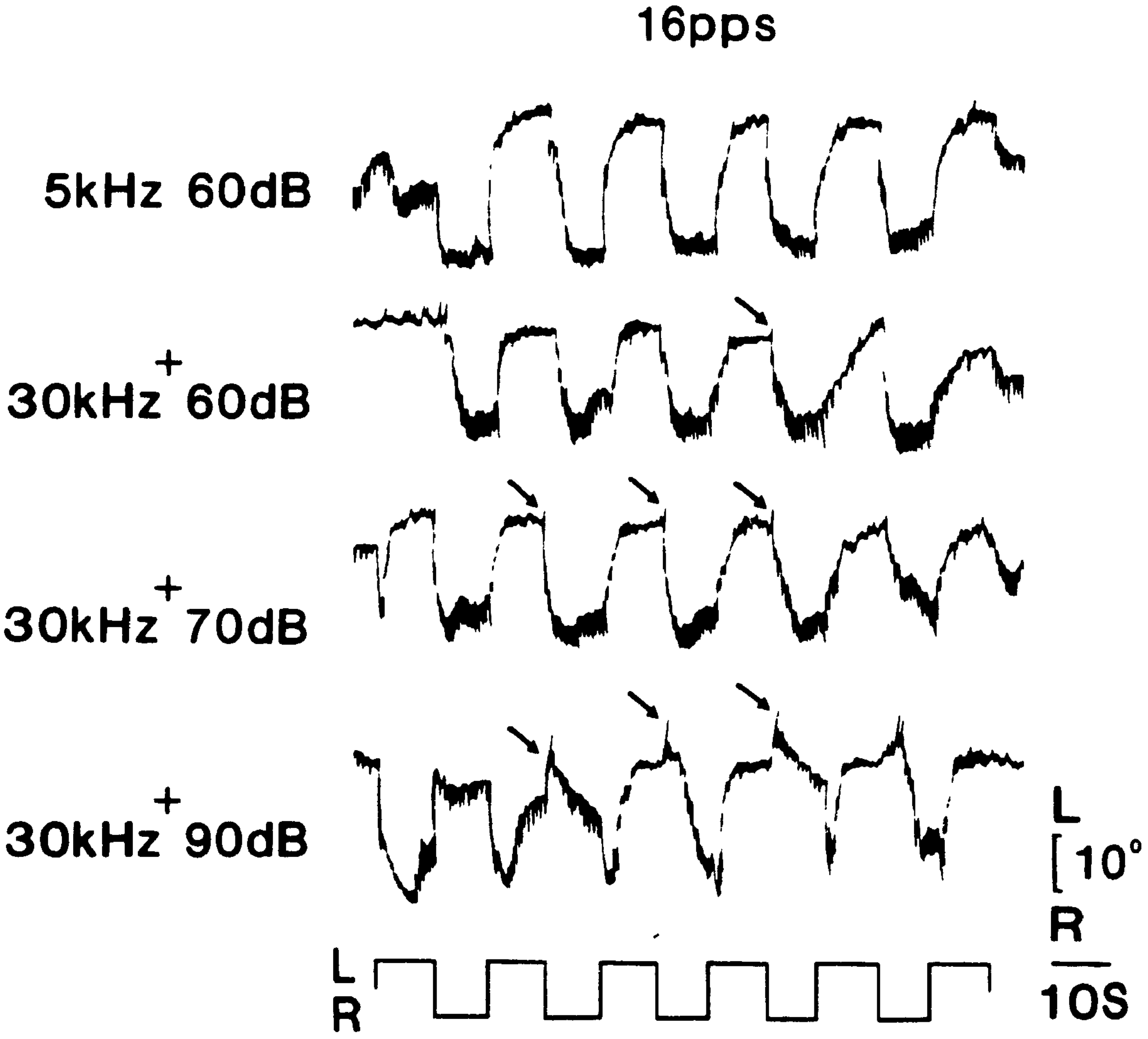

A
16vs26pps
16vs12pps
DIFFERENCE IN M.R.S.
1.0
0.8
0.6
0.4
0.2
0.0
60dB
70dB
90dB
5kHz
B
16vs26pps
16vs12pps
DIFFERENCE IN M.R.S.
1.0
0.8
0.6
0.4
0.2
0.0
5kHz60dB
5kHz60dB
30kHz70dB
5kHz60dB
30kHz90dB

Figure 4. Transient negative responses (indicated by arrows) appear with the addition of 30 kHz and are quickly replaced by positive phonotactic steering. The negative component of the response becomes more pronounced as intensity of 30 kHz is increased. (Not all the transient negative responses are indicated). Upper traces indicate abdominal movements, lower trace indicates the direction (Left or Right) of the sound.


16pps
5kHz 60dB
+
30kHz 60dB
+
30kHz 70dB
+
30kHz 90dB
L
10°
R
10S
L
R

For a transient response to occur a positive response is necessary to replace the initial negative movement. Therefore it is important to check if the total absence of transient responses at rates other than 16 pps is due to such stimuli being ineffective in inducing a positive response. To verify this possibility I quantified the occurence of each type of response (positive, negative, or zero) for the individual 10 s switches for the 30 crickets (table 2). Although positive responses are more frequent at 16 pps, they also occur at all other repetition rates. This shows that the absence of transient response is not due to a lack in motivation for positive responses.

However, further analyses, measuring and comparing the relative amplitude of positive and negative tendencies at each repetition rate, would have provided a clearer explanation for the absense of a transient response. In fact, although positive responses occur at repetition rates other than 16 pps, they are not capable of overriding the initial negative tendency. This is supported by some cases (at 12, 26 and 32 pps) which showed only a reduction in the amplitude of the negative response rather than completely taking over. Such competition between the two tendencies is also reflected at 16 pps by more frequent occurence (67%) of transient responses when, in the mixed stimulus, 30 kHz was at 70 dB compared to 33 % with 30 kHz at 90 dB. This can be explained by the persistence of negative phonotactic steering over the duration of the 10 s switch interval

Table 2. Frequency of each type of response at different repetition rates during the 300 trials . For the 30 crickets tested, the values for each of the 10 s switches are grouped under positive, negative, or zero. For each repetition rate the number of each type of response out of the 300 trials is reported. (These are the same values which were previously averaged resulting in the M.R.S. in figure 2).

STIMULUS (kHz/dB)	REPETION RATE (pps)														
	8			12			16			26			32		
	RESPONSE														
	-	+	0	-	+	0	-	+	0	-	+	0	-	+	0
5/60 + 30/70	124	86	90	73	103	124	47	209	44	53	164	83	55	162	83
5/60 + 30/90	226	30	44	152	69	79	80	155	55	116	137	57	119	140	41

when 30 kHz is played at much higher intensity than 5 kHz.

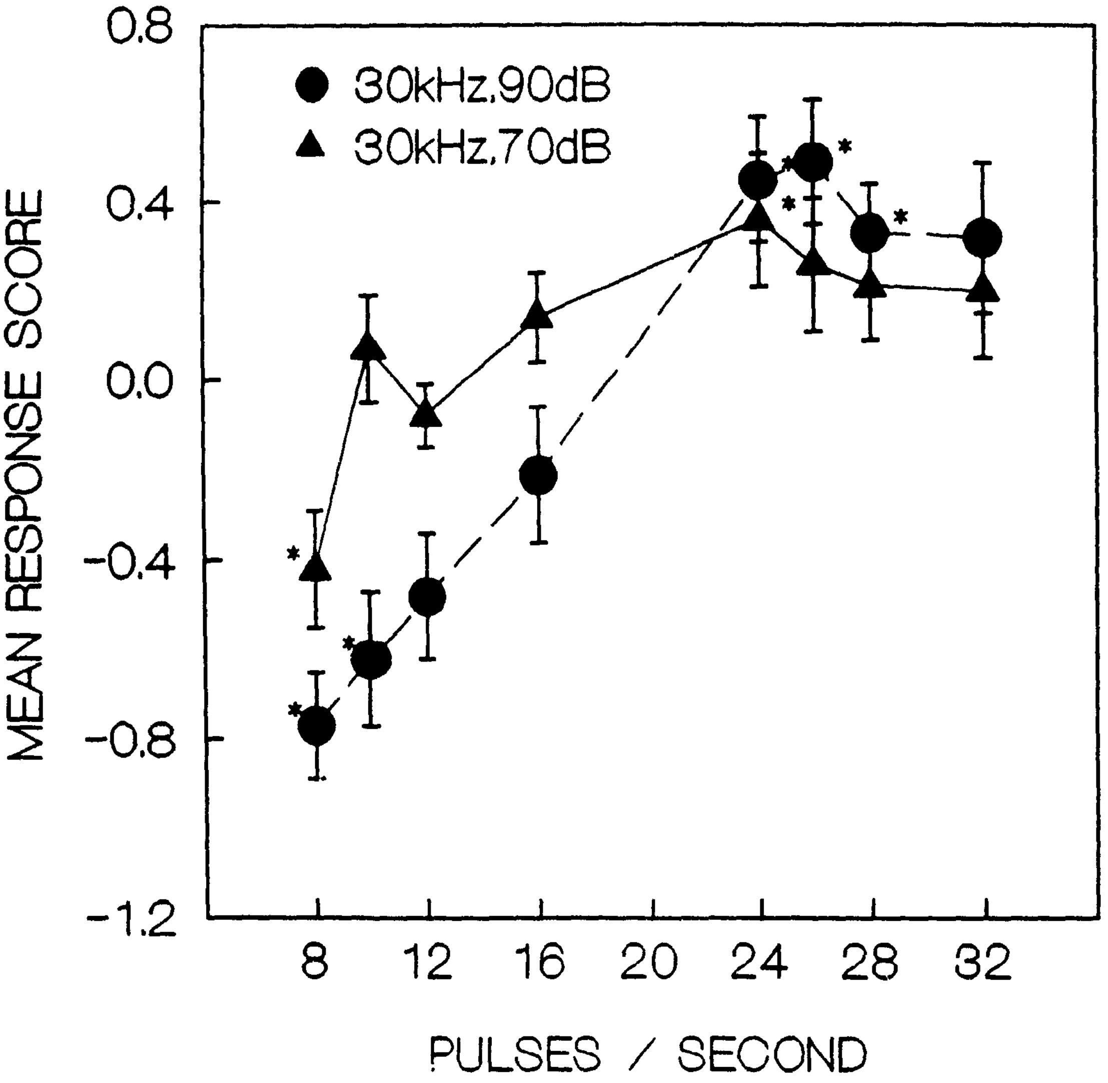
Response to pure ultrasound: dependence on the repetition rate

Positive and negative phonotaxis are considered two separate behaviors (Pollack et al. 1983; Nolen and Hoy 1986a). The first is tuned to the carrier frequency of the calling song and the second more broadly to frequencies between 20 and 100 kHz (Nolen and Hoy 1986a).

In the previous section I studied the response to 5 kHz alone, and to simultaneous presentation of 5 kHz and 30 kHz. The question I pose now is whether the response to the mixed stimuli is merely the sum of the effects of the individual stimuli. I therefore studied the response to 30 kHz at 70 and 90 dB .

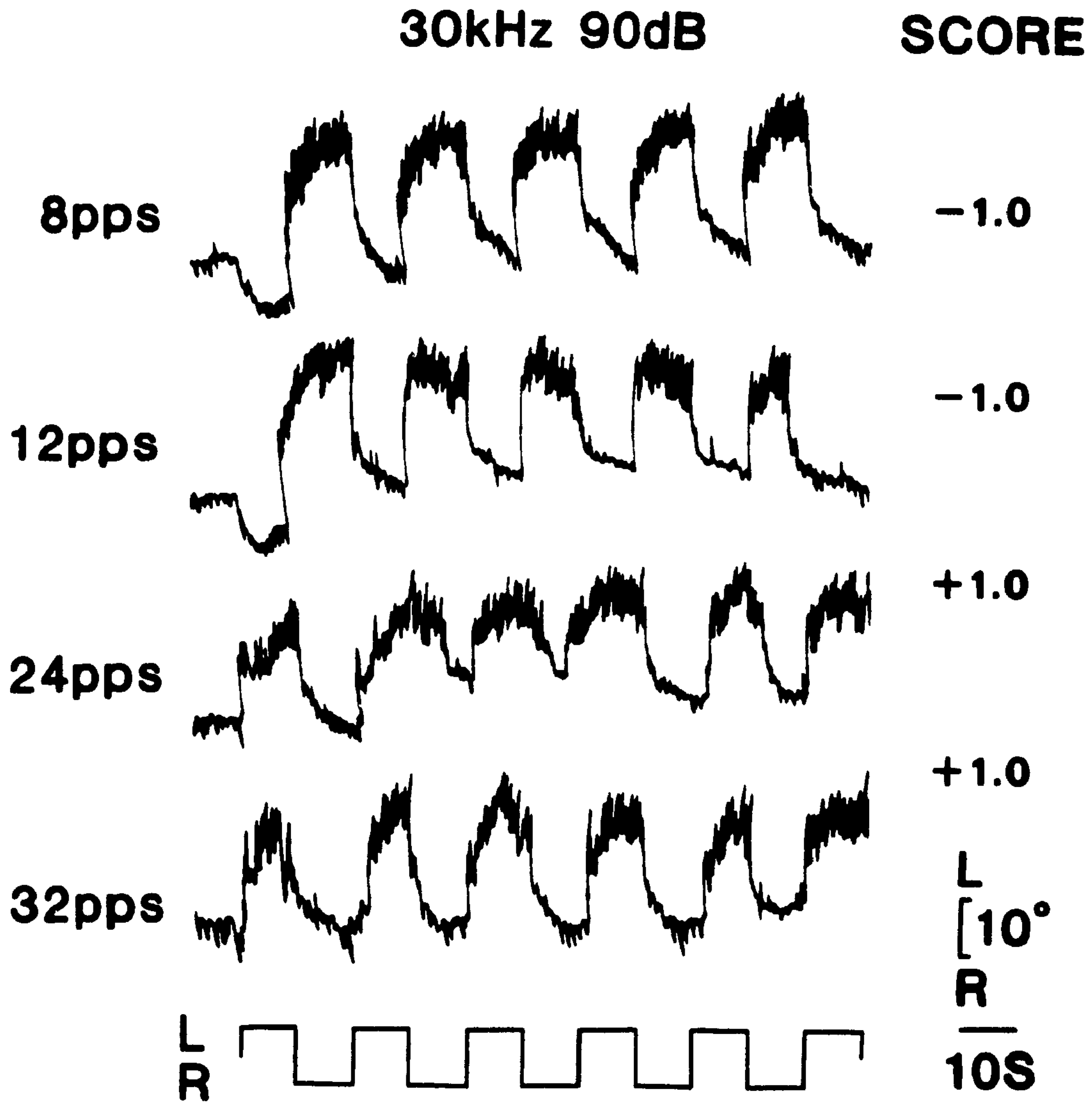
I found that phonotactic steering in response to 30 kHz (Fig. 5) depends on the repetition rate of the sound. Crickets respond with positive phonotactic steering at high repetition rates and negative steering at low repetition rates. This effect increased with intensity as indicated by the curves at 70 and 90 dB. Figure 6 shows an example in which an individual cricket responded negatively to 30 kHz at low repetition rates but positively at high repetition rates.

Figure 5. The response to 30 kHz depends on the repetition rate of the stimulus. Phonotactic steering movements are expressed as response scores (mean±standard error) indicating negative phonotaxis at low repetition rates and positive phonotaxis at high repetition rates. Asterisks indicate M.R.S. which differ significantly from 0. One group of 11 crickets was used. The sample size associated with each repetition rate is: 7 at 26 pps, 9 at 10 and 28 pps, 10 at 8 and 24 pps, 11 at 12, 16 and 32 pps.



0.8
0.4
0.0
-0.4
-0.8
-1.2
MEAN RESPONSE SCORE
● 30kHz,90dB
▲ 30kHz,70dB
*
8
12
16
20
24
28
32
PULSES / SECOND

Figure 6. An example in which an individual cricket responded to 30 kHz, 90 dB with negative phonotaxis (Score=-1.0) at 8 and 12 pps and with positive phonotaxis (Score=1.0) at 24 and 32 pps. Positive phonotactic steering movement is sometimes preceded by a negative component which is more clearly illustrated in figure 7.


30kHz 90dB
SCORE
8pps
−1.0
12pps
−1.0
24pps
+1.0
32pps
+1.0
L
10°
R
L
R
10S

Temporal pattern tuning for 30 kHz stimuli cannot explain the response to mixed stimuli. Particularly at high repetition rates, 30 kHz alone elicits positive responses whereas when added to 5 kHz it inhibits positive phonotactic steering. The response to a mixed stimulus therefore cannot be a linear addition of the responses to its components.

The initial response to 30 kHz

My results describing the response to 30 kHz are not consistent with previous studies. The dependency on temporal pattern and positive phonotactic steering at high pulse repetition rates were not expected in view of the current literature. Previous studies of phonotaxis in response to ultrasound (Moiseff et al. 1978; Nolen and Hoy 1984, 1986a) generally imply that high frequencies (>20 kHz) readily elicit negative phonotaxis.

However, no one previously used an attractive stimulus at a higher rate than that of the natural song. Most importantly, and contrary also to others who reported the direction of the movement immediately after switching the sound, I monitored changes in the position of the cricket's abdomen after a 10 sec duration of the stimulus. The cricket's response after a 10 sec interval was not necessarily the same as that directly after switching the sound. In some cases the initial response was a quick movement away from the sound source which was replaced by positive phonotaxis after a short delay (less than 2 sec). This transient negative response happened most frequently at 90 dB with 16, 24, 26 and 32 pps. An example is shown in figure 7. It seemed possible therefore that the dependence of the response to 30 kHz on the temporal pattern, and in particular positive phonotactic steering at high repetition rates, might be a consequence of the 10 sec duration of the stimulus.

The scores were therefore reevaluated considering only the initial response, i.e. comparing the position of the cricket's abdomen when the switch occured with the first response that follows within 2 sec. In the example shown in figure 7 for instance, the score of 1.0 would be changed to -0.9.

The tuning curves (Fig. 8) show that the initial response to 30 kHz is negative phonotactic steering at all repetition rates studied, at 70 and 90 dB. The initial response is not significantly dependent on the repetition rate of the sound at 70 dB (one-way Anova, $F_{7,70}=1.12$, $P>.3$). At 90 dB however, the response is dependent on the repetition rate (one-way Anova, $F_{7,69}=2.60$, $P<.02$). This shows that even when only the initial response to 30 kHz is considered the temporal patern can still affect the response. Nevertheless, even in this case only negative phonotaxis was elicited at all repetition rates.

Figure 7. A transient negative response precedes positive steering movements in response to 30 kHz, 90 dB. This transient response is an initial movement away from the sound (as indicated by the arrows) and is indicative of negative phonotactic steering in response to 30 kHz. On the other hand, positive phonotaxis built up over the 10 sec duration of the stimulus resulting in a positive response score of 1.0.

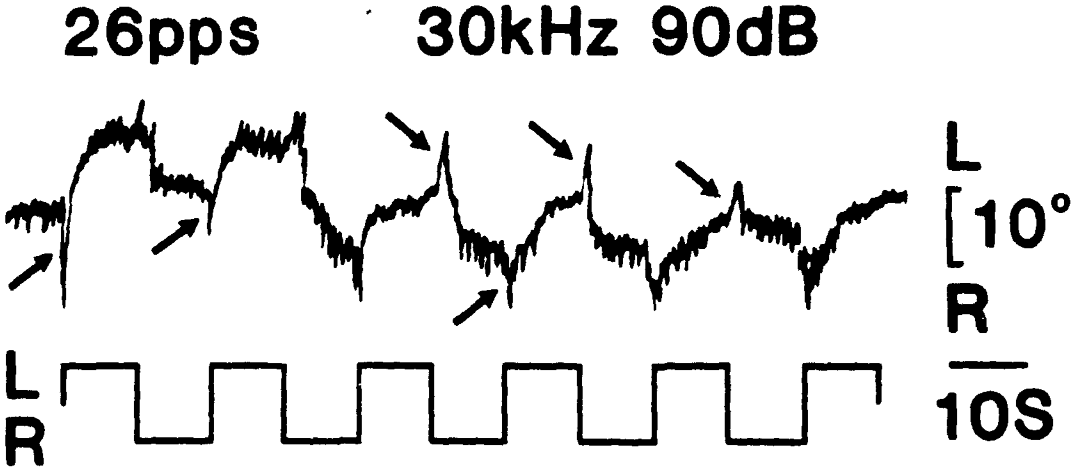
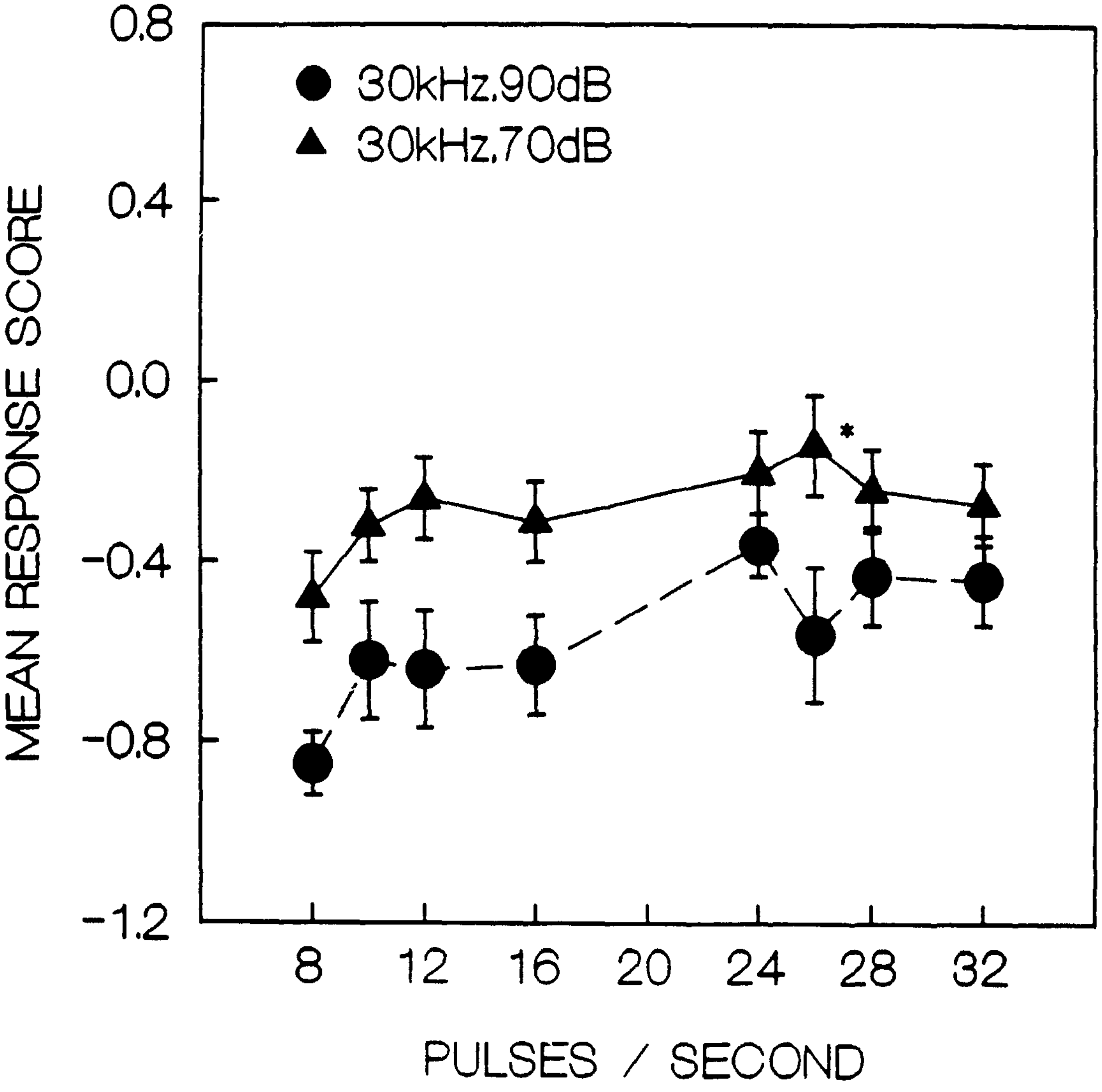

26pps
30kHz 90dB
L
10°
R
L
R
10S

Figure 8. The initial response to 30 kHz. Negative phonotactic steering is elicited at all repetition rates as expressed by negative response scores (mean ± standard error).


0.8
0.4
0.0
-0.4
-0.8
-1.2
MEAN RESPONSE SCORE
● 30kHz,90dB
▲ 30kHz,70dB
*
8
12
16
20
24
28
32
PULSES / SECOND

DISCUSSION

Behavioral studies of different cricket species have described the importance for phonotaxis of the sound spectrum (Walker 1957; Zaretsky 1972; Thorson et al. 1982; Nolen and Hoy 1986a), the temporal structure (Popov and Shuvalov 1977; Pollack and Hoy 1981; Weber et al. 1981) and intensity (Pollack and Plourde 1982; Doolan and Pollack 1985). Thus all these features of the songs play a role in determining phonotactic responses.

Temporal pattern

Popov and Shuvalov (1977) suggested that recognition of conspecifics cannot be based solely on a frequency-dependent system since many species of crickets have songs with similar carrier frequency. On the other hand, temporal parameters of the calling song vary among different species (Alexander 1962; Popov and Shuvalov 1977; Otte and Alexander 1983) and are important in conspecifics recognition(Walker 1957; Popov and Shuvalov 1977).

Different parameters of the temporal pattern are not equally important among all species. While in <u>Gryllodinus kerkennensis</u>, for example, the continuity of the song is the essential parameter, in <u>Gryllus</u> <u>campestris</u> the pulse rate and chirp duration are important. In an unidentified species (referred to as Species 2) chirp rate and pulse duration are the determining factors of the response, and in Melanogryllus desertus the pulse rate and the increase in amplitude of pulses within the chirp are the most important parameters (Popov and Shuvalov 1977).

In general, alterations of important temporal parameters reduce the attractive property of the calling song. Stout et al. (1983) reported that in A. domesticus temporal parameters become less attractive the further they deviate from those of the male's natural calling song. On the other hand, attraction can sometimes be improved by manipulating the temporal structure of the natural calling song. Weber et al. (1981) demonstrated that in G. campestris increasing the number of syllables per chirp above that of the natural calling song increases attractiveness. In T. oceanicus Pollack and Hoy (1981) demonstrated that the chirp section of the natural song is the most attractive temporal featureof the song. The calling song became more attractive when the trill section was eliminated and the chirp section extended indefinitely. The attractiveness of this 'continuous chirp' is affected by changes in pulse rate and/or pulse duration (Doolan and Pollack 1985).

In this thesis, I studied tuning of phonotactic steering movements to the pulse repetition rate of a 5 kHz 'continuous chirp'. I found that positive steering is most pronounced at repetition rates of 16-18 pps. Figure 1 indicates sharper tuning to low repetition rates (<16 pps) than to high repetition rates (>18 pps). This is similar to the findings of Thorson et al. (1982) in G. campestris and Stout et al. (1983) in A. domesticus who reported that decreases in syllable period (the inverse of syllable rate) below the modal values were less detrimental than increases in syllable period.

Intensity and temporal pattern selectivity

Intensity of the stimulus is an important factor in determining the response. With the model calling song above threshold levels, positive phonotaxis is maintained independently of stimulus intensity (Stout et al. 1982; Weber et al. 1981; Doolan and Pollack 1985). I found similar results: with the most attractive repetition rates (16 and 18 pps) positive response is maintained at its peak independently of intensity. In contrast, with altered temporal patterns the response is intensity dependent. When intensity is sufficiently increased some previously effective repetition rates become ineffective. This is reflected in response scores (Fig. 2) which, at those particular repetition rates, are not significantly different from 0 and therefore do not indicate significant phonotactic steering. As previously found by Doolan and Pollack (1985) I confirmed that temporal pattern selectivity increases with intensity. This can be seen in figure 2 where the effective range of repetition rates eliciting positive phonotaxis narrows when intensity reaches sufficiently high levels. It is further described in figure 3 (A) which shows that differences in M.R.S. at different repetition rates increase with intensity.

High frequency-tuned neurons are not behind selectivity for temporal pattern

Doolan and Pollack (1985) suggested that the increase in temporal pattern selectivity with increased intensity might involve some high frequency-tuned neurons which can also be excited by intense 5 kHz. This hypothesis suggests that the effect on selectivity of increasing the intensity of the 5 kHz stimulus might be mimicked by adding ultrasound to the stimulus. However, I have demonstrated that cessation of response to songs with abnormal temporal patterns cannot be solely due to inhibition of positive phonotaxis involving high frequency-tuned negative phonotactic pathways. I showed that contrary to the increase in temporal pattern selectivity with increased intensity the addition of 30 kHz to a 5 kHz stimulus results in a decrease in selectivity. Moreover, whereas positive phonotaxis is maintained at particularly attractive repetition rates independent of the stimulus intensity the addition of 30 kHz reduced attraction at all repetition rates. With the increase in intensity of the 30 kHz component of the stimulus positive phonotaxis was gradually eliminated and at low pulse repetition rates (8 and 12 pps) it was replaced by negative phonotaxis.

The appearance of transient negative responses when 30 kHz was added further emphasizes the difference between the effect of increasing the intensity of 5 kHz and the addition of ultrasound. In other words, the total absence of transient negative response with different intensities of 5 kHz works against the possibility that high frequency neurons are responsible for cessation of response with altered temporal pattern at high intensity.

Sound spectrum

Positive phonotaxis is best tuned to the calling song carrier (Moiseff at al. 1978) and negative phonotaxis is more broadly to high frequencies between 20 and 100 kHz (Nolen and Hoy 1986a). However, although positive and negative phonotaxis are best tuned to different frequencies the neural pathways mediating these behaviors seem to be accessible by 5 KHz and by ultrasound depending on the temporal pattern and intensity of the stimulus. Doolan and Pollack (1985) and Nolen and Hoy (1986a) reported negative phonotaxis in response to intense 5 kHz with altered temporal patterns. High frequencies, known to elicit negative phonotaxis even when played with the model calling song (Moiseff at al. 1978; Pollack et al. 1984) were reported to elicit also positive responses. Stout et al. (1983) showed positive phonotaxis in walking female

Acheta domesticus in response to very loud (95 dB) 13 kHz chirps. They suggested that very intense high frequency chirps affected acoustic neurons that normally respond only to the low frequency component of the natural chirp. Zaretsky (1972) also reported that in S. marginatus an artificial song with the specific pulse pattern played at 18 kHz elicits phonotactic responses as effectively as the natural calling song the dominant frequency of which is approximately 5 kHz. In T. oceanicus Moiseff et al. (1978) reported also some cases of positive phonotaxis in response to 25 and 70 kHz and Bourgeois (1985) reported that some tethered flying females steered toward a calling song with 30 kHz carrier.

I found that the type of response to 30 kHz depends on the temporal pattern of the stimulus: negative phonotactic steering dominates at low pulse repetition rates but negative tendencies decrease as the repetition rate increases and are replaced by positive responses at high syllable rate. The responses to 30 kHz and their behavioral significance will be further discussed below.

Negative and positive responses to 30 kHz

Previous studies of phonotactic responses to high frequency were mostly interested in the immediate response that follows the stimulus. In this context negative phonotaxis is readily elicited by ultrasound. At first sight my results looked inconsistent with previous works due mainly to two facts:

1) Although previously phonotactic responses to high frequency were studied with the model calling song, syllable rates higher than that of the natural call were not considered. Hence. it wasn't possible to assess their attractive nature.

2) Most importantly and contrary to others, my results were based on evaluating phonotactic responses after a 10 s stimulus duration. It was therefore necessary to reevaluate the data on a basis that made it comparable to others. This was possible by monitoring the first response that follows the stimulus since negative phonotaxis in response to ultrasounds is a short latency (25-35 ms) behavior (Nolen and Hoy 1986a). My results support that the initial response to ultrasound is dominantly negative phonotactic steering at all syllable repetition rates within the range studied.

However, if given enough time, negative phonotaxis is replaced by positive phonotaxis at high repetition rates. Moiseff et al. (1978) reported some cases of bimodal responses in which the cricket steers in one direction and then the other. They described these responses as "ambiguous" and did not specify the successive directions of steering. Pollack et al. (1984) also reported a biphasic response to 15 kHz song pattern in which an initial negative component of the response was supplanted by a positive component after 2-5 sec. They suggested that these biphasic responses confirm that stimuli with a carrier frequency of 15 kHz have access to the neural substrates that mediate positive and negative responses. Nolen and Hoy (1986a) more recently reported that in some cases negative phonotactic steering in response to ultraound is replaced by positive responses after a few seconds. They suggested that the neural circuitry tuned to the calling song is capable of overriding the aversive high frequency if given enough time to assess the attractive nature of the repetition rate. The neural correlates could be neurons which are tuned to the temporal pattern of the calling song and are broadly tuned to frequency. Schildberger (1984) identified such neurons in the brain of Gryllus bimaculatus.

Behavioral significance

I present two suggestions in an attempt to explain how high frequency stimuli with high pulse repetition rates might be implicated in eliciting positive responses. However, these suggestions remain tentative and speculative without further behavioral and physiological studies. The first explanation is related to the structure of the natural calling song whereas the second relates to recognition of the courtship song.

Positive phonotactic steering with high pulse repetition rates of 30 kHz might be related to the interpulse interval of the trill phrase of the calling song. This section consists, on average, of 9 doublets of pulses with an average interval of 41 ms (24.34 pps) between pulses of a pair (Bentley and Hoy 1972). Around this pulse repetition rate (at 22 and 24 pps) I found that at a high intensity of 5 kHz positive responses were maintained as indicated by the secondary peak at 70 and 90 dB (Fig. 1). Moreover, with 30 kHz positive phonotaxis was most pronounced around 24 pps (22 to 26 pps) (Fig. 7).

Positive phonotaxis in response to high frequency might also be related to the role of high frequency in identifying the courtship song. Boyan (1981) suggested that in species where songs have different frequency content song recognition relies on frequency discrimination. In _T. oceanicus_ however, the calling and courtship songs have similar carrier frequencies and harmonics (Hutching and Lewis 1984) but different temporal patterns (Loher and Rence 1978; Hill et al. 1972). Unlike the calling song, the courtship song has a high (30-40 pulses/s) syllable repetition rate (see Bentley 1977 for the structure of courtship song). Hutching and Lewis (1984) suggested that, in T. oceanicus high frequency neurons tuned to the harmonics of the songs might be involved in song coding at high intensities, especially when those neurons tuned to the calling song carrier are saturated. Distinguishing between different songs would be based on the differences in their temporal pattern and identification of the courtship song, in particular, probably relies on its fast syllable period. Hence positive responses at high intensity, high frequency, and high repetition rate might be related to these high frequency neurons which are sensitive to the temporal pattern.

The role of high frequency harmonics in eliciting copulation was also demonstrated in Gryllus bimaculatus (Murray and Hoy, 1988). In this species, the calling song has a carrier frequency of 4.5 kHz. The courtship song, on the other hand, has a more complex harmonic structure with the dominant frequency at the third harmonic (13.5 kHz). Murray and Hoy found that the 13.5 kHz component alone is necessary and sufficient to elicit copulation behavior in the female cricket. They also found that an identified high frequency neuron (HF1AN) is more strongly excited by 13.5 kHz than by 4.5 kHz. They suggested that this same neuron might be necessary and sufficient for both copulation and avoidance steering.

In T. oceanicus the calling song and courtship song have similar carrier (5 kHz) and higher harmonics (Hutching and Lewis 1984). Therefore, this species cannot rely solely on the frequency content of these two songs to distinguish between them. However, positive phonotaxis which was observed at repetition rates similar to the calling song pulse rate suggests that high frequencies may have access to the neural circuitry responsible of courtship song recognition.

Although positive responses of _T. oceanicus_ to high frequency might be of no adaptive significance since the calling and courtship songs in _T. oceanicus_ do not differ in frequency content, it might be ascribed to the process of evolution. Dumont and Robertson (1988) proposed that vestigial components of the neural system without any apparent behavioral function can be conserved through evolution. An example of such a vestigial circuitry is the flight circuitry in _Gryllus campestris_, a species that does not fly (personal communication, Pollack G.). Alexander (1967) discussed the evolutionary changes in cricket acoustic communication. It can be inferred from the degree of complexity in its calling song that _T. oceanicus_ is more evolved than _G. bimaculatus_. Hence the neural circuitry of _T. oceanicus_ might have revealed some remnant characteristics in those behavioral experiments.

Response to simultaneous stimuli is not the sum of the effects of the individual stimuli

Positive and negative phonotaxis are considered two separate behaviors (Pollack et al. 1984; Nolen and Hoy 1986a) with each tuned to a different range of frequencies (Moiseff et al. 1978; Nolen and Hoy 1986a). This led to the question of whether phonotactic responses to simultaneous presentation of the calling song carrier (5 kHz) and a high frequency (30 kHz) might be the sum of the effects of the individual stimuli. My results rule out this possibility: stimuli with a 30 kHz carrier were found capable of inducing positive responses at certain repetition rates contrary to their inhibiting effects when simultaneously presented with 5 kHz. It is now quite certain that both positive and negative neural pathways are affected by a wide range of frequencies which encompasses 5 kHz and 30 kHz. This implies that when simultaneously presented, the two frequencies together affect each phonotactic pathway.

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