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SENSORY HABITUATION OF AUDITORY RECEPTOR NEURONS: IMPLICATIONS FOR SOUND LOCALIZATION

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Summary

Auditory receptor neurons exhibit sensory habituation; their responses decline with repeated stimulation. We studied the effects of sensory habituation on the neural encoding of sound localization cues using crickets as a model system. In crickets, *Teleogryllus oceanicus*, sound localization is based on binaural comparison of stimulus intensity. There are two potential codes at the receptor-neuron level for interaural intensity difference: interaural difference in response strength, i.e. spike rate and/or count, and interaural difference in response latency. These are affected differently by sensory habituation. When crickets are stimulated with cricket-song-like trains of sound pulses, response strength declines for successive pulses in the train, and the decrease becomes more pronounced as the stimulus intensity increases. Response decrement is thus greater for receptors serving the ear ipsilateral to the sound source, where intensity is higher, resulting in a decrease in the interaural difference in response strength. Sensory habituation also affects response latency, which increases for responses to successive sound pulses in the stimulus train. The change in latency is independent of intensity, and thus is similar for receptors serving both ears. As a result, interaural latency difference is unaffected by sensory habituation and may be a more reliable cue for sound localization.

Key words: hearing, sound localization, adaptation, habituation, interaural intensity difference, interaural time difference, cricket, *Teleogryllus oceanicus*.

Introduction

Sound localization in the horizontal plane is based on binaural comparison of sound features. Sound sources that are displaced from the midline generate interaural differences in timing and intensity. Microsecond-level differences in timing result from direction-dependent differences in path length from the source to each ear, and some vertebrates can use this as a localization cue (Carr, 1993). Intensity differences arise as a result of reflection and diffraction of sound by the portion of the body between the ears and/or because of interference between sounds arriving at each ear along multiple paths (Michelsen et al., 1994; Miles et al., 1995). These physical cues for sound direction are reported to the central nervous system by auditory receptor neurons. Stimulus onset and phase are represented by spike timing, whereas stimulus intensity affects both the timing (latency) and the strength (spike rate and/or count) of responses.

The responses of sensory neurons change with time. Adaptation, a decrease in firing rate, occurs with constant stimuli (Adrian, 1928). A similar phenomenon, sensory habituation (Pasztor and Bush, 1983; also termed receptor-cell habituation: Coro et al., 1998), occurs with rapidly repeated stimuli; in addition to the declining firing rate within each response, responses to successive stimuli decline. Auditory receptors show both adaptation and habituation (Kiang et al., 1965; Esch et al., 1980; Smith et al., 1983; Sippel and Breckow, 1984; Yates et al., 1985; Coro et al., 1998; Wickesberg and Stevens, 1998). Both types of decrement increase with stimulus intensity (Eggermont and Spoor, 1973a; Sippel and Breckow, 1984; Westerman and Smith, 1984; Yates et al., 1985), and this has important implications for the neural representation of one cue for sound direction, interaural intensity difference. For a lateral sound source, intensity is greater at the ipsilateral ear, so response decrement will be more pronounced for ipsilateral receptors, resulting in a diminished interaural difference in response strength. Thus, the response decrement of auditory receptors may compromise the neural encoding of interaural intensity difference. In this study, we investigate this problem in the auditory system of crickets.

Male crickets use acoustic signals (songs) to attract mates, to induce copulation and in agonistic encounters with other males (Alexander, 1961). The songs of *Teleogryllus oceanicus* may consist of minutes-long trains of 20–35 ms sound pulses, repeated at rates ranging from 8 to 32 pulses s^{-1} (Balakrishnan and Pollack, 1996). That crickets can localize these signals is demonstrated by their behavior (for a review, see Pollack, 1998). The body of a cricket body is too small (diameter approximately 5 mm) with respect to the wavelength of the dominant frequency of its songs (approximately 73 mm) to

produce a substantial sound shadow. Substantial interaural intensity differences occur, nevertheless, because the ear functions as a pressure-gradient system; sounds reaching both the exterior and interior surfaces of the tympanum interfere in a direction-dependent manner. Measurements in another species (*Gryllus bimaculatus*) show that effective interaural intensity differences may be as high as 20–30 dB (Michelsen et al., 1994).

We describe the effects of sensory habituation on the responses of auditory receptors to cricket-song-like pulse trains, with particular emphasis on the consequences of this for sound localization. Some of this work has been reported previously in abstract form (Givois and Pollack, 1999).

Materials and methods

Animals

Teleogryllus oceanicus (Le Guillou) were raised in the laboratory on a diet of Purina cat chow (Ralston-Purina, St Louis, MI, USA) and water. Unmated females, aged 13–28 days after the final moult, were used. Crickets were mounted ventral side uppermost to a wax support after removal of their wings and middle and hind legs. The ears of crickets are in the tibiae of the fore legs. The femora of the fore legs were fixed, with a beeswax/colophonium mixture, perpendicular to the longitudinal axis, and the tibiae and tarsi were held flexed against the femora.

Electrophysiology

Branch TB of prothoracic nerve 5, which carries the axons of approximately 50 auditory receptor neurons of the ear (K. Imaizumi, personal communication) to the central nervous system, courses along the anterior-dorsal surface of the main leg trachea (Eibl and Huber, 1979). A small hole was made in the cuticle of the anterior surface of the femur using the leg trachea, visible through the cuticle, as a guide. A silver wire, insulated with Teflon except at its tip (outer diameter 114 μm; AM Systems, Carlsborg, WA, USA) was placed near the nerve. An indifferent electrode was placed proximally and ventrally in the femur.

For single-unit recordings, the cricket was prepared as described above, and the prothoracic ganglion was exposed by removing the overlying cuticle. The ganglion was supported on a stainless-steel platform and bathed in Tes Ringer (Strausfeld et al., 1983). Receptor axons were recorded with 3 mol l^{-1} KCl-filled glass micropipettes (resistance 20–50 MΩ) in prothoracic nerve 5 at its junction with the ganglion. Electrophysiological recordings and a stimulus monitor were stored on magnetic tape for off-line analysis.

Stimulus generation

Stimuli consisted of trains of trapezoid-shaped sound pulses (duration 30 ms, including 5 ms rise and fall times) generated by custom-written software. The carrier frequency was 4.5 kHz, which is the dominant frequency of *T. oceanicus* songs (Balakrishnan and Pollack, 1996). Pulse trains were

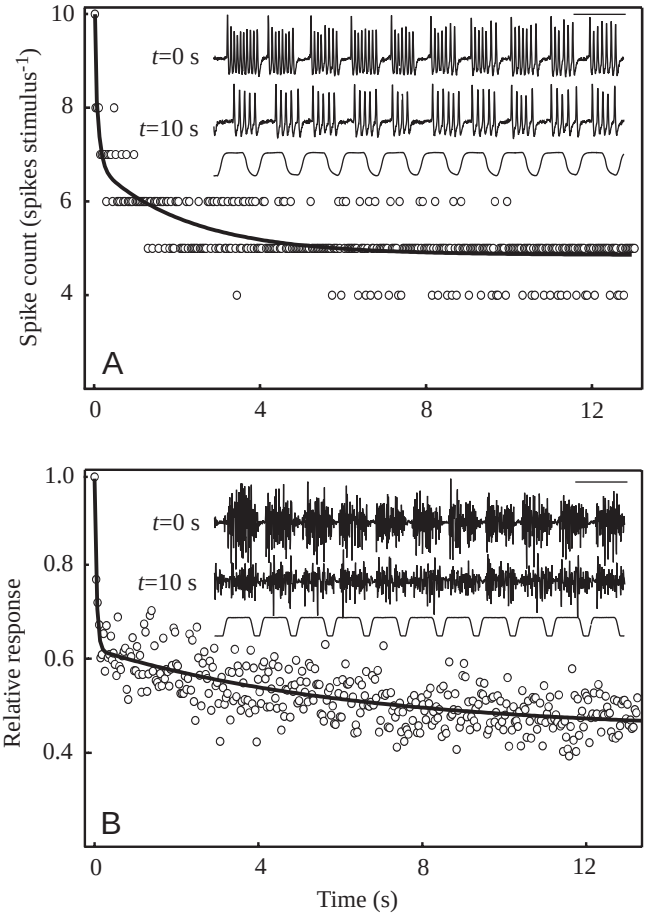

Fig. 1. Decrement of responses to rapidly repeated stimuli. (A) Spike counts of successive responses of a single auditory receptor; the stimulus was 27 pulses s^{-1}, 90 dB, ipsilateral; points are plotted at times corresponding to the onsets of the corresponding sound pulses. (B) Response strengths measured from whole-nerve recording (see Materials and methods), normalized with respect to the response to the first sound pulse of the train; 28 pulses s^{-1}, 90 dB, ipsilateral. Insets in A and B show excerpts from the beginning of stimulation (0 s) and approximately 10 s later; time scales, 50 ms. The curves are least-squares fits of equations of the form: $r(t)=r_{ss}+ae^{-t/\tau_s}+be^{-t/\tau_l}$; where $r(t)$ is response at time t, r_{ss} is the steady-state response, τ_s and τ_l are short and long time constants, and a and b are constants.

30–40 s long and were preceded by a 60 s period of silence. Stimuli were generated under computer control by a digital-to-analog circuit (ATMIO16F5, National Instruments, Austin; D/A update rate 200 kHz) and were either routed directly to the remainder of the apparatus (see below) or stored on digital audio tape (TCD-D3, Sony, Tokyo; sampling rate 22.05 kHz) for later playback. Stimuli were attenuated (350D, Hewlett Packard, Palo Alto, CA, USA, or PA4, Tucker-Davis, Gainseville, FL, USA), amplified (S1-1050G, Sanken-Allegro, Worcester, MA, USA, or D150A, Amcron, Elkhart, IN, USA) and broadcast through loudspeakers (MLXICO, Motorola) situated on the left and right of the cricket in the horizontal plane, perpendicular to the longitudinal axis, at a distance of 45 cm from the midline. The loudspeakers and cricket were

housed in a chamber lined with echo-attenuating foam wedges. Sound intensity, in dB re 2×10^{-5} N m^{-2}, was measured at the position of the cricket with a Brüel & Kjaer (Naerum, Denmark) 4135 microphone and 2610 measuring amplifier.

Data analysis

Responses were viewed and analyzed using the program SWEEPS (Pollack, 1997). Whole-nerve response strength was quantified by integrating recordings (following full-wave rectification) over a 35 ms time window beginning 2–3 ms before the apparent onset of the response, as determined by visual inspection of signal-averaged recordings. Statistical analyses were performed using the programs Statistica (StatSoft, Tulsa) and StarOffice (Sun Microsystems, Palo Alto).

Results

Characteristics of response decrement

The responses of auditory receptors decline with rapidly repeated stimulation. Fig. 1A shows the response of a single receptor to a train of sound pulses. Response strength, as measured by the number of action potentials per stimulus, declines over a period of a few seconds, approaching an asymptote that, in this example, is approximately 50 % of the initial value.

Studying the relationship between habituation and stimulus variables such as intensity and pulse rate requires a stimulus protocol consisting of many long (30–40 s) pulse trains separated by long (60 s) rest periods, which requires approximately 1 h for completion. We are only able to hold single-unit recordings of receptor axons for a few minutes (cf. Imaizumi and Pollack, 1999), and so we could not rely on single-unit recordings to characterize habituation. Instead, we analyzed the decrement of whole-nerve responses, which can be recorded for long periods. As can be seen in Fig. 1B, response decrement is also evident in whole-nerve recordings and is generally similar in form to that observed for single units.

The response decrement appears to include rapid and slower components and can be described by the sum of two exponential decays. For the example given in Fig. 1A, the time constants of the fast and slow components are 91 ms and 2.2 s, respectively; for Fig. 1B, they are 45 ms and 6.3 s, respectively. We were able to fit double-exponential models adequately to whole-nerve recordings only for responses to high intensities (90 and 100 dB) and high pulse rates (16 and 28 pulses s^{-1}). At lower intensities and pulse rates, the response decrement was less pronounced (see below) and the signal-to-'noise' ratio ('noise' in this case being ongoing activity in the nerve) was lower. As a result, curve-fitting models accounted for only a small proportion of the total variance. Within the restricted range of stimuli for which curve-fitting was feasible (arbitrarily

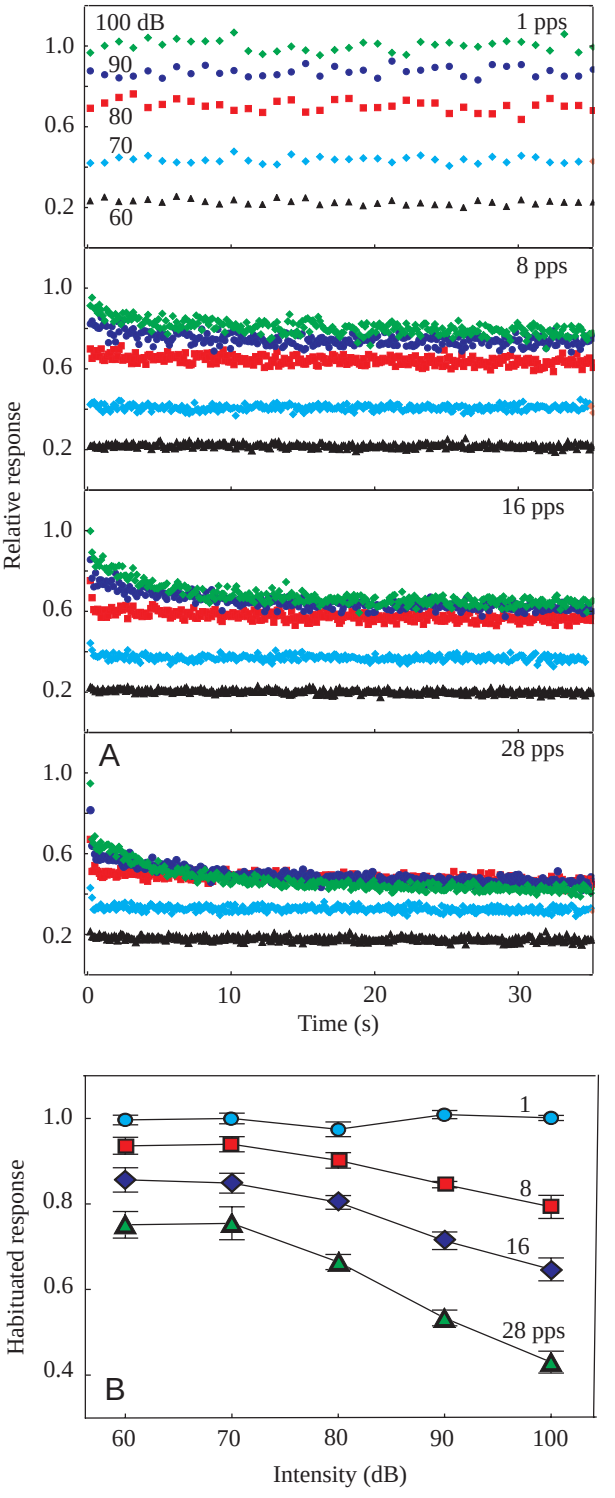

Fig. 2. Effects of sound intensity and pulse rate on response decrement. (A) Points are integrated measurements of whole-nerve responses to ipsilateral stimuli (see Materials and methods) averaged for eight crickets. For each cricket, all responses were normalized with respect to the mean of 30 responses to 1 pulse s^{-1} (pps), 100 dB. For clarity, only every second response is plotted for 16 pulses s^{-1}, and only every third response for 28 pulses s^{-1}. (B) Habituated response strength was computed as the mean response to sound pulses delivered during seconds 20–30 of the pulse train, normalized with respect to the mean of 30 responses to 1 pulse s^{-1} at the same intensity. Points are means ± S.E.M. of eight crickets.

defined as $r^2>0.3$), neither the short nor the long time constant varied with either intensity or pulse rate (analysis of variance, ANOVA, short time constant: intensity effect, $F_{1,28}=0.093$, $P=0.76$; pulse-rate effect, $F_{1,28}=1.86$, $P=0.18$; long time constant: intensity effect, $F_{1,28}=0.34$, $P=0.57$; pulse-rate effect, $F_{1,28}=2.54$, $P=0.12$). The mean short and long time constants were 280±338 ms (mean ± S.D.) and 9.7±4.5 s, respectively (pooled data for 90 and 100 dB, and 16 and 28 pulses s^{-1}, averaged across eight crickets).

Response decrement increases with stimulus intensity and pulse rate

Fig. 2 illustrates the decline in response strength for several pulse rates and intensities. When stimuli are presented at a low rate (1 pulse s^{-1}), responses are stable over time. Response decrement becomes evident at higher pulse rates and is more pronounced at higher intensities (Fig. 2A). As a result of habituation, the relationship between stimulus intensity and response magnitude becomes 'flattened'. The clear increase in response magnitude with increasing intensity, evident at 1 pulse s^{-1} as well as for the initial responses to higher pulse rates, is diminished by the convergence of responses to different intensities once habituation is established (cf. Sippel and Breckow, 1984).

The effects of pulse rate and intensity are summarized in Fig. 2B. Here, to illustrate these effects more clearly, we show the strengths of habituated responses relative to non-habituated responses at the same intensities. We measured the strength of the habituated response as the mean response strength for stimuli delivered during the period 20–30 s after the onset of the pulse train. As Fig. 2A shows, by this time (which amounts to 2–3 times the mean slower time constant of habituation; see above), responses have essentially reached an asymptote. Habituated response strength varies significantly with both intensity and pulse rate (two-way ANOVA: intensity effect, $F_{4,139}=46.55$, $P<0.0001$; pulse-rate effect, $F_{3,139}=271.12$, $P<0.0001$). Moreover, these two variables interact in their effects on response decrement; the effect of pulse rate increases with stimulus intensity (interaction: $F_{12,139}=8.23$, $P<0.0001$). Although habituation is most pronounced at high intensities, it is still evident for more moderate stimuli. For example, even at 60 dB, habituation may bring about a 25 % decrease in response strength (Fig. 2B, 28 pulses s^{-1}).

Effects of habituation on response latency

Fig. 3A shows response latency of a single receptor for successive sound pulses in a train. Latency increases over time. Like the change in response strength, the change in latency appears to comprise both rapid and slower components. In a few instances, we were able to hold single-unit recordings long enough to record habituating responses at more than one intensity. Fig. 3A shows one case, which is typical of eight others. The habituation-induced latency shift is similar at the different intensities, as indicated in Fig. 3A by the nearly parallel fitted curves. We measured the latency shift for single units by comparing the latency of the first response to a train of sound pulses (27 pulses s^{-1}) with the latency of the habituated response, defined here as the mean latency for responses occurring between 20 and 21 s after the onset of the stimulus train, by which time the latency change has approached an asymptote. We were able to make this comparison at two intensities for six receptors (for the remaining three receptors that were stimulated at more than one intensity, one of the recordings lasted less than 20 s). Four receptors were stimulated at more than two intensities; for these, we compared latency shifts for the lowest and highest intensities tested. Intensity differences ranged from 10 to 30 dB and averaged 20.8±6.7 dB. The mean latency shift for the six receptors did not differ for the two intensities (3.1±0.7 ms for the lower intensity, and 3.6±0.5 ms for the higher intensity; $P=0.21$, paired *t*-test). In contrast, the habituation-induced decrease in spike count, again measured as the difference between the first response and the mean habituated response, was significantly different for the two intensities (decrement was 2.5±1.4 spikes for the lower intensity, 4.4±0.7 spikes for the higher intensity; $P<0.01$). This analysis, although only possible for a few receptor neurons, thus confirms the impression from Fig. 3A that the change in latency during habituation is similar at different intensities.

We analyzed the effects of habituation on response latency more extensively for whole-nerve recordings. Whereas it is straightforward to measure response latencies in single-unit recordings, for which response onsets are clear, this is problematic in whole-nerve recordings, for which response onsets must be detected despite ongoing activity. The problem is that any quantitative criterion for latency is based on response amplitude, but response amplitude decreases with time. For example, response onset might be identified as the point at which the response exceeds the average background level by 2 standard deviations; but this point would occur later during the course of a habituated response than during a non-habituated response, thus imposing a systematic, time-dependent bias on the latency measurement. Rather than attempting to track the change in latency over time in whole-nerve recordings by measuring the latencies of individual responses, we instead measured the latencies of habituated responses by signal-averaging responses during the period 20–30 s after the onset of the pulse train. Averaging single responses that have been aligned according to the timing of the sound pulses reveals series of compound action potentials, which reflect the nearly synchronous firing of many receptor neurons (Pollack and Faulkes, 1998; Fig. 3B, inset). We measured latency as the time from the stimulus onset to the negative peak of the first compound action potential. A 10 s period was chosen for averaging, as opposed to the 1 s period used above, so that at low pulse rates sufficient responses would be included in the average for the compound response to be clearly defined. Fig. 3B summarizes the effect of habituation on response latency. Latency increases with repeated stimulation, and the change is more pronounced for higher pulse rates ($F_{3,112}=52.76$, $P<0.0001$). Moreover, as the single-unit recordings indicate

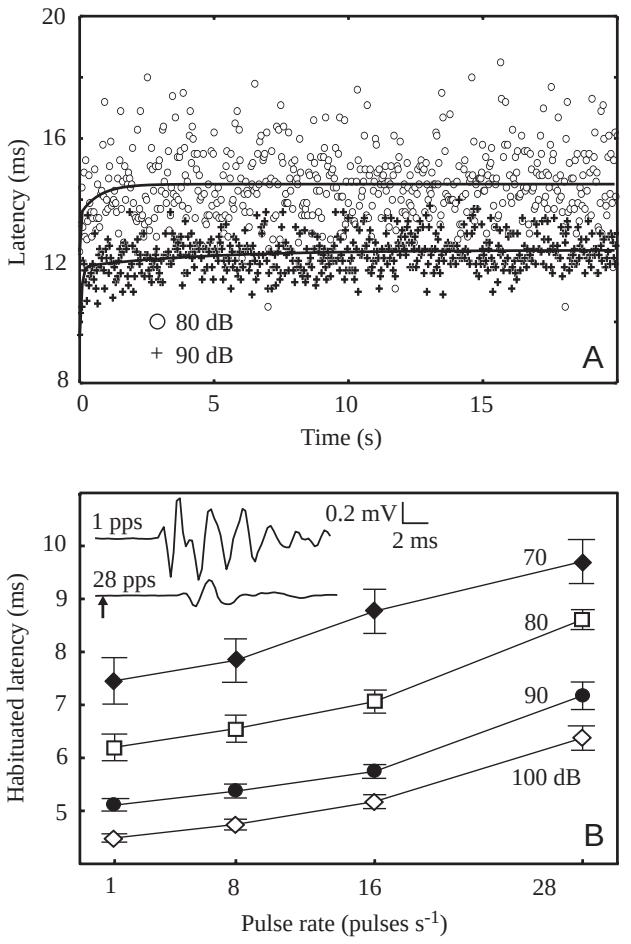

Fig. 3. Effects of repeated stimulation on response latency. (A) Points are latencies (time from sound onset to first spike) of successive responses for a single receptor neuron (27 pulses s^{-1} ipsilateral). Curves are least-squares fits of equations of the form: $l(t)=l_{min}+a(1-be^{-t/\tau_s}-ce^{-t/\tau_l})$, where $l(t)$ is the latency at time t, l_{min} is the minimum latency, τ_s and τ_l are short and long time constants, and a, b and c are constants. Similar results were obtained for eight additional receptors. (B) Change in latency increases with pulse rate, but is similar for different intensities. Latency was measured from averaged whole-nerve responses during the period 20–30 s after the onset of stimulation, except for 1 pulse s^{-1} (pps), for which all responses to a 30 s stimulus train were averaged; the inset shows averaged recordings from a single cricket to 1 pulse s^{-1} and 28 pulses s^{-1}, 100 dB. Points are means ± S.E.M. for eight crickets. Clear compound action potentials could not be resolved, and latency could not therefore be measured, for high pulse rates at intensities below 70 dB.

(Fig. 3A), although latency clearly depends on stimulus intensity, being shorter for higher intensities ($F_{3,112}$=120.36, P<0.0001), the change in latency during habituation is independent of intensity. This is evident in whole-nerve data from the absence of a statistical interaction between intensity and pulse-rate effects ($F_{9,112}$=0.39, P=0.94), as well as from the parallelism of the curves for different intensities in Fig. 3B.

Effects of habituation on interaural cues

The strong effect of intensity on the decline in response strength implies that the interaural response-strength difference (IRSD) will change as habituation builds up. For a lateral sound source, intensity is greater at the ipsilateral ear, and so the decrement in response strength should be more pronounced in receptor neurons serving that ear. As a result, the responses of both ears should converge as habituation accrues. We assessed IRSD by measuring the difference in the response of a single ear to ipsilateral and contralateral stimuli. Assuming bilateral symmetry and the absence of standing waves in the sound field, this measure is equivalent to the difference in the responses of the two ears to a single, lateral stimulus.

Interaural response-strength differences are shown in Fig. 4A. When the pulse rate is low (1 pulse s^{-1}), IRSD remains constant. For higher pulse rates, IRSD decreases with time. The decrease is more pronounced the higher the intensity and pulse rate, and for the highest values tested (28 pulses s^{-1}, 100 dB) the IRSD approaches zero. In some individuals, the sign of the interaural difference may even reverse (Fig. 4B).

These effects are summarized in Fig. 4C, which shows IRSDs for habituated responses (for the period 20–30 s after pulse-train onset). The effects of intensity and pulse rate, as well as their interaction, are all significant (two-way ANOVA: intensity effect, $F_{4,139}$=20.76, P<0.0001; pulse-rate effect, $F_{3,139}$=61.48, P<0.0001; interaction, $F_{12,139}$=5.04, P<0.0001). Although the decline in the IRSD is most pronounced at high intensities, a clear decrease is evident at lower intensities as well for 16 and 28 pulses s^{-1}.

Unlike the change in response strength, the habituation-induced change in latency is independent of intensity (Fig. 3) and so should be similar for both ears. The interaural latency difference should, therefore, remain constant despite habituation. This is confirmed in Fig. 4D: the interaural latency difference of habituated responses is independent of both stimulus intensity ($F_{2,76}$=1.51, P=0.23) and pulse rate ($F_{3,76}$=0.76, P=0.52) as well as the interaction between these variables ($F_{6,76}$0.24, P=0.96).

Discussion

Sensory habituation has been reported previously in insect auditory receptors. Esch et al. (1980) studied the auditory receptors of *Gryllus campestris* and *G. bimaculatus.* Unlike *T. oceanicus*, which produces long, uninterrupted trains of sound pulses, these species produce ‘chirps’ consisting of 3–4 rapidly occurring sound pulses, with neighboring chirps separated by silent periods lasting several hundred milliseconds. Although spike count decreased, and latency increased, for responses to successive sound pulses in a chirp, these effects reversed completely during the silent interchirp intervals (Esch et al., 1980). Coro et al. (1998) studied the responses of moth auditory receptors to long trains of ultrasound pulses similar to those produced by insectivorous, echolocating bats. They too found that responses to successive pulses decreased in spike count and increased in latency. Neither Esch et al. (1980) nor

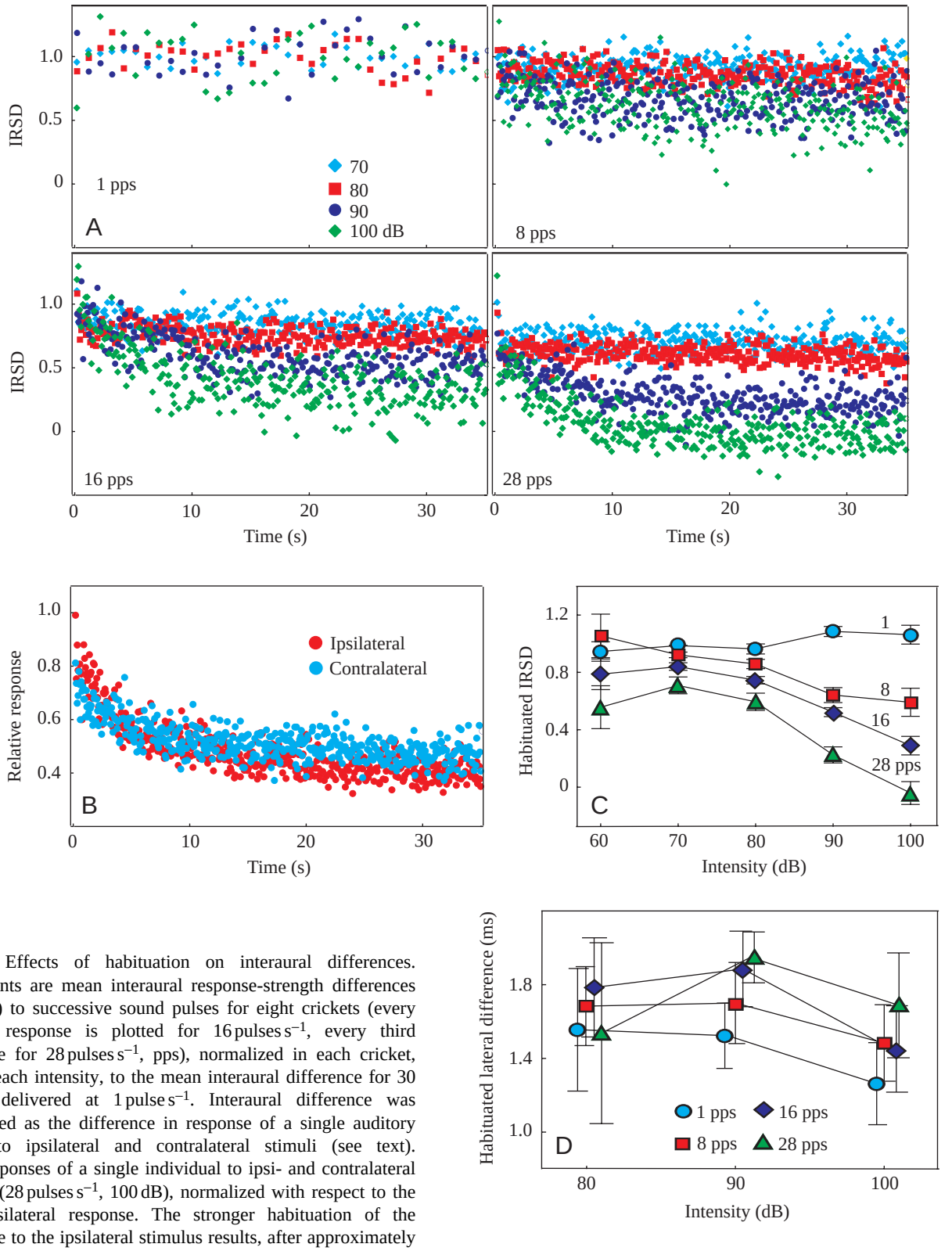

Fig. 4. Effects of habituation on interaural differences. (A) Points are mean interaural response-strength differences (IRSDs) to successive sound pulses for eight crickets (every second response is plotted for 16 pulses s^{-1}, every third response for 28 pulses s^{-1}, pps), normalized in each cricket, and at each intensity, to the mean interaural difference for 30 pulses delivered at 1 pulse s^{-1}. Interaural difference was calculated as the difference in response of a single auditory nerve to ipsilateral and contralateral stimuli (see text). (B) Responses of a single individual to ipsi- and contralateral stimuli (28 pulses s^{-1}, 100 dB), normalized with respect to the first ipsilateral response. The stronger habituation of the response to the ipsilateral stimulus results, after approximately 12 s, in a reversal of the initial directional preference. Only every second response is plotted. (C) The mean interaural difference during seconds 20–30 of the stimulus train was normalized to the mean interaural difference for 30 pulses at 1 pulse s^{-1}; points are means ± S.E.M. of these values for eight crickets. (D) The interaural latency difference measured from signal-averaged responses during seconds 20–30 of the pulse train; points are means ± S.E.M. of eight crickets. At high pulse rates, clear compound action potentials could not be resolved for contralateral stimuli below 80 dB; these intensities were therefore excluded.

Coro et al. (1998) studied the effect of stimulus intensity on these changes in response. Sippel and Breckow (1984) described similar phenomena in the auditory receptors of locusts. They investigated the effects of both pulse rate and intensity and obtained results strikingly similar to those we report here. In particular, they found that the decrement in spike count increased with both pulse rate and intensity, whereas the increase in latency depended only on pulse rate. The relevance of pulse rate to the acoustic behavior of the animal they studied, *Locusta migratoria*, is not known; thus, it is difficult to place their results in a functional context.

Response decrement also occurs in auditory receptors of vertebrates, both in responses to constant tones and in successive responses to rapidly repeated tones (Kiang et al., 1965; Eggermont and Spoor, 1973a; Smith et al., 1983; Yates et al., 1985). As in insects, the response strength decreases with time, and this effect increases with intensity. Moreover, the latency to successive pulses in a train increases with time and, as in insects, the shift in latency is similar at different intensities (Eggermont and Spoor, 1973a). These phenomenological similarities between insects and vertebrates are intriguing, given that the cellular mechanisms underlying the response changes differ in the two cases. In vertebrates, auditory transduction occurs in inner hair cells, which make chemical synapses with auditory neurons. Although adaptation is evident at the level of auditory neurons, the receptor potential of the hair cell does not adapt (Russell and Sellick, 1978). The adaptation apparent at the neural level is ascribable, at least in part, to decreased transmitter release by hair cells (Furukawa and Matsuura, 1978). In vertebrates, adaptation and habituation of auditory receptors appear to be manifestations of the same underlying processes (Eggermont and Spoor, 1973b; Smith et al., 1983; Eggermont, 1985); thus, the synapse between hair-cell and receptor neuron is the likely site of sensory habituation as well. In insects, transduction takes place within the dendrites of receptor neurons (Oldfield and Hill, 1986), pointing towards transduction and/or spike initiation as the most likely loci of sensory habituation. In other arthropod mechanoreceptors, adaptation and sensory habituation have been ascribed to Na^+ channel inactivation and to the electrogenic activity of ion-exchange pumps (Sokolove and Cooke, 1971; Pasztor and Bush, 1983; French, 1989a,b).

Response decrements, whether to continued or repeated stimulation, have been proposed to serve a number of information-processing functions. Perhaps the most commonly offered of these is to permit sensory neurons to detect changes in stimulus variables over a wide dynamic range (Laughlin, 1989) or when there are sudden changes in the stimulus (Pasztor and Bush, 1983). Additional functions that have been proposed include increasing the selectivity of neurons for the frequency spectrum (Clague et al., 1997), temporal pattern (Epping, 1990) or direction of the signal (Pollack, 1988). One possible role of the habituation we describe is in filtering of stimulus temporal pattern. Crickets are selective for stimulus pulse rate, responding behaviorally most strongly to pulse rates similar to those of conspecific signals (for a review, Pollack, 1998). The phonotactic behavior of *T. oceanicus* females displays band-pass characteristics: phonotaxis is robust for stimuli with pulse rates near 16 pulses s^{-1} and diminishes for lower and higher pulse rates (Pollack and El-Feghaly, 1993; Hennig and Weber, 1997). Previous work has identified the brain as the main site of temporal-pattern filtering in crickets (Schildberger, 1984). However, our results suggest that receptor habituation may contribute to the low-pass component of the filter. Poor behavioral responses to high pulse rates may, in part, be due to weaker input from receptor neurons. In this context, it is interesting to note that selectivity for temporal pattern increases with stimulus intensity, as does receptor habituation (Doolan and Pollack, 1985).

Because of its dependence on stimulus intensity, habituation affects the neural encoding of the interaural difference in sound intensity. This physical cue is almost certainly the basis for sound localization in crickets. The interaural difference in sound arrival time, the alternative cue, has a maximum value, for a sound source perpendicular to the midline, of 30 μs (and crickets can determine the laterality of sounds displaced from the midline by as little as 5–10 °; Pollack and Plourde, 1982). Although time differences of this order can be resolved in the brains of several vertebrates (Yin and Chan, 1990; Carr, 1993), this does not appear to be the case in the simpler nervous systems of insects. Behavioral and neurophysiological experiments on grasshoppers indicate that the minimum resolvable interaural time difference in these animals is an order of magnitude larger than the maximum value available to crickets (Rheinlaender and Mörchen, 1979; von Helversen and Rheinlaender, 1988).

There are two, non-exclusive, candidate codes for the difference in intensity at the two ears: interaural difference in response strength, i.e. spike count and/or rate, and interaural latency difference. Under natural stimulus conditions, these two measures are tightly linked. Both vary with stimulus intensity, and their individual effects are difficult to tease apart. Experiments using dichotic stimulation have shown that either cue, alone, is able to influence the responses of direction-sensitive interneurons (Rheinlaender and Mörchen, 1979; Römer and Rheinlaender, 1983). Behavioral experiments have confirmed that interaural latency difference alone can provide directional information. von Helversen and Rheinlaender (1988) stimulated grasshoppers with sounds played independently from two free-field loudspeakers, one on each side of the animal. When they played equally intense sounds from both sides, animals turned towards the leading loudspeaker with 100 % accuracy when the time difference between the two stimuli was 1.5 ms or greater. Such time differences are within the range of those generated by the intensity-dependence of receptor latency (Mörchen et al., 1978; Fig. 3). von Helversen and Rheinlaender (1988) also presented stimuli simultaneously from the two sides but at different intensities. Grasshoppers turned reliably towards the more intense stimulus when the difference was 1.6 dB. However, this result is more difficult to interpret because, even though the two stimuli were emitted simultaneously, the

intensity-dependence of response latency would have produced an interaural difference in timing of receptor activity.

Although differences in response strength and latency can both provide directional information, our results suggest that, at least for stimuli that induce strong habituation, latency difference may be a more reliable cue. The difference in response strength degrades with time, whereas the difference in latency remains stable. At moderate intensities and pulse rates, where the habituated IRSD remains reasonably large (Fig. 4C), the effect of habituation on sound localization may be only slight. If crickets were to base their phonotactic behavior solely on response-strength differences, they might be expected to underestimate the sound azimuth, perhaps resulting in turns that undershoot the target. For high rates and intensities, habituation might completely obliterate the response-strength difference or, worse, cause it to reverse (Fig. 4A–C). Although this might appear to be a severe impediment to sound localization, this may not be the case during natural behavior. For *T. oceanicus*, the high stimulus intensities that induce the strongest habituation occur only when the listener is within a few centimeters of the singer (Balakrishnan and Pollack, 1996). At such small distances, the two animals may be in visual, tactile and chemosensory contact (Balakrishnan and Pollack, 1997), and accurate sound localization might no longer be necessary. Moreover, *T. oceanicus* songs are rhythmically complex (Balakrishnan and Pollack, 1996): segments with a high pulse rate are interrupted by lower-pulse-rate segments, during which partial recovery from habituation might be possible. Finally, phonotaxis is characterized by a zig-zag path, resulting in reversals every few seconds of which ear is ipsilateral and which contralateral (Weber et al., 1981). With each reversal, a new binaural intensity difference is established, which might allow recovery from imbalances set up by prior habituation. The reversals in intended walking direction occur even under open-loop conditions, in which the orientation of the cricket is fixed with respect to the sound source and its intended walking direction is inferred from movements of a sphere that it turns beneath it (Böhm et al., 1991). Thus, the reversals are not simply consequences of the cricket having detected a sufficiently large error angle in its acoustic orientation; rather, zig-zagging appears to be intrinsic to the motor pattern for acoustic orientation and may be an adaptation that serves to minimize the distortion of localization cues by habituation.

The interaural latency difference would be most useful as a localization cue for signals that present frequent opportunities to measure this difference, i.e. either repeated trains of sounds (as in cricket song) or stimuli that contain frequent amplitude modulations. These features are characteristic of communication signals in many animal groups, including birds (Kroodsma and Miller, 1996), frogs (Gerhardt, 1991) and mammals (Fenton, 1995). Because of the phenomenological similarities of the effects of repeated stimulation in vertebrates and insects (see above), it seems possible that for vertebrates, as for crickets, interaural latency difference may be a more reliable measure of intensity difference than interaural difference in response strength. Interestingly, it has been suggested that the interaural intensity difference is computed, in mammalian brains, by comparing response latencies (Yin et al., 1985; Pollak, 1988; Eggermont, 1998). One reason for the selection of this computational strategy may be its relative immunity to the effects of habituation.

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