Effects of Inhibitory Timing on Contrast Enhancement in Auditory Circuits in Crickets (Teleogryllus oceanicus)

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Faulkes, Zen and Gerald S. Pollack. Effects of inhibitory timing on contrast enhancement in auditory circuits in crickets (Teleogryllus oceanicus). J Neurophysiol 84: 1247–1255, 2000. In crickets (Teleogryllus oceanicus), the paired auditory interneuron Omega Neuron 1 (ON1) responds to sounds with frequencies in the range from 3 to 40 kHz. The neuron is tuned to frequencies similar to that of conspecific songs (4.5 kHz), but its latency is longest for these same frequencies by a margin of 5–10 ms. Each ON1 is strongly excited by input from the ipsilateral ear and inhibits contralateral auditory neurons that are excited by the contralateral ear, including the interneurons ascending neurons 1 and 2 (AN1 and AN2). We investigated the functional consequences of ON1’s long latency to cricket-like sound and the resulting delay in inhibition of AN1 and AN2. Using dichotic stimuli, we controlled the timing of contralateral inhibition of the ANs relative to their excitation by ipsilateral stimuli. Advancing the stimulus to the ear driving ON1 relative to that driving the ANs “subtracted” ON1’s additional latency to 4.5 kHz. This had little effect on the spike counts of AN1 and AN2. The response latencies of these neurons, however, increased markedly. This is because in the absence of a delay in ON1’s response, inhibition arrived at AN1 and AN2 early enough to abolish the first spikes in their responses. This also increased the variability of AN1 latency. This suggests that one possible function of the delay in ON1’s response may be to protect the precise timing of the onset of response in the contralateral AN1, thus preserving interaural difference in response latency as a reliable potential cue for sound localization. Hyperpolarizing ON1 removed all detectable contralateral inhibition of AN1 and AN2, suggesting that ON1 is the main, if not the only, source of contralateral inhibition.

INTRODUCTION

Hearing is vital to crickets. Male crickets use song to signal their location to potential mates, during courtship and during fights with conspecific rivals (Alexander 1960, 1961). Crickets also hear and avoid ultrasound signals like those made by insectivorous echolocating bats (Moiseff et al. 1978; Nolen and Hoy 1986). Behavioral responses to sound often require localization of the sound source in the horizontal plane. One neuron that is believed to improve sound localization is Omega Neuron 1 (ON1), a large, paired, local interneuron in the prothoracic ganglion (Casaday and Hoy 1977; Popov et al. 1978; Wohlers and Huber 1982). Each ON1 has branches in both the left and right halves of the prothoracic ganglion. The processes on one side (the same side as the cell body) are mainly postsynaptic, and receive excitatory input from one ear. The processes on the other side are mainly presynaptic and provide inhibitory input to several auditory neurons that receive excitatory input from the other ear, including the mirror-image ON1 (Kleindienst et al. 1981; Silverston et al. 1985; Watson and Hardt 1996). The ON1 pair enhances contrast between the left and right sides of the auditory pathway, thus improving the ability to localize a sound source (Atkins et al. 1984; Horseman and Huber 1994b; Schildberger and Horner 1988). Wiese and Elits (1985) suggested that ON1 also tunes the auditory system to the temporal characteristics of song.

Two ranges of sound frequency, corresponding to cricket-like and bat-like sounds, are conspicuous in the nervous system of Teleogryllus oceanicus. For example, based on frequency sensitivity, auditory receptors fall into three groups, the two largest of which correspond to cricket- and bat-like frequencies (Imaizumi and Pollack 1999). Most auditory interneurons that have been identified in T. oceanicus show enhanced sensitivity to frequencies similar to those used in their songs (~4.5 kHz) (Balakrishnan and Pollack 1996; Hill et al. 1972) or to ultrasound (Atkins and Pollack 1987; Moiseff and Hoy 1983). Some neurons, including ON1, are dually tuned with enhanced sensitivity to both frequency ranges (Atkins and Pollack 1986). ON1’s threshold for 4.5 kHz is ~15 dB lower than for ultrasound (Atkins and Pollack 1986; Pollack 1986), but despite its greater sensitivity to 4.5-kHz sounds, ON1’s latency is up to 10 ms longer to low-frequency stimuli than to ultrasound stimuli of equivalent intensity (Pollack 1994). This is an unexpected finding. Generally, the sensitivity of a neuron is a measure of the amount of input (e.g., stimulus energy) needed to evoke a response. As input increases past threshold, a suite of related changes in the neuron’s response typically occurs: latency usually decreases and the number of spikes usually increases. The unusual combination of high sensitivity and long latency suggests that ON1’s longer latency for cricket-like stimuli may be functionally important in processing these sounds. We investigate this possibility in this paper.

Our first goal was to characterize the relationship between ON1 latency and sound frequency more completely by measuring ON1’s response over a broader frequency range than previously investigated (Pollack 1994). Our second goal was to learn whether ON1’s long latency to low-frequency sounds affects its inhibition of its targets and thus its effectiveness as an enhancer of binaural contrast. Among these are ascending neurons 1 and 2 (AN1 and AN2) (Wohlers and Huber 1978; see Hennig 1988 for review and further references), both of which are important for behavioral responses to sound. AN1 is
most sensitive to the frequency of conspecific songs (Hennig 1988). Crickets respond to stimulation with one type of song, calling song, by orienting toward the source (positive phonotaxis). When AN1’s response to sound is perturbed, phonotaxis is misdirected (Schildberger and Hörner 1988). AN2, which is most sensitive to ultrasound, initiates negative phonotaxis (orientation away from sound) (Nolen and Hoy 1984). AN2 also responds to cricket-like frequencies and influences the direction of positive phonotaxis (Schildberger and Hörner 1988). Harrison et al. (1988) proposed that AN2 also functions as a specific detector of a distinct signal, courtship song (but see Libersat et al. 1994).

Faulkes and Pollack (1997, 1999) are abstracts of this work.

**Methods**

**Animals**

Field crickets, *T. oceanicus* (Le Guillou, 1841), were raised in laboratory colonies where food (Purina Cat Chow) and water were available continuously. Unmated female crickets, from 1 to 3 wk of age after final molt, were used in all experiments.

**Recordings**

Crickets were anesthetized by chilling on ice. The meta- and mesothoracic legs were autotomized, and wings and antennae were removed. We affixed animals, ventral side up, to a magnetic base with wax and then used wax to hold the tibia and femur of the prothoracic legs together, with the femur positioned horizontally at right angles to the body axis. We removed the ventral thoracic cuticle to expose the prothoracic ganglion and supported the ganglion on a metal platform. The ganglion was submerged in physiological saline [(in mM) 140 NaCl, 10 KCl, 7 CaCl₂, 4 NaHCO₃, 1 MgCl₂, 5 TES, and 5 trehalose] (modified from Strausfeld et al. 1983). The main acoustic trachea, which links the two ears (Michelsen et al. 1994), was intact for experiments using free-field sound stimuli but severed during experiments using leg phones. To make extracellular recordings of ON1, we placed low-resistance microelectrodes (<10 MΩ), filled with 2 M NaCl, in the hemiganglion contralateral to the ON1 cell body (Fig. 1). We made intracellular recordings of ON1 with high-resistance microelectrodes (>30 MΩ) filled with 3 M potassium chloride (KCl). Intracellular recordings were made in the hemiganglion ipsilateral to the cell body. To make extracellular recordings from AN1 and AN2, the cervical connective was cut as far anterior as possible. The connective was desheathed using a stainless steel minutien pin, and then frayed into smaller bundles using the pin. The most medial portion of the neck connective was wrapped repeatedly around a stainless steel hook electrode (Fig. 1). The nerve and electrode were covered with a mixture of petrolatum and mineral oil. To reduce background activity, we cut the posterior connectives to the ganglion.

Microelectrode recordings were amplified by a Getting 5A microelectrode amplifier or a WPI M-707 microelectrode amplifier. Hook-electrode recordings were amplified by a Grass P15 amplifier.

**Sound stimuli**

We produced sound stimuli using STIMS, a computer program written by G. P. for the Microsoft Windows operating system. The stimuli were generated by a D/A converter (National Instruments AT-MIO-64-F5:12 bits; update rate, 250 kHz), and relayed through a programmable attenuator (Tucker-Davis Technologies PA4) and amplifier (Amercon D150A). Stimuli had trapezoidal amplitudes envelopes, with rise/fall times of either 0.2 ms (in the initial descriptive study of how ON1 latency varied with sound frequency) or 5 ms (all other experiments) and constant-intensity plateaus of 20-ms duration. Stimuli were presented at a rate of 2 s⁻¹. Sound frequency ranged from 3 to 40 kHz in free-field experiments and from 3 to 10 kHz in closed-field experiments. The frequency responses of both the free- and closed-field stimulus delivery systems (see following text) were “flattened” by the stimulus-generation software, which adjusted the attenuator separately for each sound frequency. Intensity ranged from 25 to 100 dB SPL (2 × 10⁻¹⁰ Nm⁻²; intensity measured as RMS value during constant-intensity plateau of the trapezoidal sound pulse). Stimulus intensity was calibrated with Bruel and Kjaer instruments (type 4135 microphone, type 2610 measuring amplifier, type 4230 calibrator).

Experiments were performed in an anechoic chamber (Eckel Industries: 2 × 2 × 1.5 m; energy reflectance <1% at ≥150 Hz). The table that supported the cricket, the micromanipulator, and other surfaces in the sound field were covered with echo-suppressing polyurethane foam (Illbruck, Sonex). Free-field stimuli were played through loudspeakers (Motorola piezoelectric tweeters) placed 35 cm away from the subjects, perpendicular to the long body axis. Closed-field stimuli were played through leg phones (Kleinindienst et al. 1981). Brass cylinders (13 mm ID × 5 mm length), with gaps cut in the wall for the cricket’s legs, were closed at one end with small loudspeakers (Koss earphones) and at the other with brass caps. The theoretical lowest resonant frequency for such a chamber is ~30 kHz (calculated by f = c/(2 × l)), where f is resonant sound frequency, c is sound velocity, and l is length of the cavity) (Kleinindienst et al. 1981). The gaps around the legs were sealed with wax and petrolatum. We tested the acoustic isolation of the leg phones by comparing ON1’s response to stimuli given to the intact ipsilateral ear and to the contralateral ear, the nerve from which was severed to ensure that any response of ON1 would be due to incomplete isolation of the leg phones rather than to neural input from the contralateral ear (Selverston et al. 1985). The mean threshold for ON1 at 4.5 kHz was 51.25 dB SPL (n = 4) for ipsilateral stimulation, and thresholds were ~90 dB SPL for contralateral stimulation (in 2 animals, threshold was not reached at 100 dB SPL, the maximum intensity available). We also examined each preparation for evidence of excitation timed to the contralateral sound pulse; those showing such excitation were discarded.

**Cell identification**

ON1 is unique among known prothoracic auditory interneurons in that extracellular recordings can be made readily from its proximal axonal processes in the hemiganglion opposite to its most sensitive side (Pollack 1986). Accordingly, ON1 was identified in extracellular recordings from its axon by its preference for “electrode-contralateral” stimuli (Fig. 1). Intracellular recordings were made from ON1’s large dendritic process (Wohlers and Huber 1978); cell identity was established on the basis of one-for-one correspondence between intracellularly recorded action potentials and extracellular spikes recorded simultaneously from the axonal processes. AN1 and AN2 receive excitatory input from the ear ipsilateral to their ascending axons (Wohlers and Huber 1978). We identified AN1 and AN2 by their preference for “electrode-ipsilateral” sound (Fig. 1), by their characteristic difference in spike size (AN2 >> AN1; Fig. 2, A and B), and by their frequency sensitivity (Fig. 2, C and D); AN1 is tuned to ~4.5 kHz in this species, whereas AN2 is tuned to ultrasound, with a secondary sensitivity peak around 4.5 kHz (Hennig 1988; Moiseff and Hoy 1983; Wohlers and Huber 1978). Throughout this paper, the terms “ON1-ipsilateral stimuli” and “AN-ipsilateral stimuli” refer to stimuli presented to the ear providing excitatory input to the neuron in question.

**Data analysis**

Data and event markers were recorded on a frequency-modulated tape recorder (Vetter Model D), then digitized through an analog-digital board (National Instruments AT-MIO-64-F5) using the program SWEEPS (Pollack 1997), at a 10-kHz/channel sampling rate with 12 bits of A/D resolution. SWEEPS was also used for analyzing data off-line. Statistical analyses were per-
formed using the software package Statistica for Windows 5.1 (StatSoft).

ON1 threshold was defined as the lowest sound intensity (±2.5 dB SPL) that generated a mean response of $1 \text{ spike/100-ms sampling window (30 repetitions).}$ Subthreshold activity of ON1 averaged 0.33 spike/100-ms sampling window.

Because AN1 is a small unit, recorded extracellularly in a mixed nerve, activity from other neurons with similarly sized (but differently shaped) spikes, and other “noise” (e.g., bursts of activity, where individual spikes could not be discerned), could not reliably be excluded by our software. Traces were examined individually, and potentials that were misidentified as AN1 spikes were excluded from the analysis. To minimize the effect of spontaneous AN1 activity on measures of AN1’s latency and spike counts, AN1 spikes were tallied

FIG. 1. Schematic diagram of recording situation. A hook electrode (R1) records from the axons of AN1 and AN2 (arrow) in the cervical connective, while a blunt glass microelectrode (R2) records from the axonal processes of omega neuron 1 (ON1) (see METHODS). Labeled circles represent the laterality of the cells' main auditory input, not the location of their cell bodies, which would be to the left of the midline (dotted line) for all the illustrated cells. ON1 and ascending neurons 1 and 2 (AN1 and AN2) are bilaterally paired neurons; the mirror image of the circuit shown here also occurs, but is omitted for clarity. Based on Wiese and Eltis (1985); Horseman and Huber (1994a) and Selverston et al. (1985).

FIG. 2. Identification of AN1 and AN2 in extracellular recordings. A and B: sample traces showing relative size of spikes. AN1 spikes indicated by ●. Note that the threshold of AN2 at 4.5 kHz is sufficiently higher than AN1’s that a relatively clear response for AN1 can be recorded for lower sound intensities. C: frequency/response curves for AN1. AN1’s response to higher intensity sounds is not shown because it becomes masked by AN2 response. n = 6 individuals; 5 repetitions of sound stimulus at each frequency and each intensity.

D: frequency/response curves for AN2. Although the leg phones could not generate sound above 10 kHz, we confirmed that the putative AN2 responded to ultrasound generated by jangling keys. n = 6 individuals; 5 repetitions of sound stimulus at each frequency and each intensity.

FIG. 3. ON1’s latency is longer to sound stimuli of ~4.5 kHz. Intensity is given relative to threshold at each frequency. Other sound intensities ($T + 0$, $T + 10$, and $T + 20$) were included in statistical analysis but are omitted for clarity. Latency measured from extracellular ON1 recording in response to ipsilateral free-field sound stimuli. $T + X = \text{dB above threshold.}$ Mean ± SE; $n = 5$ individuals; 30 stimulus repetitions at each frequency and each intensity.

C and D, error bars (= SE) for other intensities are similar to those shown.
only if they occurred within a “counting window” beginning \(\pm 1-2 \text{ ms}\) before the onset and after the offset of the sound-evoked response. Response onset and offset were determined, for each individual, by examining raster plots of AN1 spikes for all stimuli in the experiment. For example, the counting window for the responses shown in Fig. 4A1 was 11–47 ms, while for Fig. 4A2, the window was 13–50 ms. On average, counting windows began 13.0 ms after sound onset and terminated 47.9 ms after sound onset; all windows were \(\pm 30 \text{ ms}\) in duration. We also used interspike interval as an indicator of the onset of sound-evoked responses. Examination of a number of responses showed that instantaneous firing frequency typically rose immediately to 100 Hz shortly after stimulus onset but rarely reached this value before the stimulus. Accordingly, we identified the onset of a driven response as the time of occurrence of the first AN1 spike of the first

**FIG. 4.** A, 1 and 2: representative raster plots from 2 individuals showing AN1 response as a function of timing (shown by □ and ◁) and intensity (shown for 0-ms lag) of 4.5-kHz ON1-ipsilateral stimuli. □, “normal” timing of stimuli, i.e., simultaneous presentation of ON1- and AN1-ipsilateral stimuli. Ten stimulus repetitions at each intensity. AN1-ipsilateral stimuli were 4.5-kHz, 70-dB SPL sound pulses (30 ms) in all cases. In this and subsequent raster plots, each row is aligned to the onset of AN1-ipsilateral stimuli, which shifts within a 100-ms recording window (○; spikes may precede these markers due to small software-generated variations in the position of the recording window relative to the stimulus). B: AN1 spike counts. Mean (± SE; 85 dB SPL); \(n = 8\) individuals. C: AN1 latency. Mean (± SE; 85 dB SPL) \(n = 6\) individuals. For clarity, error bars are shown only for 85 dB SPL in B and C; they are similar for other intensities. In this and subsequent figures, negative lag values indicate ON1-ipsilateral stimuli occurred before AN1-ipsilateral stimuli, while positive lag values indicate ON1-ipsilateral stimuli occurred after AN1-ipsilateral stimuli.
pair separated by <10 ms. The analysis of AN1 latency with this method gave similar results to those using the “counting window” method; only the latter are shown. These procedures were not necessary for ON1 or AN2 because background activity was lower, and recorded signals larger, than for AN1.

RESULTS

ON1’s latency varies significantly with sound frequency [ANOVA; n = 5, F(19, 634) = 14.6, P < 0.01], and is longest near 4.5 kHz (Fig. 3). Latency decreases as sound intensity increases but remains longer around 4.5 kHz than for other sound frequencies. The difference in ON1’s latency at 4.5 kHz and ultrasound is ~5–10 ms, similar to that previously reported (Pollack 1994). The specific increase in ON1’s latency at 4.5 kHz, the dominant frequency of songs in this species, suggests that the delay might be functionally important in enhancing binaural contrast for song stimuli. We examined the effects of the timing of ON1’s response on two of its putative postsynaptic targets, AN1 and AN2, both of which have been shown to play roles in phonotaxis (see INTRODUCTION).

A particular AN1 or AN2 is excited by input from the opposite ear than the ON1 that inhibits it. For example, the left AN1/AN2 are excited by the left ear, but they are inhibited by the right ON1, which is excited by the right ear. Under free-field conditions, sound arrives at both ears nearly simultaneously (the maximum interaural delay, due to sound propagation time, is ~30 μs), and the relative timing with which ipsilaterally derived excitation and contralaterally derived inhibition arrive at the ascending neurons is determined by the characteristics of the neurons delivering these inputs. Using leg phones, however, we could manipulate the relative timing of ipsilateral, excitatory and contralateral, inhibitory inputs to AN1/AN2 by adjusting the timing of stimulation of each ear, and thereby investigate whether the normal delay in ON1’s response to cricket-like stimuli is functionally important. For example, the normal delay can be “subtracted,” or exaggerated, by advancing or retarding, respectively, the ON1-ipsilateral stimulus relative to the AN1-ipsilateral stimulus. To emulate the effects of sounds emitted at different azimuths relative to the cricket, we also controlled the stimulus intensity at each ear. Under free-field conditions, interaural sound intensity differences of up to 20 dB SPL can arise depending on sound azimuth (Michelsen et al. 1994). Accordingly, we presented ON1-ipsilateral stimuli at intensities 0–15 dB SPL greater than AN1/AN2-ipsilateral stimuli.

Figure 4 illustrates the effect of changes in the relative timing and intensity of stimuli to the two ears on the inhibition of AN1. In Fig. 4A, I and 2, two examples are illustrated as raster plots. Each of the vertically stacked sections of these figures represents a different time relationship between the stimuli at the two ears. The AN1-ipsilateral stimulus was delivered at time = 0 ms throughout (as indicated on the abscissa), and the timing of the ON1-ipsilateral stimulus is indicated by □ (except when both stimuli were simultaneous, where the ON1-ipsilateral stimulus is indicated by □). AN1-ipsilateral stimulus intensity was held constant at 70 dB SPL, and ON1-ipsilateral intensity varied as indicated for the section where the two stimuli were simultaneous.

AN1 spike count varies significantly with the relative timing of stimuli to the two ears [ANOVA, n = 6, F(8, 180) = 19.50, P < 0.01]. Although AN1’s response is usually smallest when ON1-ipsilateral stimuli were presented before AN1-ipsilateral stimuli (negative lag values; Fig. 4B), the temporal window for inhibition is fairly broad (Fig. 4A) (see also Horsemann and Huber 1994a; Wiese and Eilts 1985). Responses for lag values from ~20 to 5 ms (all ON1-ipsilateral stimulus intensities pooled) do not differ significantly from each other (Tukey HSD post hoc test on lag, P > 0.05). Increasing the intensity of ON1-ipsilateral stimuli significantly decreases the number of AN1 spikes [ANOVA, n = 6, F(3, 180) = 14.50, P < 0.01]. Although the relative timing and intensity of the two stimuli both affect AN1 spike counts, there is no significant interaction between these two effects, i.e., the effect of timing is similar at different intensities [ANOVA, n = 8, F(24, 234) = 0.37, P = 0.997].

AN1’s latency also varies significantly with the timing of the ON1-ipsilateral stimulus [ANOVA, n = 6, F(8, 170) = 25.61, P < 0.01]. AN1 latency significantly increases with ON1-ipsilateral stimulus intensity [ANOVA, n = 6, F(3, 180) = 3.26, P < 0.05], but there is no significant interaction between ON1-ipsilateral timing and ON1-ipsilateral intensity on AN1’s latency [ANOVA, n = 6, F(24, 180) = 0.58, P = 0.94]. AN1’s latency increases by ≤10 ms when the ON1-ipsilateral stimulus is advanced (Fig. 4C) compared with when ON1-ipsilateral stimuli are delayed or simultaneous with AN1-ipsilateral stimuli (and thus cannot interfere with the start of AN1’s response). The increase in latency, like the change in AN1’s spike count, occurs over a broad time window. There is a striking discontinuity between negative ON1-ipsilateral lag values, which increase AN1 latency, and all others, which do not.

In addition to increasing AN1’s latency, advancing the onset of contralateral inhibition increases the variability of latency (Fig. 5). The coefficient of variation of AN1 latency varies significantly with the timing of ON1-ipsilateral stimuli [ANOVA, n = 6, F(8, 180) = 8.22, P < 0.01]. When intensity of the ON1-ipsilateral stimulus is greater than that of the AN1-ipsilateral stimulus (as would occur for a lateral sound source), AN1 latency is, on average, most variable when the ON1-ipsilateral stimulus is advanced by 5 ms (Fig. 5). Nevertheless, a post hoc comparison on the effect of lag (pooling all ON1-ipsilateral stimulus intensities) shows the variability of 0 ms lag is not significantly different from any other lag value (Tukey HSD test, P values = 0.10–0.99). The intensity of ON1-ipsilateral stimuli does not significantly affect the variability of AN1 latency [ANOVA, n = 6, F(3, 180) = 1.21, P = 0.32].

FIG. 5. Variability of AN1 latency changes significantly with lag. Points show mean and SE for coefficient of variation of latency (n = 6 individuals). Intensity scale refers to ON1-ipsilateral stimulus; AN1-ipsilateral stimulus intensity was 70 dB SPL throughout.
nor is there any significant interaction between the timing and intensity of ON1-ipsilateral stimuli on AN1 latency [ANOVA, $n = 6$, $F(24, 180) = 0.60$, $P = 0.93$]. When the onset of AN1’s response is unaffected by contralateral inhibition (i.e., 20-ms lag), the standard deviation of AN1’s latency ranges from 0.73 to 2.92 ms (calculated separately for each of 6 crickets).

The results for AN2 parallel those for AN1. AN2 receives contralateral inhibition (Fig. 6), and the reduction in spike count is significantly altered by the timing of ON1-ipsilateral stimuli [ANOVA, $n = 6$, $F(8, 180) = 4.36$, $P < 0.01$]. The latency of AN2 is significantly altered by the timing of ON1-ipsilateral stimuli [ANOVA, $n = 6$, $F(8, 177) = 22.83$, $P < 0.01$]. AN2 latency does not significantly vary with the intensity of ON1-ipsilateral stimuli [ANOVA, $n = 6$, $F(3, 177) = 1.26$, $P = 0.29$]. Unlike AN1, however, AN2’s latency is significantly greater for ON1-ipsilateral stimuli with a lag of 0 ms than for positive lag values (Tukey HSD test, $P$ values $< 0.01$). This is because AN2 (which is most sensitive to ultrasound) (Moiseff and Hoy 1983; Nolen and Hoy 1987) responds to 4.5 kHz with a long latency, and thus contralateral inhibition arrives early enough to affect the onset of AN2 response even with 0 ms lag.

It is clear from the preceding experiments and previous work that AN1 (Horseman and Huber 1994a; Wiese and Eilts 1985) and AN2 (Moiseff and Hoy 1983; Selverston et al. 1985) are inhibited by contralateral low-frequency sounds, but the extent to which ON1 is responsible for this inhibition is not known. We recorded from AN1 and AN2 while making intracellular recordings from ON1. Hyperpolarizing ON1 with 10-nA current abolishes its spiking response (monitored both intracellu-

**FIG. 6.** A, 1 and 2: 2 representative raster plots of AN2 response. Format same as Fig. 4, except that 5 stimuli, not 10, are presented for a given intensity of ON1-ipsilateral stimuli at each lag. AN2-ipsilateral stimulus: 4.5 kHz, 80 dB SPL throughout. B: mean AN2 spike counts. $n = 6$ individuals. C: mean AN2 latency. $n = 6$ individuals. For clarity, error bars are shown only for 95 dB SPL; they are similar for other intensities.
larly and extracellularly), and reversibly increases the spike counts of AN1 and AN2 (Figs. 7 and 8, Table 1). No evidence of residual contralateral inhibition (e.g., change in latency) is visible in AN1 or AN2 when ON1 is hyperpolarized (Figs. 7 and 8). If there were other sources of contralateral inhibition besides ON1, then hyperpolarization of ON1 might be expected only partially to restore the responses of AN1 and AN2 toward their monaural response levels. In fact, in all but one case, spike counts in response to binaural stimulation, but with ON1 hyperpolarized, were slightly greater than those in response to monaural stimulation, although not always significantly so (Table 1).

**FIG. 7.** A: raster plot of AN1 response to 4.5-kHz, 70-dB SPL sound stimuli. As in Fig. 4, AN1 response varies with latency and intensity of contralateral sound stimuli; 10 stimuli for each intensity at each lag. B: raster plot of data from same individual but with ON1 spiking abolished by injecting 10-nA hyperpolarizing current (monitored by recording ON1 extracellularly). Data in A and B were recorded in an interleaved fashion (i.e., 20-ms lag without current, 20-ms lag with injected current, 15-ms lag without current, and so on), rather than sequentially. See also Table 1.

**FIG. 8.** A: raster plot of AN2 response to 4.5-kHz, 80-dB SPL sound stimuli. As in Fig. 6, response varies with latency and intensity of contralateral sound stimuli. Ten stimuli for each intensity of a given lag. B: raster plot of same individual but with ON1 spiking abolished by injecting 10-nA hyperpolarizing current (monitored by recording ON1 extracellularly). Data in A and B were recorded in an interleaved fashion. See also Table 1.
contrast enhancement of the spike counts of AN1 (Fig. 4) from ON1 on ascending cells, however, is not critical for related to processing of song. The exact timing of inhibition suggests that ON1’s long latency to low-frequency sound is important of this sound frequency in this species (i.e., songs).

DISCUSSION

ON1’s latency is greater for stimulus frequencies near 4.5 kHz than for other frequencies (Fig. 3). The known behavioral importance of this sound frequency in this species (i.e., songs) (Balakrishnan and Pollack 1996; Hill et al. 1972) strongly suggests that ON1’s long latency to low-frequency sound is related to processing of song. The exact timing of inhibition from ON1 on ascending cells, however, is not critical for contrast enhancement of the spike counts of AN1 (Fig. 4B) or AN2 (Fig. 6B) or of the latency of AN2 (Fig. 6C). For these measures, ON1 would be as effective in inhibiting its targets (if not slightly more so) if it did not have a long latency to low-frequency sound.

The most striking effect of the normal (i.e., delayed) timing of ON1’s response is that it spares any effect on AN1’s latency; inhibition from ON1 arrives too late to affect the first spike of AN1’s response. Interaural latency difference is a potential cue for sound localization. Although a cricket’s ears are too close together to produce substantial interaural differences in time of arrival of sound (the maximum difference is only ~30 μs), interaural latency differences of several milliseconds are nevertheless possible because the latency of cricket auditory receptor neurons varies with stimulus intensity (Esch et al. 1980; Givois and Pollack 1999). The effective sound intensity at the two ears may differ, in an azimuth-dependent fashion, by ≥20 dB SPL (Michelsen 1994), generating latency differences of up to several milliseconds. In acridid grasshoppers, interaural latency differences (also generated by direction-dependent interaural intensity differences) (Mörchen and Mörchen 1978) can serve as a cue for sound localization. Grasshoppers performing positive phototaxis can detect an interaural latency difference of 0.4 ms (Helversen and Rheinlaender 1988), and their auditory interneurons are likewise sensitive to latency differences of <1 ms (Rheinlaender and Mörchen 1979).

If crickets use interaural latency difference as a directional cue, it might be advantageous to maximize the latency difference produced by a given stimulus, yet ON1’s delayed response to 4.5 kHz does the opposite. When we “subtracted” the additional latency to 4.5 kHz from ON1’s response, by advancing the stimulus by 5–10 ms, the latency of the contralateral AN1 increased (Fig. 4C). If, instead of being delayed, ON1’s latency to cricket-like frequencies was similar to that for other sounds, then the latency of the contralateral AN1 (and thus the interaural latency difference) would increase by ~4–8 ms (Fig. 4C). Clearly, the delay in ON1’s response to cricket-like sound does not amplify interaural latency difference.

One possibility is that the delay in ON1’s response helps to preserve the precision of the response onset of the contralateral AN1. Advancing ON1’s response by 5 ms to compensate for the normal delay increased the variability of AN1’s latency (Fig. 5). Increased variation in the latency of one component of a binaural comparison would make a latency-based estimate of sound azimuth less accurate. Nevertheless, variability with natural timing (i.e., 0-ms lag) was not significantly lower than it would be if ON1’s response were not delayed.

Although we could find no strong advantage, for binaural contrast enhancement, of the delay in ON1’s response, we note that neither does this delay substantially degrade the binaural difference in spike counts of the ascending neurons. Thus even if the delay is not advantageous, it may at least be tolerable.

If functional considerations cannot fully explain ON1’s long latency to low-frequency sounds, then mechanistic explanations might prove more illuminating. Several mechanisms could explain ON1’s increased latency to low-frequency sound. Pollack (1994) suggested that auditory receptors tuned to low frequencies might differ in conduction velocity compared with receptors tuned to high frequencies, but Pollack and Faulkes (1998) could find no evidence for this. The remaining hypotheses are, briefly, that there is frequency-specific inhibition of ON1’s response, either postsynaptic or presynaptic; that integrative properties of ON1 create differences in excitatory postsynaptic potential (EPSP) shape and spike onset; and that one or more interneurons are interposed between ON1 and receptors that are tuned to cricket-like sounds. These hypotheses are examined in detail elsewhere (Faulkes and Pollack 1998). We focus here only on the last of these, which is favored by experimental evidence. Input to ON1 from receptors tuned to bat-like sounds is direct, whereas input from cricket-song-tuned receptors appears to be mainly polysynaptic (Faulkes and Pollack 1998). The putative interneurons interposed between receptors and ON1 might serve several roles in addition to delaying the delivery of ON1’s inhibition to its targets. For example, they may be required for the apparently more complex processing of communication signals, which entails, e.g., analyzing the temporal pattern of sound pulses (Pollack 1998).

The separation of inputs from cricket- and bat-tuned pathways might also allow their independent regulation, e.g., by neuromodulators targeted to the interposed interneurons. This could be advantageous considering that ON1 is dually tuned to sound frequencies that evoke disparate behaviors (i.e., positive and negative phototaxis). The delay in ON1’s response to cricket-like sound might thus be an unavoidable by-product of other circuitry requirements rather than a specialization to permit accurate measurement of interaural latency difference.

We demonstrate for the first time that ON1 can account for all of the contralateral inhibition of AN1. We also confirm that ON1 inhibits AN2 (and is apparently the only source of contralateral inhibition), in agreement with Selverston et al. (1985), but in contrast to Harrison et al. (1988). In most instances, AN1 and AN2 responded more strongly to binaural stimulation, with ON1 hyperpolarized, than to monaural stimulation, with ON1 left undisturbed. One possible explanation for this is that ON1 releases transmitter tonically, and that this is suppressed by hyperpolarization. This is consistent with an earlier suggestion of nonspiking transmission by ON1 (Selverston et al. 1985). A second possibility is that removal of ON1-mediated inhibition unmasked contralateral excitatory in-

| Table 1. ON1 can account for contralateral inhibition to AN1 and AN2 |
|----------------|----------------|
| **Spike Counts** |
| **Cell** | **Animal** | **Normal binaural** | **ON1 hyperpolarized** | **Monaural** |
| AN1 | I | 8.9 ± 0.80 | 12.10 ± 0.74 | 11.06 ± 0.29 |
| | 2 | 7.0 ± 0.70 | 8.30 ± 0.70 | 8.86 ± 0.39 |
| | 3 | 7.42 ± 0.32 | 12.36 ± 0.30 | 8.96 ± 0.30 † |
| AN2 | I | 6.1 ± 0.57 | 7.80 ± 0.33 | 6.52 ± 0.16 † |
| | 2 | 7.62 ± 0.24 | 10.60 ± 0.32 | 8.80 ± 0.38 † |

Values are means ± SE. * AN1-ipsilateral stimuli = 70 dB SPL, 4.5 kHz. AN2-ipsilateral stimuli = 90 dB SPL, 4.5 kHz. ON1-ipsilateral stimuli = 80 dB SPL, 4.5 kHz. † Significantly different from “ON1 hyperpolarized;” t-test, P < 0.05.

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puts to AN1/AN2 (Selverston et al. 1985), which might contribute to the increase in their response.

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