Fast Inhibition Alters First Spike Timing in Auditory Brainstem Neurons

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INTRODUCTION

The physiological characteristics of T stellate neurons located in the first station of the central auditory pathway, the ventral cochlear nucleus (VCN), make them well-suited to code frequency information in terms of their discharge rate. They have dendritic arborizations contained within isofrequency laminae (Doucet and Ryugo 1997; Oertel et al. 1990), are narrowly tuned to tonal frequency, and their discharge rates are monotonically related to the spectral energy near their characteristic frequency (CF) (Blackburn and Sachs 1990; Yu and Young 2000). In response to CF tone bursts, these cells (termed choppers) exhibit an intrinsic and regular discharge pattern that is unrelated to stimulus frequency. Their discharge regularity is either maintained throughout a tone or decreases shortly after tone onset (sustained (C_S) and transient (C_T) regularity is either maintained throughout a tone or decreases pattern that is unrelated to stimulus frequency. Their discharge (termed choppers) exhibit an intrinsic and regular discharge and Young 2000). In response to CF tone bursts, these cells characteristic frequency (CF) (Blackburn and Sachs 1990; Yu discharge rate in T stellate cells is unlikely to be suppressed during depolarization. This resulted in a delay in the initial action potential produced by T stellate cells. This ability of fast inhibition to alter the first spike timing of a T stellate neuron was confirmed by electrically activating the D stellate cell pathway that arises in the contralateral cochlear nucleus. Delay was also induced when two tones were presented: one at CF and one outside the frequency response area of the T stellate neuron. These findings suggest that the traditional view of lateral inhibition within the VCN should incorporate delay as one of its principle outcomes.

METHODS

Experiments were performed on 9 Long-Evans rats and 12 Hooded Wistar rats weighing 240–350 g. Details of the experimental protocol have been outlined previously (Paolini and Clark 1999). Briefly, animals were anesthetized with intraperitoneal urethane in water (20% wt/vol; total dose 2.6 g/kg; Sigma, Sydney, Australia), and supplemental doses were administered during the experiment if a corneal or paw reflex was observed. These procedures were in accordance with the Royal Victorian Eye and Ear Hospital Animal Research Ethics Committee guidelines (Project 95037). Following a craniotomy, the cerebellum was aspirated to expose a portion of the brain stem with the cochlear nucleus (CN) at the lateral external, enabling recording electrodes to be inserted into the VCN under visual control. Electrodes were oriented in a caudal-rostral direction away from the dorsal CN and octopus cell area of the posterior VCN. In some animals, a stimulating electrode was also inserted into the contralateral CN to activate commissural D stellate cell inhibition, as detailed by Needham and Paolini (2003). In this study, D stellate inhibition was induced by electrical stimuli consisting of 300–μA pulses.

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Microelectrodes (quartz thin-walled; 1.0 mm OD; Sutter Instrument, Novato, CA) were filled with 1 M potassium acetate (70–80 mΩ) and advanced through the VCN in 2-μm steps by a motorized microdrive (Sutter Instrument), while presenting 70–80 dB white noise bursts (bandwidth, 0.45 Hz–50 kHz, 50-ms duration, 5-ms rise–fall time, repetition interval of 500 ms). Stable intracellular impalements were signaled by a prolonged (>3 min), stable drop (>30 mV) in the DC level and the presence of synaptic or large APs (>25 mV) with monophasic rise and fall times. Intracellular record-ings typically lasted 20–40 min (maximum, 150 min).

Acoustic stimuli were synthesized digitally and generated by a Beyer DT48 transducer (Beyerdynamic, Farmingdale, NY) and controlled by a Tucker-Davis signal generator (Tucker-Davis Technologies, Gainesville, FL). The acoustic system was calibrated using a Bruel and Kjaer measuring amplifier (type 2606, Bruel and Kjaer, Naerum, Denmark) and a 0.5-in condenser microphone, coupled to a small probe tube positioned within the ear bar tube ~3 mm from the tympanic membrane. This enabled acoustic input to be measured in decibel sound pressure level (SPL).

Neurons were recorded intracellularly using an Axoclamp 2B amplifier (Axon Instruments, Union City, CA). The department’s “Neurophysiology Laboratory System” (NLS software, by R. E. Millard), run on a PC, was used to control the Tucker-Davis unit and collate AP time information. A MacLab 4S data acquisition system (AD Instruments, Sydney, Australia) was used to store membrane potential records (traces) at a bandwidth of 20 or 40 kHz. Once impaled, the neuron’s CF and input–output function at CF were determined (Liberman 1978). CF tone bursts (50-ms duration, 5-ms rise–fall time, repetition interval of 100 ms, usually 50 repetitions) were delivered in 5- or 10-dB steps in a sequential manner from subthreshold SPLs to SPLs that produced discharge rate saturation.

Classification of neurons was possible on the basis of their intracellular response profile. Averaging and superimposition of intracellu-lar traces also aided in classification of neurons as did poststimulus time, interspike interval, and period histograms that were constructed on-line. The regularity of chopping behavior was determined, where possible, by calculating the CV (to suprathreshold CF tones) derived from the interspike interval histogram (Blackburn and Sachs 1989; Young et al. 1988). This measure differentiates between two chopper subtypes, both of which are recorded from T stellate cells. Neurons with low, consistent CV values (<0.2) were classified as C_T while C_S neurons were those with an initial, low CV (<0.2) that increased during the tone (0.2 < CV < 0.5). Contour plots derived from the average intracellular traces over 25 stimulus repetitions were used to examine the relationship between stimulus SPL and membrane po-tential during CF tone presentation. The intracellular response profile of all chopper neurons recorded in this study were distinct from that of bushy cells (Paolini and Clark 1999; Paolini et al. 1997).

R E S U L T S

Intracellular recordings were made from 29 stellate neurons: 20 showed a C_T or C_S response (T stellate cells) and 9 showed an O_C response (D stellate cells). While all neurons had similar average resting potentials (~60.9 ± 1.4 mV), AP heights (39.8 ± 1.9 mV), and CFs (9.1 ± 1.0 kHz), O_C neurons had significantly higher depolarization and AP thresholds than C_S/T neurons. Specifically, the minimal SPLs that evoked depolarization in C_S/T and O_C neurons were 31.7 ± 2.9 and 45.6 ± 2.7 dB, respectively (t25 = 3.02, P < 0.05), and the minimal SPLs that evoked AP generation were 41.8 ± 3.8 and 56.6 ± 2.2 dB, respectively (t25 = 2.5, P < 0.05).

Figure 1, A–H, shows that neurons with low CV are distinguishable from those with high CV. In particular, the neuronal response of the two subtypes is quite different. Neurons with low CV (Fig. 1A–D) had higher thresholds, both neuron types (C_S/T and O_C) had similar latencies for depolarization across a range of SPLs; however, a significant difference was observed at 30 dB above threshold (t18 = 2.1, P < 0.05).

In response to CF tones, the activation threshold for O_C cells was reached relative to C_S/T cells (Fig. 1, D–G, vertical dashed lines), but similar depolarization and first AP latencies occur in both cell types (Fig. 1, H, and I). Thus at CF, inhibition would arrive first in C_S/T cells because there is a short synaptic delay. However, we would predict that the relative timing of inhibition and excitation would be different to off-CF tonal frequencies because D stellate neurons are broadly tuned. At frequencies removed from CF, O_C cell activation threshold would be lower than T stellate cells with similar CFs. In this situation, it would be possible for D stellate cells to inhibit T stellate cells before initiation of their first AP.

This expectation was confirmed by examination of the intracellular traces and contour plots depicting regions of hyperpolarization and depolarization in C_S/T neurons to off-CF tones. In the two neurons shown in Fig. 2, A–H, fast, short-duration inhibition is apparent at frequencies removed from CF and occurs before generation of the first spike. Inhibition of this type at frequencies removed from CF was observed in 60% (12/20) of T stellate cells tested at 80 dB SPL. Figure 2E shows that, if inhibition arrives during depolarization in response to an off-CF tone, the course of depolarization is altered (asterisk) and it takes longer for the cell to reach AP firing threshold (arrow); that is, the first AP is delayed. The time course of this inhibition precisely matched that previously described for D stellate inhibition produced by contralateral click stimulation (Fig. 2D, top, red trace) (Needham and Paolini 2003). In these same two cells, a long time base reveals an additional long latency, slow and sustained hyperpolarization to off-CF tones (Fig. 2, I and J, top and middle). Sustained hyperpolarization of this type was observed in all T stellate cells in which fast inhibition was also observed. In addition, it was sometimes observed in the absence of fast, short-duration inhibition (Fig. 2, I and J, bottom). These two hyperpolarization events have different latencies and time courses, and this is particularly
When the short-duration inhibition at tone onset is compared with the slow time course of the return to RMP following tone cessation (Fig. 2J), the influence of O \( \text{C} \) (D stellate) inhibition on the timing of sound-evoked APs in C\( \text{S/T} \) (T stellate) cells was examined directly by electrically stimulating the contralateral cochlear nucleus (Needham and Paolini 2003). This paradigm evokes an inhibitory postsynaptic potential (IPSP) in ipsilaterally recorded CS/T neurons (Fig. 3A\( \text{top} \), *). By manipulating the interval between click and electrical stimulation, an IPSP can occur prior to AP generation in T stellate cells and significantly delay it (Fig. 3, A and B).

Figure 4 shows the effect on the latency of T stellate cell activation when two-tone stimuli were presented. When a CF tone is presented in conjunction with a second tone that fell outside the cell’s response area and within the lateral inhibitory domain, the first AP is delayed. The inhibition evoked by the second tone interacts with the excitation and has the overall effect of increasing the duration of depolarization before spike activation (Fig. 4, B, C, E, and F). This effect was observed when the second tone was presented at a SPL that did not in itself produce inhibition (Fig. 4, C and D). This is consistent with the fact that D stellate cells are facilitated by two-tone stimuli (Jiang et al. 1996). The timing of inputs from the auditory nerve does not appear to be altered in this two-tone paradigm because there are similar depolarization latencies observed under all conditions (Fig. 4, A–C). This indicates that AP delay is not simply a reflection of two-tone suppression effects that occur within the cochlea (Ruggero et al. 1992). A decrease in spike regularity associated with two-tone stimulation also suggests that inhibition mediates this delay in AP activation rather than it being a property of peripheral suppression (Blackburn and Sachs 1992).

**Discussion**

We have shown that the main inhibitory neurons of the VCN—D stellate cells—can delay the first AP produced by one of the major VCN projection neurons, the T stellate cells. We showed that discrete regions of hyperpolarization mark the edges of many T stellate cells, consistent with the traditional view of lateral inhibition in this cell type (Ferragamo et al. 1998; Fujino and Oertel 2001; Rhode and Greenberg 1994). These regions of hyperpolarization were short-lived and fol-
FIG. 2. Relationship between inhibition and excitation to tonal frequencies near the edge of T stellate cells’ frequency response areas. Intracellular responses of 2 T stellate (C7) neurons (A–C and D–H, respectively) to tones presented over a range of frequencies. Vertical dashed lines indicate minimum depolarization latency; i.e., the point of upward deflection in the average membrane potential at CF. Average traces (A), single traces (B), and a contour plot (C) indicate the presence of short-duration inhibitory postsynaptic potentials (IPSPs) when tones at the low-frequency edge of this neuron’s response area were presented (70 dB). Average traces (D) and a contour plot (H) show the presence of similar IPSPs when tones at the high-frequency edge of a 2nd neuron’s response area were presented (80 dB). Time course of the inhibition observed at 8 kHz (D) matches that previously described in a T stellate cell and is shown to originate from a contralateral D stellate cell that was stimulated with 60-dB clicks (average trace in red from neuron 715–007) (Needham and Paolini 2003).

E–G: average membrane potential changes as a function of time collapsed over the frequency ranges indicated in H (e–g). In E, the arrow indicates AP threshold, and the asterisk indicates the change in the course of depolarization when inhibition is present. E: contour plots (with a long time base) of 3 T stellate neurons showing the extent of hyperpolarization and depolarization over a range of frequencies during and after tone presentation. J: corresponding average intracellular traces to tones just outside the excitatory frequency response area of the neurons shown in I. On this time base (top and middle), a slower hyperpolarization (indicated as 2) is seen in addition to the fast IPSP (indicated as 1). This slower hyperpolarization was also observed in the absence of a fast IPSP in some cells (bottom). All contour plots show membrane potential from resting (RMP, green) in a depolarizing and hyperpolarizing direction. Key in H applies to C, E–G, and I (C, RMP = −63 mV, range −5 to +4 mV; H, RMP = −63 mV, range −5 to +5 mV; I, bottom). RMP = −56 mV, range −5 to +4 mV). Average traces consist of ≥20 repetitions of a given stimulus. Cal.D: max amp = 1.8, 3, 25 mV, respectively.
followed the known time course of D stellate inhibition (Needham and Paolini 2003). At the edge of a T stellate cell’s frequency response area, fast inhibition occurred during initial depolarization and was associated with longer AP latencies to off-CF tones. When a CF tone was presented in conjunction with a tone that fell outside a cell’s response area, a delay in the T stellate cell’s first AP was produced. This delay was not a result of a shift in threshold because the intracellular traces indicated that the threshold of depolarization was very similar in the two-tone and CF tone alone conditions. Therefore

**FIG. 3.** Latency changes in click-evoked APs of T stellate neurons resulting from D stellate inhibition. *A:* effect of electrical stimulation (S; 300-μA pulses) of the contralateral CN at 2 time points on a C T cell’s AP latency to ipsilaterally-presented clicks (90 dB). This paradigm evokes inhibition in T stellate cells, shown to be mediated by contralateral O C neurons (Needham and Paolini 2003). Three overlaid traces are shown. Top: inhibition (asterisk) occurs after cell’s AP. Bottom: inhibition prolongs depolarisation (arrow) and AP generation (vertical line). *B:* change in average latencies (15 repetitions) for 2 C T cells in response to interaction of electrically evoked inhibition with ipsilaterally induced APs. White bars indicate where ipsilateral APs were induced prior to the presentation of contralateral electrical stimulation (no interaction of stimuli). Black bars depict average latencies when excitation was timed to occur simultaneous to the contralaterally derived IPSP (overlap of stimuli). Horizontal line indicates overall mean in 1st condition; differences were statistically significant (Mann-Whitney rank-sum test, *P* < 0.05).

**FIG. 4.** Neural delay resulting from 2-tone stimulation in a C T neuron. *A–C:* color contour plots showing membrane potential as a function of CF tone SPL and time from stimulus onset for a CF (3.5 kHz) tone alone (*A*) and a CF tone combined with a 7-kHz tone at either 80 (B) or 60 dB (C). Plots show average membrane potential (25 repetitions) from resting (RMP = −63 mV, white) in a depolarizing direction (key in *A* shows range from 0 to +20 mV). *D:* average intracellular traces showing neuron’s response to a 60- and 80-dB 7-kHz tone alone: an IPSP was observed in response to the latter SPL only. Vertical solid line indicates the beginning of the 1st AP to an 80-dB CF tone (*A*) and corresponds with the latency of the IPSP shown in *D*. Vertical dashed line indicates the beginning of the 1st APs in the 2-tone conditions (*B* and *C*), when the CF tone was presented at 80 dB. Time between vertical lines indicates the delay of the 1st AP resulting from 2-tone stimulation. *E:* average membrane potential as a function of time for the 3 conditions when the CF tone was presented at a single SPL (80 dB). These average traces are equivalent to a horizontal “slice” through each of the contour plots in *A–C*. Black trace shows the response to an 80-dB CF tone alone; red trace shows the response to an 80-dB CF tone combined with an 80-dB 7-kHz tone; green trace shows the response to an 80-dB CF tone combined with a 60-dB 7-kHz tone. *F:* same format as *E* but with the CF tone at 65 dB SPL, showing that the delaying effect of the 2nd tone is consistent across CF SPL.
activation of a lateral inhibitory domain can delay a T stellate cell’s response to an optimal (CF) tone.

In addition to the fast, short-duration inhibition, we showed that a longer latency, sustained hyperpolarization was evoked by off-CF tones in the same cells. The latter type of hyperpolarization would explain the sustained suppression of spontaneous activity (or activity evoked by continuous background noise) by off-CF tone bursts in chopper cells (Blackburn and Sachs 1992; Rhode and Greenberg 1994; Smith and Rhode 1989). This sustained component may reflect input from the subset of D stellate cells that show low level sustained activity throughout a tone burst (Paolini and Clark 1999; Rhode and Smith 1986; Winter and Palmer 1995). Alternatively, the different latencies and time courses of these two inhibitory events might reflect different sources. This conclusion is supported by the fact that sustained inhibition was sometimes observed in isolation and that T stellate cells receive inhibitory inputs from the DCN and other brain stem regions (Benson and Potashner 1990; Oostapoff et al. 1997; Wickesberg and Oertel 1990; Wickesberg et al. 1991). Regardless of the source of this slower inhibitory component, the presence and effects of fast, short-duration inhibition described here indicate that the classic view of lateral inhibition in VCN T stellate cells should include first spike delay as one of its outcomes.

The effects we observed are consistent with the broad frequency tuning of D stellate cells and the response latency data that indicated D stellate cells can be activated before T stellate cells to frequencies at the edge of a T stellate cell’s response area. We used the fast, monosynaptic connection between D stellate and T stellate cells in the opposite cochlear nucleus (Needham and Paolini 2003) to confirm that, when inhibition occurred during depolarization in a T stellate cell, the course of this event was altered, and the initial AP was significantly delayed. Overall, these results indicate that inhibition can alter the timing of tone-evoked APs in neurons at the lowest level of the auditory brain stem. The importance of the interaction between inhibition and excitation in determining spike timing has been revealed in previous studies of the auditory brain stem, including the inferior colliculus (Casseday et al. 2000; Faure et al. 2003) and the medial superior olive (Brand et al. 2002; Grothe et al. 1992).

Our results (Fig. 1), and those of other groups (Rhode and Smith 1986; Winter and Palmer 1995), indicate that D stellate cells have higher CF tone thresholds than T stellate cells. Therefore in response to a CF tone, inhibition would arrive after the first AP produced by a T stellate cell. In auditory cortex, inhibition following initial excitation improves temporal precision by reducing the spike time variability associated with the initial “barrage” of excitatory inputs that impinge on cortical neurons (Wehr and Zador 2003). A similar effect may be occurring in T stellate cells and is consistent with the findings of Young et al. (1988) who found very low first spike variability in VCN chopper neurons.

The classic view of lateral inhibition in T stellate cells depicts a wideband inhibitor (D stellate cell input) superimposed on its narrow frequency tuning curve (Ferragamo et al. 1998; Fujino and Oertel 2001). In higher auditory centers, surround inhibition can improve spectral selectivity (Jen et al. 2002; Yang et al. 1992); however, in T stellate cells, this does not appear to be the case. When glycinergic transmission is blocked (by iontophoretic application of the antagonist strychnine), there is very little or no change in the width of frequency tuning of T stellate neurons (Caspari et al. 1994). T stellate neurons also have similar widths of frequency tuning to primary-like neurons, another of the major projection neurons that show less extensive and intensive lateral inhibition than T stellate cells (Rhode and Greenberg 1994; Rhode and Smith 1986). In response to pure tones, our data indicate that the main effect of D stellate inhibition is to delay the response to tones at the edge of a cell’s frequency response area. This presumably results in a greater difference in the response latency to CF versus “edge” tones than would exist in the absence of inhibition. This outcome could form the basis of an improvement in spectral selectivity if neurons at the next level of the pathway were sensitive to the time of arrival of inputs. For example, a coincidence detector receiving tonotopically mapped inputs will be optimally activated by the subset of inputs that arrives during an initial, narrow time window. Empirically, and in principle, the relative timing of inputs can be just as crucial as response magnitude to feature extractors at various levels of the auditory pathway (Brand et al. 2002; Cariani 1994; Faure et al. 2003; Park and Pollak 1993; Wehr and Zador 2003).

Thus far, we have interpreted our results in terms of their pure tone responses, and these are not particularly relevant to the spectrally and temporally complex sounds that animals encounter in their environment. D stellate cells receive input from auditory nerve fibers across isofrequency lamina and are not optimally activated by pure tones. They can show more sustained responses and lower thresholds to broadband compared with narrowband sounds, although they do retain their prominent and precise onset response (Winter and Palmer 1995). D stellate cells also respond vigorously and precisely to amplitude modulated sounds, including synthetic vowels (Palmer and Winter 1993; Rhode 1994; Wang and Sachs 1994). Therefore the extent to which D stellate cells can alter the timing and/or discharge rate of T stellate neurons would depend on the complexity of the stimulus. Finally, the effect of D stellate cell activation would not be restricted to T stellate cells with matching CFs because these inhibitory neurons are known to project quite widely within the VCN (Palmer et al. 2003; Smith and Rhode 1989). Thus they have the potential to alter the timing of the first spike response of T stellate cells in other isofrequency lamina.

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