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Powerful, Onset Inhibition in the Ventral Nucleus of the Lateral Lemniscus

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Nayagam, David A. X., Janine C. Clarey, Antonio G. Paolini. Powerful, Onset Inhibition in the Ventral Nucleus of the Lateral Lemniscus. J Neurophysiol 94: 1651–1654, 2005. First published April 7, 2005; doi:10.1152/jn.00167.2005. The function of the ventral nucleus of the lateral lemniscus (VNLL), a secondary processing site within the auditory brain stem, is unclear. It is known to be a major source of inhibition to the inferior colliculus (IC). It is also thought to play a role in coding the temporal aspects of sound, such as onsets and the periodic components of complex stimuli. In vivo intracellular recordings from VNLL neurons (n = 56) in urethane anesthetized rats revealed the presence of large-amplitude, short-duration, onset inhibition in a subset of neurons (14.3%). This inhibition occurred before the first action potential (AP) elicited by noise or tone bursts, was broadly tuned to tonal frequency and was shown to delay the first AP. Our data suggest it is a result of an intrinsic circuit activated by the octopus cell pathway originating in the contralateral cochlear nucleus; this pathway is known to convey exquisitely timed and broadly tuned onset information. This powerful inhibition within the VNLL appears to control the timing of this structure’s inhibitory output to higher centers, which has important auditory processing outcomes. The circuit also provides a pathway for fast, broadly tuned, onset inhibition to the IC.

INTRODUCTION

Various lines of evidence indicate that inhibition plays as important a role as excitation in controlling spike timing in auditory nuclei (Brand et al. 2002; Casseday et al. 2000, 1994; Wehr and Zador 2003). One of the major inhibitory pathways within the auditory brain stem originates in the ventral nucleus of the lateral lemniscus (VNLL) (Saintmhairé and Baker 1990; Zhao and Wu 2001), a nucleus thought to play a role in temporal pattern processing (Covey and Casseday 1999; Oertel and Wickesberg 2002). This structure is a crucial integration site for a subset of fibers from the lower auditory brain stem en route to the inferior colliculus (IC) (Covey and Casseday 1999; Oertel and Wickesberg 2002; Wu 1999). VNLL neurons receive convergent excitatory input from a variety of cell types within the contralateral cochlear nucleus (CN) (Glendenning et al. 1981; Zhao and Wu 2001), including an exclusive projection from the octopus cell area (OCA) of the contralateral CN (Adams 1997; Schofield and Cant 1997). Octopus cells give rise to thick axons that terminate in large calyx-like synapses, akin to endbulbs of Held in other auditory nuclei (Adams 1997; Schofield and Cant 1997). These characteristics suggest that this pathway provides fast and faithful transmission of timing information (Batra and Fitzpatrick 1999; Zhao and Wu 2001). Octopus cells respond to the onsets of sounds with exquisitely timed responses (Godfrey et al. 1975; Rhode and Smith 1986), termed onset-ideal (O). This response pattern is a result of the detection of synchrony in auditory nerve fiber inputs representing a wide range of characteristic frequencies (CFs) (Golding et al. 1995; Oertel et al. 2000). The function of these cells and their projection to the VNLL is unknown, although they seem well suited to encode onsets, transients, and temporal features of complex, periodic stimuli (Oertel and Wickesberg 2002). The role of the VNLL’s projection to the IC (Kelly et al. 1998; Merchant and Berbel 1996), which is inhibitory (Batra and Fitzpatrick 2002; Oertel and Wickesberg 2002) is equally uncertain. We used in vivo intracellular recordings to investigate how VNLL cells utilize this well-timed onset information.

METHODS

Experiments were performed on 26 male Hooded Wistar (pigmented) rats weighing between 250 and 360 g. Animals were anesthetized with intraperitoneal aqueous urethan (20% wt/vol; total dose: 2.6 g/kg; Sigma, Sydney, Australia), and supplementary doses were administered during the experiment if a corneal or paw reflex was observed. Some animals were initially sedated with isoflurane (4–5% in 2% O2) prior to injection of urethan. The animal’s temperature was maintained at ~37.5°C by a thermostatically controlled heating pad. At the end of the recording session, the animal was intra-cardially perfused with 10% formalin. Brains were removed, postfixed in 10% formalin, and sectioned on a freezing sledge microtome (100 μm sections). Sections were stained for Nissl substance using thionin, and standard histological procedures were used to reconstruct and verify the location of electrode tracks and recorded units (Paolini et al. 2001). Reconstructed recording depths were referenced to the cortical surface as well as the point at which the electrode broke when it hit the pia underlying the ventral brain stem (Paolini et al. 2001). A dramatic drop in microelectrode impedance indicated this breaking point. All procedures were in accordance with the Royal Victorian Eye and Ear Hospital Animal Research Ethics Committee guidelines (project approval codes 95/037 and 04/104A).

Once the animal was deeply anesthetized, it was placed in a stereotaxic frame, and a pinhole craniotomy (area: ~4 mm2) was made using stereotaxic coordinates (Paxinos and Watson 1998) and skull suture landmarks as guides. Recording micropipettes were inserted dorsoventrally into the left hemisphere and traversed the cerebral cortex and IC before encountering the lateral lemniscus and its dorsal and ventral nuclei.

Intracellular neural responses were recorded using quartz glass micropipettes (1.0 mm OD, 0.7 mm ID, Sutter Instruments, Novato, CA), filled with 1 M potassium acetate and with impedances ranging from 30 to 70 MΩ. The amplified (Axoclamp 2B amplifier, Axon Instruments, Novato, CA) and filtered signal (output bandwidth = 1 kHz) from the microelectrode was played through a speaker and displayed on a Tektronix 465 storage oscilloscope (Beaverton, OR).

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The microelectrode was advanced remotely, using a motorized microdrive (MPC-100; Sutter Instruments), in small steps (2 μm) once the VNLL was encountered, but occasionally intracellular recordings were made from the IC (n = 14).

Stimuli presented to both ears were 50 ms in duration with 5 ms rise-fall times and a 500 ms repetition interval; the left and right transducer outputs were offset by 200 ms. Responsive neurons were detected using a “search” stimulus of 80 dB noise bursts. Intracellular impalements were signaled by a sudden and stable drop (>25 mV) in the DC level and the presence of synaptic or large action potentials (>15 mV). Intracellular recordings typically lasted 2 min (maximum: ∼30 min); however, some recordings lasted for shorter periods, and only responses to noise bursts were obtained. This limitation was due to the difficulty in maintaining an in vivo impalement at recording depths of 5–8.5 mm from the brain surface. A Maclab 4-s 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 rate-level function at CF were determined. Other data (e.g., binaural response properties) were also sometimes collected as part of a larger study; however, such data are not presented in this report.

Acoustic stimuli were synthesized digitally and generated by either Beyer DT48 transducers (Beyerdynamic, Farmingdale, NY) or Tucker-Davis Technologies (TDT, Gainesville, FL) EC1 electrostatic speakers in concert with a TDT ED1 speaker driver. All transducers were controlled by a TDT signal generator (TDT System 2) and coupled to the end of each hollow earbar. The department’s PC based “Neurophysiology Laboratory System” (program by R. E. Millard) was used to control all parameters of acoustic stimulation and data collection. The acoustic system was calibrated using a Bruel and Kjaer 2606 measuring amplifier (type 2606, Bruel & Kjaer, Naerum, Denmark). Beyer transducers were calibrated with a B&K 0.5-in condenser microphone, coupled to a small probe tube positioned within the ear bar tube ∼3 mm from the tympanic membrane. The TDT speakers were calibrated using a 0.25-in B&K condenser microphone inserted into a custom built acoustic coupler designed to simulate the rat’s ear canal at the end of the hollow earbar (designed and built by R. E. Millard). Both these methods allowed acoustic input to be measured in dB sound pressure level (SPL; referenced to 20 μPa). The noise bandwidth generated by TDT EC1 speakers was measured with a Stanford Research Systems dynamic signal analyzer (SR785, Sunnyvale, CA) and found to be spectrally flat at 80 ± 10 dB between 20 Hz and 60 kHz. The Beyer transducers had a nominal rise-fall times and a 500 ms repetition interval; the left and right transducer outputs were offset by 200 ms. Responsive neurons were detected using a “search” stimulus of 80 dB noise bursts. Intracellular impalements were signaled by a sudden and stable drop (>25 mV) in the DC level and the presence of synaptic or large action potentials (>15 mV). Intracellular recordings typically lasted 2 min (maximum: ∼30 min); however, some recordings lasted for shorter periods, and only responses to noise bursts were obtained. This limitation was due to the difficulty in maintaining an in vivo impalement at recording depths of 5–8.5 mm from the brain surface. A Maclab 4-s 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 rate-level function at CF were determined. Other data (e.g., binaural response properties) were also sometimes collected as part of a larger study; however, such data are not presented in this report.

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**RESULTS AND DISCUSSION**

Intracellular recordings from 56 VNLL neurons revealed that a subset (14.3%; 8/56) showed contralaterally presented noise bursts (80 dB RMS; 20 trace overlay, lighter gray) and a characteristic frequency (CF) tone burst (80 dB SPL; single trace, darker gray). Horizontal dashed line indicates the mean resting membrane potential (RMP; −47 ± 4 mV). The arrow indicates membrane hyperpolarization and the horizontal bar indicates stimulus duration. B: single traces to off-CF tone bursts. Horizontal bar indicates time scale from stimulus onset. C: spontaneous inhibitory postsynaptic potentials (IPSPs) were also observed in this cell (black trace) and are compared with the onset component of a single noise response (gray trace). Asterisks indicate truncated action potentials (APs). D: distribution of latencies of first AP (black bars) and maximum hyperpolarization (white bars) for cells with onset inhibition. E: first spike latency (FSL) distribution of VNLL OI neurons. Vertical dashed lines indicate the average of each sample (see text). Bin widths are 0.25 ms.

Several questions arise from these observations, including what is the source of this onset inhibition and what is its function? The short and consistent latency of the IPSPs, its prominence at noise or tone onset, and its broad frequency tuning suggest the involvement of the octopus cell pathway. However, this pathway is excitatory (Adams 1997; Oertel and Wickesberg 2002), and therefore we hypothesized that this inhibition resulted from the activation of another VNLL neuron that received octopus cell inputs (Fig. 2A). For this to be the case, there must be a subset of VNLL neurons that respond at short latency and prior to the observed inhibition. Figure 1E shows that such a cell group exists (n = 10), responding with a mean FSL of 4.6 ± 0.3 ms. Importantly, these cells showed an OI response pattern to contralateral acoustic stimulation (Fig. 2A), consistent with an octopus cell input. The proposed circuitry is also consistent with the anatomical description of collateral branches from VNLL cells that form local and presumably inhibitory circuits within this structure (Zhao and Wu 2001) (Fig. 2, gray axon). Another possible candidate for the source of this inhibition is the glycineric principal cell of the MNTB that is known to project to the VNLL (Glendenning et al. 1981). However, the narrow frequency tuning and sus-
tained responses of these neurons (Paolini et al. 2001) would suggest that this is unlikely.

Excluding OI cells, a comparison of cells with and without \( \text{onset inhibition} \) shows that the former group had longer mean FSLs than the latter group (not shown; respectively, 8.5 vs. 7.3 ms). Therefore this inhibition delays the first AP in a subset of VNLL neurons. This conclusion is supported by the data presented in Fig. 2b1; this cell did not exhibit onset inhibition in a small subset of stimulus presentations (black traces) and the presence of inhibition (gray traces) resulted in a longer FSL (means of 6.2 vs. 8.5 ms, respectively).

The amplitude and time course of the fast inhibition described in the preceding text is similar to that observed within the MNTB (Awatramani et al. 2004). Awatramani and colleagues (2004) proposed that fast inhibition may control the excitatory drive from a calyx. However, in the VNLL the calyx appears to trigger fast inhibition in a local circuit that produces neural delays within the VNLL and therefore its output to the IC. The ramifications of this, and the reason that it occurs in only a subset of VNLL cells, are unknown but there are several possible outcomes at the IC. VNLL neurons receiving onset inhibition showed either a sustained or onset response to noise (Fig. 2b, 2 and 3, respectively). The response pattern of these VNLL neurons will determine the pattern of inhibition in the IC. Delayed inhibition from VNLL cells with a sustained response may create phasic responses in the IC (Fig. 2c1), while delayed inhibition from VNLL onset cells may mediate pauser IC responses (Fig. 2c2).

The proposed VNLL circuitry predicts the presence of fast inhibition preceding excitation in the IC (Fig. 2d) because the inhibitory projection within the VNLL (Fig. 2, gray axon) is a collateral of an axon projecting to the IC. Note that the VNLL neuron (Fig. 2b) and the IC neuron (Fig. 2d) receive the same pattern of inputs, and therefore they would both be expected to exhibit similar onset inhibition. This prediction is supported by our intracellular recordings from IC neurons (Fig. 2d) because the inhibitory projection within the VNLL (Fig. 2, gray axon) is a collateral of an axon projecting to the IC. Note that the VNLL neuron (Fig. 2b) and the IC neuron (Fig. 2d) receive the same pattern of inputs, and therefore they would both be expected to exhibit similar onset inhibition. This prediction is supported by our intracellular recordings from IC neurons (Fig. 2d).
is mediated by glycinergic interneurons with onset responses, and results in the delay of the first AP to off-CF tones (Paolini et al. 2004). These descriptions of fast, onset inhibition at several levels of the auditory pathway suggest that it may be a widespread means of controlling first spike timing.

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GRANTS

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REFERENCES


