Physiological Properties of Neurons in the Ventral Nucleus of the Lateral Lemniscus of the Rat: Intrinsic Membrane Properties and Synaptic Responses

SHU HUI WU
Laboratory of Sensory Neuroscience, Institute of Neuroscience, Carleton University, Ottawa, Ontario K1S 5B6, Canada

Wu, Shu Hui. Physiological properties of neurons in the ventral nucleus of the lateral lemniscus of the rat: intrinsic membrane properties and synaptic responses. J. Neurophysiol. 81: 2862–2874, 1999. The physiological properties including current-voltage relationships, firing patterns, and synaptic responses of the neurons in the ventral nucleus of the lateral lemniscus (VNLL) were studied in brain slices taken through the young rat’s (17–37 days old) auditory brain stem. Intracellular recordings were made from VNLL neurons, and synaptic potentials were elicited by electrical stimulation of the lateral lemniscus ventral to the VNLL. Current-voltage relations and firing patterns were tested by recording the electrical potentials produced by intracellular injection of positive and negative currents. There were two types of VNLL neurons (type I and II) that exhibited different current-voltage relationships. In response to negative current, both type I and II neurons produced a graded hyperpolarization. Type I neurons responded to positive current with a graded depolarization and multiple action potentials the number of which was related to the strength of the current injected. The current-voltage relations of type I neurons were nearly linear. Type II neurons responded to positive current with a limited depolarization and only one or a few action potentials. The current-voltage relations of type II neurons were nonlinear near the resting potential. The membrane properties of the type II VNLL neurons may play an important role for processing information about time of onset of a sound. Type I neurons showed three different firing patterns, i.e., regular, onset-pause and adaptation, in response to small positive current. The onset-pause and adaptation patterns could become sustained when a large current was injected. The regular, onset-pause, and adaptation patterns in type I neurons and the onset pattern in type II neurons resemble “chopper,” “pauser,” “primary-like,” and “on” responses, respectively, as defined in in vitro VNLL studies. The results suggest that different responses to acoustic stimulation could be attributed to intrinsic membrane properties of VNLL neurons. Many VNLL neurons responded to stimulation of the lateral lemniscus with excitatory or inhibitory responses or both. Excitatory and inhibitory responses showed interaction, and the output of the synaptic integration depended on the relative strength of excitatory and inhibitory responses. Neurons with an onset-pause firing pattern were more likely to receive mixed excitatory and inhibitory inputs from the lower auditory brain stem.

INTRODUCTION

The ventral nucleus of the lateral lemniscus (VNLL) is the most ventral neuronal group of the nuclei of the lateral lemniscus. In contrast to the dorsal nucleus of the lateral lemniscus (DNLL), which receives inputs from the brain stem bilaterally and is concerned primarily with binaural processing, the VNLL receives inputs mainly from the contralateral ventral cochlear nucleus (VCN) with smaller projections arising from the ipsilateral VCN and superior olivary complex (SOC) (Brown and Webber 1975; Covey and Casseday 1986; Friauf and Ostwald 1988; Glendenning et al. 1981; Helfert et al. 1991; Huffman and Covey 1995; Schofield and Cant 1997; Schwartz 1992; Spangler et al. 1985; Warr 1982; Zook and Casseday 1985). Physiological responses of VNLL neurons to acoustic stimulation and functional organization of the VNLL have been studied in cat (Aitkin et al. 1970), and extensively in echo-locating bat (Covey and Casseday 1986, 1991; Metzner and Radtke-Schuller 1987; Vater et al. 1997). VNLL neurons mainly are influenced by sound presented to contralateral ear and respond to tone bursts with different temporal discharge patterns. The VNLL mainly is concerned with monaural processing and may have an important role in acoustic temporal discrimination (Aitkin et al. 1970; Covey and Casseday 1991; Guinan et al. 1972a,b; Metzner and Radtke-Schuller 1987).

In the bat, the nuclei of the lateral lemniscus including the VNLL are greatly expanded and highly developed. The bat VNLL can be divided into two subdivisions, the columnar area (VNLLc) and the multipolar area (VNLLm), based on the cytoarchitecture, synaptic arrangement, afferent tonotopic projections and physiological properties (Covey and Casseday 1986, 1991; Huffman and Covey 1995; Vater et al. 1997). The connections of the VNLLc are organized in sheets that are precisely related to the tonotopic organization of its afferents from the anterior ventral cochlear nucleus (AVCN) and its efferents from the inferior colliculus (Covey and Casseday 1986). Cells in the VNLLc are very similar to spherical bushy cells in the AVCN and are contacted with large calyx-like synaptic terminals in addition to conventional bouton terminals. They are broadly tuned with no spontaneous activity and respond with one spike per stimulus and with constant latencies to stimulus onset. The VNLLc is thought to be specialized to encode the onset of a sound (Covey and Casseday 1991). Cells in the VNLLm are multipolar in shape. They respond to tone bursts with various temporal patterns, which are distinguished by the shape of the single unit’s PSTH and histogram, i.e., tonic (discharge at a constant high rate through the duration of the tone burst), chopper (distinct and regularly spaced peaks of discharge throughout the duration of the stimulus), primary-like (discharge throughout the stimulus duration, but firing rate diminished after an initial transient on response), or pauser (initial discharge followed by a silent period and then

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
firing at a diminished rate) but without the single-spike constant-latency response pattern seen in the VNLL. The VNLLm may play a role in encoding ongoing properties of a sound (Covey and Casseday 1991).

A variety of mammalian species including bat, cat, rat, mouse, gerbil, mole, ferret, guinea pig, and opossum, the VNLL sends efferent projections to the ipsilateral inferior colliculus (IC) (Adams 1979; Bruno-Bechtold et al. 1981; Colemans and Clerici 1987; Druga and Syka 1984; Frisina et al. 1998; Kudo 1981; Kudo et al. 1990; Majorossy and Kiss 1994; Moore 1988; Nordeen et al. 1983; Ross et al. 1988; Schofield and Cant 1997; Schweizer 1981; Strutz 1980; Whitley and Henkel 1984; Willard and Martin 1983; Willard and Ryugo 1983; Zook and Casseday 1987). Immunocytochemical studies have shown that most of VNLL neurons are glycineergic (Vater et al. 1997); half of this projection is also $\gamma$-aminobutyric acid (GABA) immunoreactive (Saint Marie and Baker 1997). After injection of tritiated glycine into the IC many VNLL neurons are retrogradely labeled (Saint Marie and Baker 1990). After injection of horseradish peroxidase (HRP) or Fluorogold into the IC, many of the retrogradely labeled neurons in VNLL show positive immunostaining with GABA antibody (González-Hernández et al. 1996; Zhang et al. 1998). All these results suggest that the VNLL is a major source of inhibitory input to the IC and may play a significant role in inhibitory processing there.

Physiological studies on VNLL neurons in terrestrial mammals (nonchiro-locating species) are few and the function of the VNLL is still largely unknown (Adams 1997; Aitkin et al. 1970; Batra and Fitzpatrick 1997; Guinan et al. 1972a,b). A better understanding of the function of the VNLL can be gained by examining the membrane properties of individual neurons and synaptic transmission in the VNLL. Because there have been no previous intracellular studies of VNLL neurons, I am presenting here basic information concerning intrinsic membrane properties and discharge patterns of VNLL neurons in a rat brain slice preparation. Synaptic responses of VNLL neurons also have been investigated. Preliminary results of these studies have been presented previously in the form of abstracts (Wu 1996, 1997).

**Methods**

Brain slices were obtained from young albino rats (Wistar, Charles River, Quebec) between 17 and 37 days of age in this study. The animals first were anesthetized with halothane and then were killed by decapitation. The whole brain was removed and submerged in a warm (30°C), oxygenated saline solution. The auditory midbrain was blocked by making cuts rostral and caudal to the lateral lemniscus and was mounted on the stage of a tissue slicer using a cyanoacrylate adhesive. Coronal sections were taken at 400-$\mu$m sections. Finally the sections were reacted with diaminobenzidine (DAB) and counted with a Leitz dissecting microscope. The outlines of the VNLL, intermediate nucleus of the lateral lemniscus (INLL), and DNLL were clearly visible (Fig. 1A). A single recording electrode was inserted into the VNLL, and a stimulating electrode was placed on the lateral lemniscus ventral to the VNLL. The placement of the stimulating electrode is indicated in a cresyl-violet-stained frontal section shown in Fig. 1A.

Intracellular recordings were made with glass microelectrodes filled with 4 M potassium acetate. The electrode impedances were between 120 and 160 MΩ. Once the electrode reached the surface of the tissue, it was advanced in $\mu$m steps through the brain slice with a Burleigh piezoelectric driver (Inchworm). Application of an oscillating current (buzzing) was used to facilitate intracellular penetration. An Axo-probe 1-A amplifier was employed for all recordings. Experimental data were stored with a Nicolet Benchtop Waveform Acquisition System 400 and plotted later for analysis. The statistical analysis was conducted on Microsoft Excel, running one-way ANOVAs at a criterion level of $\alpha = 0.05$ on applicable data. The changes in membrane potential elicited by intracellular injection of positive and negative currents were obtained by measuring between the resting membrane potential and the plateau depolarization and between the resting membrane potential and the peak hyperpolarization, respectively. The measured values were used to plot the curve of current-voltage relationship. The slope of the curve was calculated from a range of the hyperpolarization that was approximately linear. The average data are presented as means ± SD.

Synaptic responses were elicited by electrical stimulation of the lateral lemniscus ventral to the VNLL (see Fig. 1A). The bipolar stimulating electrode was constructed from paired tungsten wires with a tip separation of ~50 $\mu$m. Electrical stimuli were square waves, 100 $\mu$s in duration, obtained from a Grass S-8800 stimulator and stimulus isolator. The rate of stimulation was one stimulus per second during the initial search for synaptic responses. When neural responses were encountered, the stimulus strength was adjusted within a range from the threshold to the suprathreshold level to produce graded postsynaptic responses.

**Results**

**Integrity of the lateral lemniscus**

The ascending neural pathway activated by stimulation of the lateral lemniscus ventral to the VNLL was confirmed anatomically in several experiments by injecting a small amount of biocytin (Vector, 2% in 2 M potassium acetate) into the slice at a position along the lateral lemniscus ventral to the VNLL. A period of 6–8 h was allowed for transport to take place. The tissue then was fixed by 4% paraformaldehyde and imbedded in agar for cutting into 80-$\mu$m sections. Finally the sections were reacted with diaminobenzidine (DAB) and counterstained with cresyl violet (Wu and Kelly 1995). As can be seen in Fig. 1, C and D, an injection of biocytin into the lateral lemniscus ventral to the VNLL resulted in labeling of large-diameter fibers and large specialized axon terminals, the calyces of Held in the medial part of the VNLL (Fig. 1C), small-diameter fibers, and bouton terminals throughout the VNLL (Fig. 1D). Some of thin fibers gave rise to collaterals which turned at a right angle and ran perpendicularly with respect to the fibers of the main stream of the lateral lemniscus. These
results demonstrate that some afferent pathway of the lateral lemniscus to the VNLL remains intact enough to transport biocytin in the frontal slice preparation.

Membrane properties and firing patterns

Membrane properties of the VNLL neurons were investigated by recording intracellular potentials in response to injection of current into the cell through the recording electrode. The intracellular recordings were accepted based on several criteria: a resting potential of at least $-57.4 \pm 4.3$ mV, narrow and full-size action potentials, and stability of the recording over a period of 0.5–4 h. Current-voltage relations were examined in 54 VNLL neurons from 43 animals. The relations between current strength and magnitude of voltage change provided a measure of the input membrane resistance ($mean = 51.6 \pm 16.8 \, M\Omega$, $n = 54$). The current-voltage (I-V) curve and temporal firing pattern were used to characterize and distinguish cell types in the VNLL.

TYPE I CELL. Figure 2 shows examples of the effect of injecting current into four cells with type I characteristics. Injection of negative current for 60 ms led to a hyperpolarization of the cell membrane that was roughly proportional to the amount of current injected (Fig. 2A, 1–3). The I-V curves of these cells were essentially linear over the $-0.1$- to $-0.8$-nA current range (Fig. 2B, 1–3). With continued application of current, the hyperpolarization was followed by a ‘sag’ of the membrane potential toward the resting level. A depolarization that elicited two to three action potentials immediately followed the end of the hyperpolarization (Fig. 2A1, bottom). The sag and afterdepolarization were more apparent with long-duration current pulses. Figure 2A4 shows an example of a cell that was injected with 100-ms intracellular current. The sag and afterdepolarization were seen clearly when the cell was hyperpolarized with $-0.3$- and $-0.5$-nA currents. The afterdepolarization lasted for 30–35 ms and was large enough for generation of a train of action potentials (Fig. 2A4, bottom 2 traces). The sag and afterdepolarization probably reflect activation and deactivation, respectively, of an inward hyperpolarization-activated current ($I_h$). Activation of $I_h$ was found in 48.8% (21/43) of type I cells.

Injection of positive current into type I VNLL neurons produced a depolarization and action potentials. Smaller cur-

FIG. 1. A: photomicrograph of a cresyl-violet-stained frontal section through the midbrain and lateral lemniscus to illustrate the position of stimulating electrode relative to the ventral nucleus of the lateral lemniscus (VNLL). Scale bar: 500 μm. B: higher-power photomicrograph of the same section as in A to show cell morphology of different VNLL neurons. Scale bar: 100 μm. C: photomicrograph of the VNLL of a rat after injection of biocytin into the lateral lemniscus ventral to the VNLL. Tissue was fixed and processed with diaminobenzidine (DAB) after the injection. Thick axon (A) and specialized synaptic terminal, a calyces of Held (C), were labeled by biocytin reaction product. Scale bar: 100 μm. D: photomicrograph of the same section as in C. Medial-sized and thin axons (A), and puncta representing bouton terminals (T) are seen throughout the VNLL. Scale bar: 100 μm.
rent injection into the neuron produced a few potentials at the onset of injection. As the current was increased a steady and regular train of action potentials was produced throughout the entire period of current injection (Fig. 2A1). This form of neuronal activation has been operationalized as “regular pattern” activity. Such a definition of neuronal unit activity is achieved when the ratio of the last interspike interval (ISI) to the initial ISI is less than or equal to 1.2 at a minimal frequency of 8–13 spikes in response to 60-ms positive current injection. If the initial ISI is reduced relative to the remaining ISIs (in some cells), the subsequent ISI is employed to calculate the ratio. Cells with regular firing pattern were found in 41.9% (18/43) of type I cells. The action potentials in some type I cells were characterized by a single negativity immediately after the spike, which is here referred to as a “single undershoot” (Fig. 2A, 1–4). The action potential with a single undershoot was found in 72.1% (31/43) of type I cells.

Figure 2A2 is an example of another firing pattern observed in VNLL neurons. Positive current injection produced a graded depolarization and a train of action potentials, the number of which increased with current strength. With smaller current (0.4 nA), two action potentials were generated at the onset of the depolarization. They were followed by a long pause (~38 ms), which then was followed by another action potential. With more current (0.5 nA), three action potentials appeared at the beginning of the injection; there was then a short pause (~28 ms) followed by a single action potential. With a stronger current (1 nA), a train of action potentials was produced throughout the entire period of injection without any pause. Cells with this onset-pause firing pattern usually had one to three action potentials at the onset of current injection following a silent period before a train of action potentials. As current injection was increased, the silent period became shorter and shorter. With larger current, the firing became sustained without any pause. Figure 2A3 shows a typical onset-pause firing pattern of a VNLL neuron. The onset-pause pattern was found in 25.6% (11/43) of type I cells.

Although the firing pattern of the cell shown in Fig. 2A2 was sustained in response to a larger current (1 nA), a regular pattern of activity was not in evidence (top). Action potentials were more frequent during the onset of current injection but were progressively diminished during the remaining current injection interval. This pattern of neuronal firing is reminiscent of “adaptation.” Such a definition of neuronal activity is achieved when the ratio of the last ISI to the initial or subsequent ISI is larger than 1.2, at a similar firing frequency...
The firing pattern of the onset-pause neurons was changed when the cell membrane potential was altered. Figure 3 shows how the membrane potential affected the onset-pause firing pattern. A stable recording initially was obtained from this neuron at the resting potential of $-59 \text{ mV}$. The cell responded to a 0.4-nA current injection of 60 ms with one action potential at the onset of the injection (Fig. 3B, left), to a 0.6-nA current with a typical onset-pause pattern (Fig. 3B, middle) and to a 0.8-nA current with a sustained train of action potentials (Fig. 3B, right). When the cell was depolarized by a continuous DC current to $-54 \text{ mV}$ and tested again with the same current pulses (0.4, 0.6, and 0.8 nA), the cell no longer showed the onset-pause pattern but only a sustained firing (Fig. 3A). When the cell was hyperpolarized by a DC current to $-64 \text{ mV}$, the threshold for generating an action potential became higher. The cell fired only one action potential to a current injection of 0.6 nA (Fig. 3C, middle). But the onset-pause pattern still was seen in response to 0.8-nA injection (Fig. 3C, right).

Some type I neurons in the VNLL responded to positive current injection with sustained firing, but their action potentials were followed by two negative components. One component was a sharp hyperpolarization, and the other was a longer-lasting negativity, which was especially clear in response to current injection at threshold level for eliciting action potential (Fig. 4D). This pattern of action potential here is referred to as ‘double undershoot’ and was observed in 27.9% (12/43) of type I neurons. Most of cells with a double undershoot action potential fired very regularly with little adaptation in response to pulse current injection. Their $I-V$ curve was also linear near the resting potential (Fig. 4G).

Figure 5A shows three examples of the interspike interval,
normalized to the duration of the first interval, as a function of time into the current pulse. As shown in Fig. 5A, 1 and 2, for these two neurons, which showed a regular firing pattern, there were only slight changes in interspike interval over the 60-ms positive current injection. Cells that showed adaptation fired action potentials with increased interspike interval as function of injection time. As shown in Fig. 5A3, the ISI gradually became longer and longer after the first 10 ms of the current injection. A summary of type I cells with different firing patterns and undershoot types of action potential is shown in Table 1.

In spite of different firing patterns, type I neurons, even onset-pause and adaptation types, discharged continuously for the duration of the stimulus in response to more intense current injection. The firing rates usually increased as the amount of current increased. Figure 5B shows the discharge rate as a function of the amount of current injected into neurons from the regular, onset-pause and adaptation groups, respectively. The firing rates between regular and adaptation groups were similar, ~200–400 Hz to a 1-nA current injection (Fig. 5B, 1 and 3). Some neurons had even higher firing rates in response to current injections >1 nA (not shown). Neurons with an onset-pause pattern fired fastest, >400 Hz to a 1-nA current, although some neurons had a lower discharge rate. Some onset-pause neurons could fire very rapidly, >600 Hz to currents >1.4 nA (Fig. 5B2, top). The ranges of the spike width at the half-amplitude for regular, onset-pause, and adaptation types were 0.25–0.67, 0.22–0.66, and 0.26–0.71 ms, respectively. It seems that the onset-pauser had no distinctly shorter spike duration than other two types, although it could fire faster than they could.

In summary, type I cells responded to a smaller positive current with multiple action potentials, showing regular, onset-pause or adaptation patterns, and to a larger current with sustained firing. The onset-pause pattern could be changed to a continuous firing pattern when the cell was more depolarized. The current-voltage relation of type I cells was linear near the

<table>
<thead>
<tr>
<th>Undershoot Type</th>
<th>Firing Pattern</th>
<th>Regular</th>
<th>Onset-Pause</th>
<th>Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Double</td>
<td>8</td>
<td>11</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>11</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

The total recordings were obtained from 43 cells. As two cells with a single undershoot had both onset-pause and adaptation patterns, the total number of cells in this table became 45.

FIG. 5. A: interspike intervals during 60 ms positive current injection for 3 VNLL neurons. Intervals are normalized to the first interval during the train. 1: interspike intervals for a neuron with double undershoot action potentials and regular firing pattern. 2: interspike intervals for a neuron with single undershoot action potentials and regular firing pattern. 3: interspike intervals for a neuron with single undershoot action potentials and adaptation firing pattern. B: firing rates as a function of the strength of positive current injected into VNLL neurons with regular (1), onset-pause (2), and adaptation (3) firing patterns. Each curve represents 1 cell.
resting potential. Neurons with type I membrane properties were found in 76.8% (43/56) of the sample. The resting potential of type I cells was $-57.1 \pm 4.2 \text{ mV}$ ($n = 43$) and the cell membrane input resistance was $54.6 \pm 17.0 \text{ M} \Omega$ ($n = 40$).

**TYPE II CELL.** The other type of neuron in the VNLL, the type II cell, had a different current-voltage relation and firing pattern from that of the type I cells. Type II cells responded to positive current with only one action potential and exhibited a nonlinear current-voltage relation as shown in Fig. 6. Negative current ($-0.2$ and $-0.4 \text{ nA}$) led to a graded hyperpolarization of the cell membrane (Fig. 6, $D$ and $E$). Both sag and afterdepolarization with action potentials were found in 53.8% (7/13).

**FIG. 6.** Current-voltage relation for a type II VNLL neuron. $A$–$C$: injection of $0.4$-, $0.3$-, and $0.2$-nA positive currents for 60 ms produced limited depolarization and only 1 action potential at the beginning of injection. $D$ and $E$: responses to injections of $-0.2$- and $-0.4$-nA current for 60 ms. $F$: relation between current and voltage as measured by the peak shift in membrane potential. Resting potential for this cell was $-55 \text{ mV}$.

**FIG. 7.** Postsynaptic responses of 4 VNLL neurons ($A$–$D$) to electrical stimulation of the lateral lemniscus ventral to the VNLL. ↑, time of the stimulus and resulting artifact.
of the type II cells. Injection of suprathreshold positive current elicited one action potential at the onset of the injection (Fig. 6, A–C). Most type II cells responded with one action potential (2–3 spikes in some cells) as current strength was increased ≥1 nA. Following the spike(s), the membrane potential of the cell remained near rest and quickly returned to the resting potential after the end of the current pulse. Equal amounts of depolarizing and hyperpolarizing current injection had a different effect on the change in membrane potential. Negative current resulted in a larger voltage shift than positive current. The current-voltage relation was nonlinear (Fig. 6F). Neurons with type II membrane characteristics were found in 23.2% (13/56) of the cells in the sample. The mean resting potential of the type II cell was −58.2 ± 4.6 mV (n = 13) and the input resistance was 42.5 ± 12.6 MΩ (n = 13), not significantly different from type I cells (P > 0.05). The type I and II cells were not segregated into separate regions within the VNLL. They were found throughout the VNLL.

**Synaptic physiology**

The synaptic physiology of the VNLL was investigated by examining synaptic potentials of VNLL neurons in response to electrical stimulation of the lateral lemniscus ventral to the VNLL. Figure 7 shows several examples of synaptic responses recorded from four VNLL neurons to stimulation of the lateral lemniscus. In Fig. 7A, the neuron responded to near-threshold stimulation with a very small excitatory postsynaptic potential (EPSP) that was followed by a prominent inhibitory postsynaptic potential (IPSP; bottom). At a slightly higher stimulus level, both EPSP and IPSP became larger (middle), and a single action potential could be evoked with sufficient stimulus strength (top). A similar response pattern with more predominant excitation was observed in some neurons. An example is shown in Fig. 7C. For this neuron, near-threshold stimulation elicited a small IPSP, and higher level of stimulation always evoked a suprathreshold response. In some neurons no suprathreshold response was observed although both EPSP and IPSP could be evoked. An example is shown in Fig. 7B. For this neuron, a lower level of stimulation (12.2 and 14 V) elicited EPSPs only. A higher stimulus level (16 V) evoked either EPSP or IPSP from trial to trial but did not elicit a suprath-

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** Distribution of proportions of type I and II VNLL neurons responding to stimulation of the lateral lemniscus with excitatory postsynaptic potential (EPSP) alone, inhibitory postsynaptic potential (IPSP) alone, both, and none.

![Figure 9](http://jn.physiology.org/)

**FIG. 9.** Distribution of proportions of type I VNLL neurons with regular, onset-pause, and adaptation firing patterns responding to stimulation of the lateral lemniscus with EPSP alone, IPSP alone, both, and none.
residual depolarization or hyperpolarization of the cell membrane. Injection of positive or negative current led to proportional depolarization or hyperpolarization of the cell membrane and generated a threshold response (action potential). In other cases, the threshold for the IPSP was lower than that for the EPSP, and inhibition dominated the synaptic response, as illustrated in Fig. 7D. This neuron responded to a low stimulus level (7 V) with an IPSP only. When the stimulus was increased to 19 V, a small EPSP was evoked. But the response could become an IPSP again at the same (19 V) or a much higher stimulus level (40 V). No suprathreshold response could be elicited at any stimulus level tested in this neuron.

In summary, many VNLL neurons, 35/43 (81.4%) of the type I and 11/13 (84.6%) of the type II neurons, responded to electrical stimulation of the lateral lemniscus with EPSPs or IPSPs or both. The latencies of most EPSPs and IPSPs were short (mean = 1.17 ms). Some type II cells had EPSPs with shorter latency (0.6–0.8 ms), larger amplitude and shorter duration, which resembled the characteristics of EPSPs observed in bushy and octopus cells in the cochlear nucleus (Golding et al. 1995; Oertel 1983). Of 43 type I cells, I observed only EPSPs in 7 neurons (16.3%), only IPSPs in 14 neurons (32.6%), and both EPSPs and IPSPs in 14 neurons (32.6%). Of 13 type II cells, I saw only EPSPs in 5 neurons (38.5%), only IPSPs in 2 neurons (15.4%), and both EPSPs and IPSPs in 4 neurons (30.8%). The distribution of the EPSPs and IPSPs in type I and II VNLL neurons is shown in Fig. 8.

A further analysis of the distribution of EPSPs and IPSPs among the three groups of neurons with regular, onset-pause, and adaptation firing patterns is shown in Fig. 9. Synaptic responses were observed in neurons of all three groups, but the responses were most common in the onset-pause (90.9%) compared with the adaptation (71.4%) and regular (85%) groups. Many more onset-pause neurons (63.6%) responded to stimulation of the lateral lemniscus with combined EPSPs and IPSPs, compared with the adaptation (14.3%) and regular (30%) groups.

Discussion

The purpose of this study was to obtain basic information about the membrane characteristics and synaptic responses of VNLL neurons. I investigated the intrinsic membrane properties of VNLL neurons in response to intracellularly injected current and synaptic potentials in response to electrical stimulation of the lateral lemniscus. There were two types of VNLL neurons, type I and II, which exhibited different current-voltage relations. Three different firing patterns were generated in type I neurons. Excitatory and inhibitory synaptic potentials could be evoked by stimulation of the lateral lemniscus fibers.

Correlation of current-voltage relation with cell morphology

VNLL neurons sampled in this study showed two different responses to intracellular injection of current. I named these type I and II cells on the basis of their membrane properties. In type I cells, injection of positive current produced a proportional depolarization of the cell membrane and generated a sustained discharge of action potentials, the number of which reflected the magnitude of the depolarization. Both regular and irregular firing patterns were found depending on the neuron from which recordings were made. For type I cells, intracelluar injection of positive or negative current led to proportional depolarization or hyperpolarization of the cell membrane. The magnitude of the initial depolarization and hyperpolarization was an approximately linear function of current strength. In contrast, type II cells responded to positive current injection with a limited depolarization and only one or a few action potentials at the onset of the current injection regardless of the intensity of the positive current. These cells responded to negative current injection with graded hyperpolarization. The current-voltage relation of the type II cells was nonlinear near the resting potential. The distinction between type I and II cells in the VNLL closely resembles that previously reported between stellate and bushy cells in the mouse VCN (Wu and Oertel 1984) and that between cells in the lateral superior olive (LSO) and medial nucleus of the trapezoid body (MNTB) (Wu and Kelly 1991). The nonlinear current-voltage relation of the type II cells in the VNLL is also very much like that of the octopus cells in the posteroventral cochlear nucleus (PVCN) (Golding et al. 1995) and principal cells in the medial superior olive (MSO) (Smith 1995).

The membrane properties of type II cells in the VNLL, bushy cells in the VCN, octopus cells in the PVCN, and principal cells in the MNTB and MSO are different from those of other types of neurons but similar to each other. These cells all have nonlinear current-voltage relationships. They respond to injection of positive current with only one or a few action potentials. For VNLL type II neurons, the input membrane resistance measured from the amplitude of voltage changes with injected hyperpolarizing current was 42.5 ± 12.6 MΩ (this study), which is similar to that for bushy cells (30–50 MΩ) (Oertel 1991). However, when the cell is excited, the cell input resistance becomes lower. The consequence of the reduced input resistance is a limitation in generation of action potentials and a faster repolarization in response to excitatory inputs. These properties are very important for precisely encoding auditory temporal information (Oertel 1991, 1997). The population of type II neurons in the rat VNLL may also precisely signal the time of onset of a sound, like VNLLc neurons in the big brown bat (Covey 1993). Voltage-clamp investigations have demonstrated that a low-threshold (about −70 mV) K+ conductance is responsible for the highly rectifying current-voltage relationship of bushy cells in the VCN, and principal cells in the MNTB (Brew and Forsythe 1995; Forsythe and Barnes-Davies 1993; Manis and Marx 1991). Whether or not type II VNLL neurons have K+ channels similar to those in bushy cells in VCN and principal cells in the MNTB requires further investigation.

In the VCN and SOC, the cell types based on membrane properties are associated closely with the cell types for cytoarchitecture. Intracellular labeling studies have shown that cells with linear current-voltage relationships correspond to stellate cells in VCN and principal cells in LSO as they appear in Golgi-impregnated material (Wu and Fu 1998; Wu and Oertel 1984). These cells typically have multiple long dendritic branches extending away from all sides of the neuron. In contrast, neurons with nonlinear current-voltage relationship correspond to bushy cells in VCN (Wu and Oertel 1984) and principal cells in the MNTB (Banks and Smith 1992; Wu and Kelly 1991). Bushy cells have fewer and shorter dendritic branches than stellate cells. Principal cells in the MNTB, like bushy cells, have compact or tufted dendrites. In this study, I did not attempt to label the neurons from which the recordings were made. Nevertheless, the previous anatomic studies have
shown that there are various morphological types of cells in the VNLL (Adams 1979; Covey and Casseday 1991; Schofield and Cant 1997; Willard and Ryugo 1983; Zook and Casseday 1982). The morphology of oval or globular cells in cat, guinea pig, and mouse VNLL and bat VNLLc is very much like that of bushy cells in the VCN or principal cells in the MNTB (Adams 1979; Covey and Casseday 1991; Helfert and Aschoff 1997), whereas the anatomical features of other types, mostly multipolar cells, in the VNLL are similar to stellate cells in the VCN and principal cells in the LSO.

Another similarity of some neurons in the VNLL, bushy cells in the VCN, and principal cells in the MNTB is that these neurons receive large terminals from afferent fibers. Bushy cells are covered by end bulbs of Held that arise from auditory nerve fibers (Lenn and Reese 1966; Lorente de Nó 1976, 1981; Ryugo and Sento 1991; Schwartz and Gulley 1978). Principal cells of the MNTB are covered by calyces of Held that come from the axons of the globular bushy cells in the contralateral VCN (Friauf and Ostwald 1988; Morest 1968a,b). Some VNLL neurons in cat and mouse and VNLLc neurons in bat also receive large calyceal endings that resemble the end bulbs of Held in the VCN and MNTB (Adams 1979, 1983; Covey 1993; Covey and Casseday 1986; Vater and Feng 1990; Willard and Ryugo 1983; Zook and Casseday 1985). In this study, biocytin injection into the lateral lemniscus labeled thick axons coursing along the fibers of the lateral lemniscus and terminating with calyceal endings in the VNLL. Those morphological similarities suggest that neurons that receive large synaptic terminals in the VCN, MNTB, and VNLL may have common physiological properties. Bushy cells in VCN and principal cells in MNTB, indeed, show very similar intrinsic membrane properties. As shown in this study, type II neurons exhibited membrane properties, viz., nonlinear current-voltage relations, which resembled those for VCN bushy cells and MNTB principal cells. One might expect that type II VNLL neurons would have bushy-like morphological features. But further study using intracellular labeling combined with physiological recording methods is required to test this hypothesis.

**Firing characteristics**

Type I VNLL neurons responded to depolarizing current injection with sustained discharges. At low levels of current injection, three different firing patterns, i.e., regular, onset-pause, and adaptation, could be discerned. Type II VNLL neurons responded to positive current injection with only one, or a few spikes at the onset of the injection. These different firing patterns may correspond to different poststimulus time histograms (PSTHs) recorded from VNLL neurons in vivo in response to acoustic stimulation. For example, in the bat, neurons in two distinct divisions, VNLLc and VNLLm, respond to tone or noise bursts differently (Covey and Casseday 1991). In the VNLLc area the majority of neurons respond to a tone burst with one spike at short and constant latencies. This “onset” type of neuron also is present in the VNLL of other mammals but appears to be intermingled with other cell types (Vater et al. 1997). The response pattern of type II VNLL cells in this study resembles the phasic type in the VNLLc of big brown bat (Covey and Casseday 1991) and the VNLL of horseshoe bat (Metzner and Radtke-Schuller 1987), and the “onset” cell type in cat VNLL (Adams 1997; Guinan et al. 1972a). The phasic or onset response observed in in vivo studies could be attributed to the type II intrinsic membrane characteristics. Type II VNLL neurons in the rat, perhaps like VNLLc neurons in the bat (Casseday and Covey 1995), probably transmit information about stimulus onset.

Previous physiological studies in vivo have shown that many VNLL neurons respond to sounds with sustained discharges. In the early physiological studies of cat VNLL, most neurons were found to have sustained firing patterns, some with a silent period during the firing (Aitkin et al. 1970), and some with primary-like or chopper patterns (Guinan et al. 1972a). In a later study, neurons in the VNLLm of the big brown bat were found to respond to short tone bursts with four sustained firing patterns, i.e., chopper, tonic, primary-like, or pauser, which were classified according to criteria established by Pfeiffer (1966) (Covey and Casseday 1991). Similar discharge types, i.e., tonic and phasic-tonic patterns, were found in VNLL neurons in the horseshoe bat (Metzner and Radtke-Schuller 1987). Neurons with regular, onset-pause, or adaptation firing patterns demonstrated in the present study possibly correspond to cells with chopper, pauser, and primary-like types, respectively. Although synaptic events must drive VNLL neurons to generate these firing patterns in vivo, the intrinsic properties of the cell membrane also could contribute to the distinct discharge patterns. The firing patterns of the VNLL neurons shown in the present study also depend on the resting level of the cell membrane. For example, an onset-pause type could be converted to a sustained type by holding the cell membrane potential at a more depolarized level than the resting potential. Similar results were obtained from an in vitro intracellular study of the dorsal cochlear nucleus (DCN) (Manis 1990). In vivo studies of the DCN also have shown that response patterns can be changed by hyperpolarizing the cell membrane in some DCN neurons (Rhode and Smith 1986; Rhode et al. 1983). All these results support the idea that intrinsic membrane properties and the resting membrane conductance are involved in generation of different firing characteristics in auditory neurons (Manis 1990) including VNLL neurons (this study).

**Synaptic inputs**

In this study stimulation of the lateral lemniscus elicited EPSPs or IPSPs or both in many VNLL neurons. The lateral lemniscus is the main route of ascending fibers to the VNLL from the trapezoid body and lower brain stem (Glendenning et al. 1981; Helfert and Aschoff 1997). In this study many bouton terminals of thin fibers are labeled after injection of biocytin into the lateral lemniscus ventral to the VNLL. Some thicker fibers that may originate from the octopus cells in the PVCN (Schofield and Cant 1997) enter the ventral VNLL, run obliquely within the VNLL and terminate with the calyces of Held in the ventral or middle region of the VNLL (observation from this study). Therefore stimulation of the lateral lemniscus immediately ventral to the VNLL in the brain slice probably activates many afferent inputs that make synapses onto VNLL neurons.

Which pathways contribute to these responses in the VNLL? Most of the afferents to the VNLL originate in the contralateral VCN, with a very small projection coming from the ipsilateral VCN (Adams and Warr 1976; Browner and Webster 1975; Covey and Casseday 1986; Friauf and Ostwald 1988; Glen-
The projection from the MNTB to the LSO is inhibitory and shown to be immunoreactive for glycine (Helfert et al. 1989). Large terminals, the calyces of Held, found in the VNLL very likely originate from thick axons of octopus cells in the PVCN (Adams 1997; Schofield and Cant 1997; Thompson 1998; Vater and Feng 1990). Calyceal endings are known to be excitatory; neurons that receive calyceal terminals, such as bushy cells in the VCN, principal cells in the MNTB and globular cells in the VNLL, are excited by acoustical stimulation (Adams 1997; Rhode et al. 1983; Smith et al. 1998). A recent electron microscopic (EM) study about synaptic organization of VNLLc neurons in the big brown bat suggests that VNLLc neurons receive excitatory inputs that may arise from large calyces derived from neurons, probably octopus cells or large multipolar neurons, in the PVCN (Vater et al. 1997). I suggest that some excitatory synaptic responses with shorter latency, larger amplitude, and shorter duration observed in type II cells may represent excitatory inputs from the octopus cells in the PVCN. But further study definitely is required to clarify the characteristics of excitatory synaptic potentials of type I and II cells in the VNLL with pharmacological manipulation to eliminate the IPSPs that can obscure the EPSPs in response to stimulation of the lateral lemniscus.

In contrast to the calyceal endings from thick axons of octopus cells, the medium-sized bead-like boutons probably originate from collaterals of thinner axons derived from bushy and/or stellate (multipolar) cells in the AVCN and PVCN, which pass through the VNLL toward the IC (Covey 1993; Friafia and Ostwald 1988; Iwahori 1986; Schofield and Cant 1997; Schwartz 1992). The EM study about projections to the IC from the AVCN has shown that axon terminals of AVCN neurons have small, round synaptic vesicles and make asymmetric synaptic contacts on IC neurons (Oliver 1987). The results suggest that synaptic inputs from the AVCN to the IC are excitatory. Because VNLL receives axon collaterals of AVCN neurons the main axons of which ultimately terminate in the IC, it is very likely that the AVCN provides excitatory inputs to the VNLL as well.

Neurochemical studies further support the idea that projections from the AVCN to the VNLL are excitatory, probably glutamatergic. Suneja et al. (1995a) reported that the VNLL manifested high-affinity uptake and release of D-[3H] aspartate, which suggested the presence of synaptic endings that may use glutamate or aspartate as an excitatory neurotransmitter. Furthermore, ablation of the cochlear nucleus resulted in depression of D-[3H] aspartate release in the VNLL, indicating that glutamate or aspartate may be a transmitter for the CN-VNLL synapses (Suneja et al. 1995b). The VNLL also receives minor inputs from the ipsilateral MNTB and periolivary nuclei (lateral and ventral nuclei of the trapezoid body, and ventral periolivary nucleus) (Elverland 1978; Glendenning et al. 1981; Huffman and Covey 1995; Spangler et al. 1985; Vater and Feng 1990; Warr and Beck 1996). The projection from the MNTB to the VNLL comes from axon collaterals of MNTB principal neurons that give rise to efferents to the LSO. MNTB principal neurons have been shown to be immunoreactive for glycine (Helfert et al. 1989). The projection from the MNTB to the LSO is inhibitory and glycinergic (Moore and Caspary 1983; Wu and Kelly 1991). The projection from axon collaterals of MNTB neurons to the VNLL is probably inhibitory and glycinergic as well. Immunocytochemical studies have shown that neurons in the periolivary region, especially in the VNNTB and LNTB, are GABAergic (Adams and Mugnaini 1990; González-Hernández et al. 1996; Helfert et al. 1989; Moore and Moore 1987; Roberts and Ribak 1987; Vater et al. 1992; Winer et al. 1995). These neurons are another possible source of inhibition to the VNLL. The presence of glycine- and GABA-immunoreactive puncta and flattened synaptic vesicles associated with inhibitory synapses in the VNLLc of the big brown bat (Vater et al. 1997), and the existence of glycine- and GABA-immunoreactive perisomatic puncta in the VNLL of cat (Saint Marie et al. 1997) further support the concept that VNLL neurons receive both glycinergic and GABAergic inputs.

Intracellular labeling of VNLL neurons reveals that axons of some VNLL neurons give rise to collaterals some of which terminate within the VNLL (Zhao and Wu 1998). Certainly these VNLL neurons can act as interneurons and may exert inhibitory influence on other VNLL neurons. Therefore the IPSPs elicited by electrical stimulation of the lateral lemniscus may originate from VNLL interneurons that also were activated by electrical stimulation of the lateral lemniscus at the same time that the recording was made. But the short-latency IPSPs observed in some cases seem not to originate from the interneurons. In addition, preliminary pharmacological data showed that the IPSPs recorded from VNLL neurons still could be seen after blockade of the EPSPs (Wu 1997), indicating that in these neurons the IPSPs were elicited by theafferent fibers of the lateral lemniscus directly rather than elicited by the lateral lemniscus through interneurons within the VNLL.

In the present study there were similar proportions of type I and II cells (81.4 and 84.6%) that responded to stimulation of the lateral lemniscus with either EPSP or IPSP or both. The results suggest that many type I and type II VNLL neurons may receive and integrate excitatory and inhibitory afferent inputs from the auditory lower brain stem. Although the response patterns seen in this study are not necessarily the patterns produced in vivo by acoustical stimulation, the results demonstrate the possible synaptic interaction in one neuron. The output of synaptic integration from one neuron can depend on relative strengths of excitatory and inhibitory inputs that impinge on it (Fig. 7).

In the type 1 neuron group, many onset-pause, regular, and adaptation neurons responded to ascending inputs of the lateral lemniscus with either a single type of synaptic response or a combination of EPSPs and IPSPs. There is so far no information available about what kind of synaptic inputs that project to the neurons with different firing patterns. The incidence of both excitatory and inhibitory synaptic responses recorded from the neurons with different firing patterns. The incidence of both excitatory and inhibitory synaptic responses recorded from the neurons with different firing patterns.
brain stem with higher centers and that it plays multiple roles in auditory processing.

I thank Dr. J. B. Kelly for a critical reading of the manuscript and many helpful comments. I also thank B. van Adel for making Fig. 1.

This research was supported by the Natural Sciences and Engineering Research Council of Canada.

Address for reprint requests: S. H. Wu, Life Sciences Research Bldg., Institute of Neuroscience, Carleton University, 1125 Colonel By Dr., Ottawa, Ontario K1S 5B6, Canada.

Received 9 October 1998; accepted in final form 1 March 1999.

REFERENCES


