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**Morphology and Physiology of Neurons in the
Young Rat's Ventral Nucleus of the Lateral Lemniscus**

by

Min Zhao B. Med./M.Sc.

A thesis submitted to the faculty of Graduate Studies and
Research in partial fulfillment of the requirements of the
degree of

Master of Science
Specialization in Neuroscience

Department of Biology

Ottawa-Carleton Institutes of Biology and Neuroscience

Carleton University

Ottawa, Ontario

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the Faculty of Graduate Studies and Research
acceptance of the thesis,

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Min Zhao. B.Med./M.Sc.

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Chair, Department of Biology

Thesis Supervisor

Thesis Co-Supervisor

Carleton University
December, 1998

Abstract

To determine the correlation of physiological with morphological neuron types in the ventral nucleus of the lateral lemniscus (VNLL), whole-cell patch-clamp recording and intracellular recording were made to examine the intrinsic membrane properties and postsynaptic responses of VNLL neurons in brain slices of the young rat, and iontophoretically intracellular injection of neurobiotin was made to examine the morphological features of individual VNLL neuron that had been recorded from. VNLL neurons could be recognized as two distinct morphological groups: bushy-like cells and stellate cells. Bushy-like cells contained neurons with three types of intrinsic firing patterns: onset, regular I and onset-pause. Stellate cells contained neurons with two types of intrinsic firing patterns: regular II and burst. Both bushy-like cells and stellate cells responded to stimulation of the lateral lemniscus with excitatory and/or inhibitory synaptic potentials. The results suggest that different VNLL neurons may play different roles in processing information of a sound.

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List of abbreviations

ACSF	artificial cerebrospinal fluid
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxalolepropionate
AVCN	anteroventral cochlear nucleus
BDA	biotinylated dextran amine
CN	cochlear nucleus
DAB	3,3'-diaminobenzidine tetrahydrochloride
DCN	dorsal cochlear nucleus
DNLL	dorsal nucleus of the lateral lemniscus
EPSP	excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
GAD	glutamic acid decarboxylase
GluR	glutamate receptor
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HRP	horseradish peroxidase
HTS	high-threshold Ca^{2+} -spike
IC	inferior colliculus
I_h	inward current activated by hyperpolarization
INLL	intermediate nucleus of the lateral lemniscus
IPSP	inhibitory postsynaptic potential
LNTB	lateral nucleus of the trapezoid body
LSO	lateral superior olive
LTS	low-threshold Ca^{2+} -spike
MNTB	medial nucleus of the trapezoid body
mRNA	messenger ribonucleic acid
MSO	medial superior olive
PSTHs	post-stimulus time histograms
PVCN	posteroventral cochlear nucleus
SOC	superior olivary complex
SPSS	statistics package of social science

TTX	tetrodotoxin
VCN	ventral cochlear nucleus
VLLa	anterior subdivision of the VNLL
VLLd	dorsal subdivision of the VNLL
VLLv	ventral subdivision of the VNLL
VNLL	ventral nucleus of the lateral lemniscus
VNLLc	columnar cell area of the VNLL
VNLLm	multipolar cell area of the VNLL
VNTB	ventral nucleus of the trapezoid body

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Introduction

Anatomical Definition of the Ventral Nucleus of the Lateral Lemniscus

The ventral nucleus of the lateral lemniscus (VNLL) is the most ventral group of neurons lying within the auditory ascending pathway, the lateral lemniscus, which connects the lower brainstem and the midbrain. The VNLL is a distinct neuronal group in all non-primate mammalian species, such as cat (Goldberg and Moore 1967; Adams 1979; Glendenning et al. 1981; Whitley and Henkel 1984), bat (Zook and Casseday 1979; Covey and Casseday 1986, 1991), rat (Druga and Syka 1984; Spiera and Davis 1988; Merchán and Berbel 1996), ferret (Moore 1988), Guinea pig (Tokunaga 1988; Saint Marie and Baker 1990; Schofield and Cant 1997), opossum (Willard and Martin 1983), chinchilla (Saint Marie and Baker 1990) and rabbit (Batra and Fitzpatrick 1997). However, the VNLL is indistinct in New World monkeys, is difficult to define in apes and is very poorly developed in the human (Moore 1987).

Traditionally, the VNLL was defined as a group of neurons ventral to the dorsal nucleus of the lateral lemniscus (DNLL) (Tanaka et al. 1985; Friauf 1992; Wynne et al. 1995; Merchán and Berbel 1996). The DNLL and the VNLL are separated from each other by a narrow band of tightly grouped neurons referred to as the horizontal cell group of the lateral lemniscus (Bajo et al. 1993; Caicedo and Herbert 1993). However, recently several groups (Moore and Moore 1987; Caicedo and Herbert 1993; Wynne et al. 1995; González-Hernández et al. 1996; Ito et al. 1996; Saint Marie 1996; Kelly et al. 1998; van Adel et al. 1998) have distinguished the dorsal part of the VNLL as a separate nucleus, the intermediate nucleus of the lateral

lemniscus (INLL), and considered that the VNLL is located ventral to the INLL. In the human it seems no separate INLL can be recognized (Moore 1987).

Afferent Projections to the VNLL

The primary input to the VNLL comes from the cochlear nucleus (CN) and superior olivary complex (SOC). The VNLL receives inputs mainly from the contralateral ventral cochlear nucleus (VCN), with smaller projections from the ipsilateral VCN and SOC, and a very slight projection from the contralateral dorsal cochlear nucleus (DCN) (Browner and Webster 1975; Adam and Warr 1976; Glendenning et al. 1981; Warr 1982; Spangler et al. 1985; Zook and Casseday 1985; Covey and Casseday 1986; Friauf and Ostwald 1988; Helfert et al. 1991; Schwartz 1992; Huffman and Covey 1995; Schofield and Cant 1997; van Adel 1998).

Glendenning et al. (1981) reported that in cat after injection of a tract tracer, horseradish peroxidase (HRP), into the VNLL, 90% of the retrogradely labeled neurons were found in the contralateral CN and the remaining 10% were found in the ipsilateral SOC. Within the CN less than 1% of the labeled neurons were found in the dorsal division of the CN. The number of labeled cells in the anteroventral cochlear nucleus (AVCN) was more than twice that found in the posteroventral cochlear nucleus (PVCN). These authors concluded that the ventral division of the contralateral CN, specifically the AVCN, is the major contributor of ascending afferents to the VNLL. A negligible fraction of cells in the ipsilateral CN and contralateral DCN projects to the VNLL, targeting a small part of the ventrocaudal VNLL (Glendenning et al. 1981). Schofield and Cant (1997) used tract tracing techniques and demonstrated that in the guinea pig the VNLL receives bilateral inputs from the CN, with contralateral inputs greatly

outnumbering ipsilateral inputs. A very recent study by van Adel (1998) showed that following injection of a fluorescent dye, Fluorogold, into the rat VNLL most retrogradely labeled neurons were found in the contralateral AVCN and PVCN. Friauf and Ostwald (1988) used the intra-axonal HRP staining technique and demonstrated that in rat the contralateral VCN sends many main axons to the VNLL, with some small collaterals to both sides of the SOC. All these studies support the idea that the VNLL receives direct and major projections from the contralateral VCN.

Vater and Feng (1990) described the functional organization of ascending connections between CN and VNLL in the horseshoe bat. They found that neurons from the AVCN and PVCN respectively projected to two distinct divisions of the VNLL. The AVCN projections were confined to the lateral subdivision. The tonotopic representation in this area was from low to high frequencies along a medial to lateral gradient. In contrast to the AVCN, the PVCN projection supplied both the lateral and medial subdivisions of the VNLL. In the lateral region, small synaptic boutons were observed whereas in the medial region, large synaptic terminals, calyces, were found. Within the medial region of the VNLL, the PVCN projection was organized with increasing frequencies represented in the dorsoventral direction. In the bat there was an extended high-frequency representation in the lateral VNLL, but not in the medial VNLL (Vater and Feng 1990).

Cells identified as bushy, multipolar and octopus cells in the VCN are the major sources of the afferents to the VNLL (Friauf and Ostwald 1988; Schofield and Cant 1997; Thompson 1998; Warr 1972, 1982). 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).

Some researchers (van Noort 1969; Warr 1969, 1982; Adams and Warr 1976) demonstrated that octopus cells of the PVCN send thick axons in the intermediate acoustic stria to the VNLL. Adams (1983) reported that in cat octopus cell axons terminate in calyx endings. Wu (1998) also observed that there are thick axons with calyx endings in rat VNLL. In other mammals such as the mouse and bat, some VNLL neurons also receive large calyceal endings (Willard and Ryugo 1983; Zook and Casseday 1985; Covey and Casseday 1986; Vater and Feng 1990). Terminals of the calyceal type are larger than conventional bouton terminals and are also found in two other auditory brainstem nuclei, the AVCN and the medial nucleus of the trapezoid body (MNTB).

The VNLL 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, which give rise to efferents to the LSO.

Multiple horizontally oriented axon arbors that came from the ascending fibers in the VNLL were described in the cat and bat (Glendenning et al. 1981; Zook and Casseday 1985; Covey and Casseday 1986). In the rat horizontally oriented axonal arbors arise from VCN neurons which have primary-like physiological responses very similar to those of the auditory nerve, whereas vertically oriented axonal arbors arise from neurons with “on” responses and little spontaneous activity (Friauf and Ostwald 1988). The calyx endings come from thick axons of the PVCN neurons and end in the medial part of the VNLL of the horseshoe bat, and in the columnar cell area of the VNLL (VNLLc) in the big brown bat and mustache bat (Zook and Casseday 1985; Covey and Casseday 1986). The medium-sized (2-3.3 μm) bead-like

buttons originate from collaterals of other axons arising in AVCN and PVCN, and end in the lateral VNLL (Vater and Feng 1990).

The VNLL also receives descending projections from the ipsilateral and contralateral inferior colliculus (IC) (Syka et al. 1988). However, Malmierca et al. (1996) doubted these results and considered that the projection to the VNLL from the IC was most probably due to retrograde rather than anterograde transport of HRP from IC since in their own study labeled fibers in the VNLL were always found with retrogradely labeled neurons. But Iwahori's (1986) Golgi study has shown that the descending pathway from the IC runs within the lateral lemniscus, gives off axon collaterals which end in the DNLL, and travels ventrally, possibly sending collaterals to the VNLL. A recent study by van Adel (1998) has demonstrated that injection of biocytin into the central nucleus of the IC results in anterogradely labeled terminals in the VNLL, and restricted injections of Fluorogold into the VNLL retrogradely labels neurons in the IC. Although both antero- and retrograde labeling was sparse, van Adel's study combining retrograde with anterograde labeling gave more convincing evidence. These results all support the suggestion that there are some descending inputs to the VNLL from the IC. Another source of descending fibers to the VNLL may come from collaterals of the commissure of Probst, which is the efferent projection from the DNLL to the contralateral DNLL and IC (Iwahori 1986).

In addition, Glendenning et al. (1981) suggested that there was no input to the VNLL from the contralateral nuclei of the lateral lemniscus in cat. But using intracellular and extracellular labeling techniques in brain slices of the gerbil and bat, Tao et al. (1998) illustrated that labeled cells sent small but distinct axonal projections from the VNLL through

the commissure of Probst to the contralateral VNLL as well as to the contralateral DNLL and IC.

Efferent Projections of the VNLL

In contrast to the afferent projections to the VNLL, anterograde and retrograde tract tracing studies have shown that efferent projections of the VNLL mainly project to the ipsilateral IC in many mammalian species, including the cat, bat, rat, mouse, guinea pig, opossum and ferret (Goldberg and Moore 1967; Beyerl 1978; Adams 1979; Brunso-Bechtold et al. 1981; Glendenning et al. 1981; Kudo 1981; Zook and Casseday 1982; Nordeen et al. 1983; Willard and Martin 1983; Whitley and Henkel 1984; Tanaka et al. 1985; Moore D.R. 1988; Ross et al. 1988; Shneiderman et al. 1988; Saint Marie and Baker 1990; Huston et al. 1991; Covey and Casseday 1995; Ito et al. 1996; Merchán and Berbel, 1996; Frisina et al. 1998; Kelly et al. 1998). In addition to this major projection, there are ascending projections from the ipsilateral VNLL to the DNLL (Bajo et al. 1993; Labelle and Kelly 1996; Yang et al. 1996) and the medial geniculate body (Whitley and Henkel 1984). Whitley and Henkel (1984) reported that in the cat there are smaller descending projections of the VNLL ending mainly in the dorsomedial periolivary region and ventral nucleus of the trapezoid body (VNTB), and a few fibers in the ipsilateral CN. Winter et al. (1989) also observed that the VNLL sent some projections to the CN ipsilaterally in guinea pig. However, there was not much evidence for the contralateral projection of the VNLL to the IC (Brunso-Bechtold et al. 1981; Glendenning et al. 1981; Ito et al. 1996; Merchán and Berbel 1996; Kelly et al. 1998).

Cytoarchitectonic Features of VNLL Neurons

With regard to anatomical subdivisions of the VNLL, there is a great deal of controversy. According to cytoarchitectonic criteria, the VNLL in various mammals has been recognized as one single division (Kudo 1981; Nordeen et al. 1983; Willard and Martin 1983; Saint Marie and Baker 1990; Hutson et al. 1991; Merchán and Berbel 1996), two subdivisions (Brunso-Bechtold et al. 1981; Glendenning et al. 1981; Zook and Casseday 1982; Willard and Ryugo 1983; Covey and Casseday 1986; Kudo et al. 1990; Huffman and Covey 1995; Ito et al. 1996; Vater et al. 1997; Kelly et al. 1998) or three subdivisions (Adams 1979; Schofield and Cant 1997). In following paragraphs I will review the detailed cytoarchitecture of the VNLL in five different species of mammals, i.e., rat, mouse, bat, cat and guinea pig. Certainly, different cytoarchitectonic features of VNLL neurons exist among these species.

In the rat, the VNLL was recognized as a single division which contains only flat stellate neurons (Merchán and Berbel 1996). These authors analyzed cell morphology by examining Nissl stained and retrogradely biotinylated dextran amine (BDA) labeled VNLL neurons. They suggested that the VNLL is a rather homogeneous structure as it is composed of neurons of the stellate type only. In their study the labeled flat stellate neurons and fibers were oriented in parallel and formed fibrodendritic laminae. Each VNLL lamina formed a continuous ventrodorsal structure which resembled a helicoid. These authors suggested that the VNLL could not be subdivided based on the continuous and homogeneous pattern of cells seen in their 3-D reconstructions. But, they proposed future research correlating the morphology, electrophysiology, immunoreactivity and connectivity of neurons to map more definite cytoarchitecture in the rat VNLL.

In the mouse, the VNLL could be subdivided into a lateral part and a medial part according to Willard and Ryugo (1983). The lateral part is composed of a compact

accumulation of neurons. The constituent neurons have small rounded cell bodies and long, relatively thin dendrites that tend to be oriented parallel to the trajectory of the lemniscal fibers. These cells receive large, axosomatic calyceal endings, seen in Protargol preparations. In contrast, the medial part is composed of large multipolar neurons that are loosely scattered among the lemniscal fibers. These neurons have darkly staining cytoplasm, filled with coarse granules of Nissl substance and a round, pale-staining nucleus. In Golgi preparations, these neurons display long, less branched dendrites that radiate away from the cell body, orthogonal to the trajectory of the lemniscal fibers.

In the bat, the VNLL is greatly expanded and highly developed. The bat VNLL can be recognized as two subdivisions, the columnar cell area and the multipolar cell area (VNLLm) (Covey and Casseday 1986; Huffman and Covey 1995; Vater et al. 1997). In the VNLLc, virtually every neuron is of a single type. They are very similar to spherical bushy cells in the AVCN in that they are round to oval in shape and have one large, thick dendrite that branches profusely from the cell body, and are contacted with large calyx-like synaptic terminals in addition to conventional bouton terminals. Some of these branches appear to extend parallel to the ascending fibers. These cells are tightly packed in a columnar arrangement between bundles of fibers, so called “columnar nucleus” (Zook and Casseday 1982; Covey and Casseday 1986, 1991). In the VNLLm, most neurons are multipolar in shape. They are larger than VNLLc neurons and have several sparsely branching thick dendrites. The cell bodies and dendrites of neurons in VNLLm have no consistent orientation relative to the fibers of the lateral lemniscus (Covey and Casseday 1986; 1991). The connections of the VNLLc are organized in sheets that are precisely related to the tonotopic organization of afferents from the AVCN and efferents from the IC.

In the cat, the VNLL was divided into 3 zones (dorsal, middle and ventral) by Adams (1979). Even though the boundaries between zones are not distinct and regions of overlap commonly occur, each zone contains cytologically distinct cell types. Cells of the dorsal zone are large and multipolar in shape with darkly staining Nissl substance. In the middle zone, there are medium-sized multipolar cells, horizontal cells and darkly staining small cells. The predominant type in the ventral zone is the oval cell, which is covered with large end bulbs or calyces of Held in Protargol preparations. In addition, large multipolar cells, lightly staining round cells, elongate cells and lightly staining small cells are also found in the ventral zone.

In the guinea pig, based on cytoarchitecture and patterns of inputs from the cochlear nucleus, three subdivisions can be distinguished in the VNLL: ventral (VLLv), dorsal (VLLd) and anterior (VLLa) (Schofield and Cant 1997). The VLLv is the largest and most complex subdivision in the VNLL. It is characterized by the presence of globular cells and giant cells, and alternating bands of densely and loosely packed cells. In cresyl violet preparations, the globular cell is small and round, and stains very darkly throughout much of the subdivision. The high packing density of the globular cells gives the VLLv a characteristically dark appearance. Lightly staining multipolar cells are scattered among the globular cells. The giant cells are most common in the posterior and medial part of the VLLv. They usually have an elongated profile and stain darkly. The VLLd is populated primarily by lightly staining multipolar cells. Some of these cells are elongated along the horizontal axis. The giant cells can be seen in the VLLd, even though they are much less than the multipolar cells. There are two types of lightly staining cells in the VLLa, vertical cells and multipolar cells. The vertical cell has an elongated soma with its long axis parallel to the fibers of the lateral lemniscus. The evidence from both anterograde and retrograde tract tracing studies suggests that the thick

axons are present only in the VLLv, which originate from contralateral octopus cells of the VCN, whereas the thin axons throughout in the VNLL arise from both ipsilateral and contralateral multipolar cells and spherical bushy cells of the VCN.

Neurophysiological Properties of VNLL Neurons

To understand the fundamental function of the VNLL it is important to know the neurophysiological properties of VNLL neurons in addition to their anatomy. Neurophysiological studies of the VNLL have demonstrated that more than 90% VNLL neurons are functionally monaural, responding only to contralateral sounds (Aitkin et al. 1970; Guinan et al. 1972a, b; Covey and Casseday 1986, 1991; Metzner and Radtke-Schuller 1987; Vater et al. 1997). These electrophysiological properties are consistent with neuroanatomical evidence of the afferent inputs to the VNLL in mammals: the VNLL displays mainly monaural response properties and receives its main projections from the contralateral cochlear nucleus. However, recent studies suggest that a few neurons in the medial part of the VNLL are specialized for binaural processing (Batra and Fitzpatrick 1997; Batra 1998).

VNLL neurons respond to tone bursts with different temporal discharge patterns. In the cat, 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 the bat, cells in the VNLLc are broadly tuned with no spontaneous activity, and respond with one spike per stimulus and with constant latency to stimulus onset, so the VNLLc is thought to be specialized to encode the onset of a sound (Covey and Casseday 1991). Cells in the VNLLm respond to tone bursts with various temporal patterns, such as tonic, chopper, primary-like or pauser, but without the onset single-spike response pattern

found in the VNLLc. Therefore, the VNLLm is supposed to play a role in encoding ongoing properties of a sound (Covey and Casseday 1991). In the unanesthetized rabbit, some VNLL neurons respond phasically whereas others respond in a sustained manner (Batra 1998). The phasic neurons are of two types: onset and transient. Onset neurons typically respond to each tone with one action potential or two action potentials that fire in a chopping pattern. Transient neurons respond with a burst of four or more action potentials but without pronounced chopping pattern.

Using intracellular recording from rat brain slices, Wu (1998) found that there are two types of neurons in the VNLL, type I and type II, based on intrinsic electrical membrane properties. Type I neurons respond to intracellular injection of positive current with a graded depolarization and multiple action potentials. These neurons show three different firing patterns: regular, onset-pause and adaptive. The current voltage relations of type I neurons are nearly linear. Type II neurons respond to positive current with a limited depolarization and only one or a few action potentials. The current voltage relations of type II neurons are non-linear near the resting potential. It is supposed that the membrane properties of the type II VNLL neurons may play an important role for processing information about time of onset of a sound.

Neurochemical Properties of VNLL Neurons

Neurochemical properties of VNLL neurons, with respect to putative neurotransmitters and synaptic receptors, have been studied in various species. Several studies of the neurotransmitter system of the VNLL have demonstrated that gamma-aminobutyric acid (GABA) and glycine are the major inhibitory amino acids in the VNLL. Using antibodies

against to glutamic acid decarboxylase (GAD, a GABA synthesizing enzyme found in GABAergic neurons) or GABA, the VNLL is found to contain both immunoreactive terminals and neurons in owl and bat (Carr et al. 1989; Vater et al. 1992). After injection of [^3H]glycine into the IC many VNLL neurons in chinchillas and guinea pig were retrogradely labeled (Saint Marie and Baker 1990). Injecting horseradish peroxidase (HRP) or Fluorogold into the IC, many of the retrogradely labeled neurons in the rat VNLL showed positive immunostaining with GABA antibody (González-Hernández et al. 1996; Zhang et al. 1998). Saint Marie et al. (1997) further detailed that in the cat the VNLL contained 81% glycine-immunoreactive neurons, and about half of these were also GABA-immunoreactive. The remaining neurons were either nonimmunoreactive (8%) or GABA-immunoreactive only (11%). Riquelme et al. (1998) used both immunohistochemistry and *in situ* hybridization to show that most neurons in the ventral portion of the rat's VNLL contained glycine-like immunoreactivity and labeled for GAD mRNA, whereas the dorsal portion had a relatively low proportion of glycine-containing neurons. GABA immunoreactive neurons were scarce throughout the VNLL, except in the ventral portion where neurons were labeled for glycine as well as GABA. In the dorsal portion of the VNLL there was a large population of neurons that was immunonegative for both GABA and glycine. Riquelme et al. (1998) suggested that the VNLL neurons can be divided in two main categories: those that contain GABA as well as glycine, and those that contain neither of these transmitters. They also pointed out that a distinct gradient in the number of immunopositive neurons is apparent, with the highest concentration in the ventral portion of the VNLL. Similar results were obtained in the rat by Oliver and Bishop (1998) using a GABA and glycine double-labeling immunofluorescence procedure. Wynne et al.'s study (1995) also showed that only neurons in the ventral division of the VNLL were labeled

for GAD mRNA. Whether or not neurons immunonegative for GABA and glycine in Riquelme et al.'s, and Oliver and Bishop's studies contained excitatory neurotransmitters is not clear. So far there has been no information about excitatory neurotransmitters contained in or released by VNLL neurons. But the studies of inhibitory transmitters suggest that many VNLL neurons contain either GABA or glycine or both. The VNLL is a major source of inhibitory input to the IC and may play a significant role in inhibitory processing in the auditory brainstem.

Neurochemical studies further support the idea that projections from the VCN 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). Levin et al. (1997) found subunits of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxalolepropionate) receptor, GluR1-4, in the VNLL neurons and high levels of GluR4 in the dendrites.

The projection from the MNTB to the VNLL comes from axon collaterals of MNTB principal neurons that also project 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 VNTB 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 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. Using an *in situ* hybridization method, Wynne et al. (1995) reported that the mRNA for $\alpha 1$, $\alpha 2$ and $\alpha 3$ GABA_A subunits of receptor was detectable in the VNLL. Fubara et al. (1996) used a receptor autoradiographic ligand binding procedure and observed glycine and GABA_A receptors, and sparse GABA_B receptors in the VNLL. In addition, there is an insulin-like growth factor type 2 receptor seen in the VNLL, whose biological significance is unknown (Nagano et al. 1995).

All these anatomical, physiological and neurochemical results suggest that the VNLL is a structure containing a heterogeneous population of neurons. These neurons have different morphological features, exhibit different physiological properties and use different neurotransmitters and synaptic receptors for synaptic transmission.

Purpose of the Present Study

Although some information about the neuroanatomy, neurophysiology and neurochemistry of the VNLL in various species of mammals have been available, physiological studies on VNLL neurons in terrestrial mammals (non-echo-locating species) are few and the function of the VNLL is still largely unknown. There has been only one intracellular study of the physiological properties of VNLL neurons (Wu 1998). In that study

each VNLL neuron could be classified as having one of two intrinsic membrane properties, viz., either a non-linear or a linear current relation. Cells with linear current voltage relations fire multiple action potentials, which exhibit different temporal patterns in different neurons. Cells with non-linear current voltage relation fire only one action potential. These two distinct characteristics are also found in the other auditory neuronal groups, such as the VCN and SOC. Intracellular recording combined with intracellular labeling from neurons of these groups have revealed that the neurons with two different membrane properties have distinguishable morphological features. Cells with non-linear current voltage relations are bushy cells in the AVCN and octopus cells in the PVCN (mouse, Wu and Oertel 1984; Golding et al. 1995), principal cells in the medial superior olive (MSO) (guinea pig, Smith 1995) and principal cells in the MNTB (rat, Wu and Kelly 1991). Cells with linear current voltage relation are stellate cells in the VCN (mouse, Wu and Oertel 1984) and principal cells in the lateral superior olive (LSO) (rat, Wu and Kelly 1991, Wu and Fu 1998). As VNLL neurons exhibit two distinct types of membrane properties similar to those found in the VCN and SOC, we proposed a hypothesis: specific physiological properties of VNLL neurons should be correlated with their distinct morphological features. In order to test this hypothesis, whole-cell patch-clamp recordings or intracellular recordings were used to examine the membrane properties and synaptic responses of individual VNLL neurons. At the same time, neurobiotin, was iontophoretically injected to label the cells and identify their morphological types. Thus, the physiology and morphology of each individual VNLL neuron could be directly compared. The objective of correlating the physiology with the cell morphology in this study was to study the relation between the function and structure of VNLL neurons, and to compare our data with the data from other studies about the VNLL as well as other auditory

neurons. Finally, with our results we would be able to try to understand how different VNLL neurons which have distinct physiological and morphological characteristics process different auditory information, and what role the VNLL may play in auditory processing.

In addition, most previous anatomical studies have investigated the cell morphology of the VNLL by examining Nissl stained or Golgi stained cells. These methods allow one to stain only the cell body and partial dendrites. There has been no previous study about dendritic field as well as axonal projection of individual VNLL neurons. With intracellular labeling, especially with whole-cell patch recording procedure, the tracer is easily diffused from the recording pipette into the cytoplasm, thus the whole profile of the dendrites as well as the axon and its collaterals can be labeled. The data about the cytoarchitectonic features of VNLL neurons will give us detailed information about not only the soma, i.e., the size, shape and location, but also the dendritic field and axonal morphology and projection patterns. Information of the size and orientation of the dendritic field will help us to understand how VNLL neurons receive afferent inputs. Orientation of the axon and collaterals are especially important for us to understand the VNLL output, which is closely related to the function of this nucleus.

Methods and Materials

Brain Slice Preparation

Albino rats (Wistar) between 12 to 24 days of age were used for brain slice preparations. The animals were decapitated after being anesthetized with halothane and their brains were then dissected in 30 °C oxygenated artificial cerebrospinal fluid (ACSF). Brain slices of 400 μm thickness were cut in frontal plane through the auditory midbrain with a Vibratome tissue slicer. Normally, one or two slices that contained the VNLL were obtained from each rat. The best slice was selected and transferred to a recording chamber. The slice was held in the chamber with two pieces of nylon mesh and completely submerged in the warm (32-34 °C) ACSF, which was circulated through the chamber at a flow rate of 10-12 ml/min. The ACSF consists of (in mM) 129 NaCl, 3 KCl, 1.2 KH_2PO_4 , 2.4 CaCl_2 , 1.3 MgSO_4 , 20 NaHCO_3 , 3 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and 10 glucose in distilled water and was saturated with 95% O_2 -5% CO_2 before circulation through the slice chamber. The pH was 7.4 after the ACSF was fully saturated with the mixture of O_2 - CO_2 . The slice was kept in the recording chamber for about one hour before any recording was made.

Electrophysiological Methods

Intracellular Recording

The brain slice was illuminated from below by light passed through a darkfield condenser and the structures of the auditory brainstem were identified with a Leitz dissecting microscope. The VNLL was clearly visible. A single recording electrode was inserted into the VNLL and a bipolar stimulating electrode constructed of insulated tungsten wires with a tip separation of

50 μm was placed on the ventral area of the VNLL.

Recordings were made with glass microelectrodes which were pulled with a Flaming/Brown micropipette puller (Model P-80/PC). The glass microelectrodes were made from 1.0 mm OD and 0.5 mm ID thin-walled glass capillaries containing a microfilament (Borosilicate with filament, Sutter Instrument Co.). The microelectrode was filled with 2% neurobiotin (Vector Laboratories) in 2 M potassium acetate. The electrode impedance was typically between 120 and 180 M Ω . Once the electrode was positioned over the VNLL it was advanced in 2- μm steps through the brain tissue with a Burleigh piezoelectric driver (Inchworm). Application of an oscillating current (buzzing) was used to facilitate intracellular penetration. Recordings were made with an Axon Instruments Axoprobe 1-A amplifier and data were stored with a Nicolet Benchtop Waveform Acquisition System 400 for subsequent analysis. Resting potentials were monitored continuously with a chart recorder.

Synaptic responses were elicited by electrical stimulation of the ventral area of VNLL (Fig.1). Electrical stimuli were either positive or negative square waves, 100 μs in duration, obtained from a Grass S-8800 stimulator and stimulus isolation unit. When neural responses were encountered, the current strength was adjusted to produce reliable postsynaptic responses. The rate of stimulation was 1 stimulus per second.

Whole-cell Patch-clamp Recording

The brain slice chamber was illuminated by light from below, so that the lateral lemniscus and the VNLL were visible under the Zeiss microscope. The recordings were made using a blind whole-cell patch procedure. The patch pipettes were fabricated with a

Flaming/Brown micropipette puller. The pipette was made from 1.1 mm OD and 0.8 mm ID thin-walled glass tubing (Kimax-51, Kimble). The pipette was filled with a solution of the following composition (in mM): 130 K gluconate, 2 MgCl_2 , 5 KCl, 0.3 GTP, 2 ATP, 0.6 EGTA, 10 HEPES. In all experiments, neurobiotin (0.5%) was added to the internal solution for intracellular staining. The impedance of the electrode was 5-7 $\text{M}\Omega$. The blind whole-cell patch was obtained using a blow and suck procedure. First, the electrode was lowered with a gross three-dimensional manipulator towards the surface of the VNLL. An appropriate positive pressure was applied by mouth to the electrode to prevent it from being clogged. Once the tissue was touched, which was indicated by increase in impedance of the electrode, the electrode was finely advanced into the tissue with a Narishige hydraulic micromanipulator. A whole-cell patch clamp was achieved by applying a sudden negative pressure to the electrode through suction by the experimenter. An Axonpatch 200A amplifier was used for whole cell patch-clamp recordings. The data were collected and analyzed with the pClamp6 software package.

Post-synaptic potentials were elicited by electrical stimulation to the ventral area of the VNLL. Bipolar stimulating electrodes were constructed from two insulated tungsten wires with a tip separation of 50 μm . A stimulation pulse that was triggered by the computer waveform was generated by a Grass S-8800 stimulator and delivered through an isolator to the tissue. The width of the pulse was 0.1 ms. The stimulation was adjusted to an appropriate level to just evoke an apparent response of the neuron.

Morphological Methods

For each brain slice, one to three neurons in the VNLL were labeled by intracellular

injections of neurobiotin. The injections in the VNLL were placed far enough apart so that correlation between cell morphology and physiological characteristics could be made from histological reconstruction. For intracellular recording, neurobiotin was iontophoretically injected into the neuron by passing positive current through the electrode tip. A 1.5 nA current was injected in 120 ms pulses at a frequency of 1.6 Hz for 15-30 min. For patch-clamp recording, positive pulse current (20-1200 pA, 120-350 ms) was routinely injected for obtaining current-voltage relation and firing patterns, which were usually sufficient for producing a successful intracellular labeling. One hour to three hours after the injection the brain slice was fixed by immersion in 4% paraformaldehyde with 0.2% picric acid. The fixed slices were embedded in a 2.5% solution of agar dissolved in 10% formaldehyde and were cut by a Vibratome into 60 μ m sections. The sections were rinsed in 0.1 M phosphate buffer and incubated overnight with an avidin/biotin-horseradish peroxidase solution provided by the VECTASTAIN ABC KITS (Vector Laboratories). The large glycoprotein, Avidin, binds strongly and irreversibly to the small molecule, biotin. Biotin, which has been previously conjugated with horseradish peroxidase, is linked in a 3-dimensional array with avidin. Each avidin molecule has 4 binding sites for biotin, and the macromolecular complex retains at least one open biotin binding site. This site is filled by a neurobiotin molecule, thus retaining the ABC complex at the injection site. The sections were then rinsed in phosphate buffer and reacted with 3,3'-diaminobenzidine tetrahydrochloride (DAB) using the nickel-cobalt intensification method (Adams 1981). In the presence of peroxidase with the addition of hydrogen peroxide, DAB was rapidly oxidized into an insoluble, amorphous brown substance. In order to increase the darkness of the color, nickel and cobalt salts were applied to create the formation of blue-black polymers. Finally, the sections were cleared, mounted and then

counterstained with cresyl violet.

Data Analysis

Physiology

When a cell was isolated, current was passed gradually from negative current to positive current through the recording electrode to observe the changes of the membrane potential. The voltage was measured by the peak or maximum shift in membrane potential and was plotted as a current-voltage curve. The input resistance of neurons could be obtained from calculating the slope of the linear part of the current-voltage curve.

The time constant was obtained by measuring the duration from the beginning of the hyperpolarization shift down to 63% of the whole hyperpolarization.

Firing rate (Hz) was calculated by the equation: spike number $\times$ 1000/duration of injected current (ms).

Postsynaptic potentials were elicited by gradual increase of electrical stimulation to the ventral area of the VNLL. The duration of synaptic potentials was measured from the beginning of synaptic responses to the end of repolarizations.

Anatomy

The dendritic morphology of labeled cells was reconstructed by camera lucida drawings of serial sections at $\times 63$ or $\times 40$ magnification. Images were projected onto drawing paper and labeled processes were superimposed for each section taken through the labeled neuron. The reconstruction of individual cells was superimposed on camera lucida drawings of the VNLL.

made with a $\times 10$ objective. Thus the position of each neuron within the VNLL was precisely identified.

To determine the orientation of the cell body, the angle between the long axis of the cell body and the parallel fibers of the lateral lemniscus passing through the VNLL was measured. If the angle was between $0-45^\circ$, the cell was defined as a vertical cell. If the angle was between $46-90^\circ$, the cell was defined as a horizontal cell. If the cell body had no preferential long axis, the cell was defined as a non-orientated cell.

The area of cell bodies was measured by the Commercial Imaging Software Package (MCID Imaging Research).

Statistics

All data measured from recordings or drawings were expressed as their actual value. Groups' numerical data presented in the text are expressed as the mean and standard error of mean (Mean $\pm$ SEM) or percentage.

The normality of sample distributions was examined by testing the skewness. It is showed that the data of each group were normally distributed. So, two-tailed student's independent t test was used to evaluate whether or not differences between the different groups were statistically significant. A P value <0.05 was considered significant.

All statistical analysis of the results was performed using SPSS (Statistics Package of Social Science) for IBM windows.

Results

Physiological Properties of the VNLL Neurons

Whole-cell patch-clamp recordings were made from 59 VNLL neurons in 43 brain slices. The physiological properties of neurons in the VNLL were examined using the neurons' intrinsic membrane characteristics and synaptic responses.

Intrinsic Membrane Characteristics

The intrinsic membrane characteristics of the VNLL neurons were investigated using injection of intracellular current. The current-voltage relationship and firing pattern of each neuron were analyzed to identify the physiological class of the neurons.

Current-Voltage Relations

The VNLL neurons recorded in this study were classified into two groups on the basis of their current-voltage relationship as either “linear” or “non-linear”.

Most of the neurons (73%, 43 of 59 neurons) belonged to the linear group. Injection of negative current produced a graded hyperpolarization of the cell membrane. In some neurons, the hyperpolarization occurred with a gradual “sag” of the membrane potential toward the level of resting potential with continued application (shown as traces 4 and 5 in Fig. 3B; traces 5 and 6 in Fig. 4B). Positive current injections produced a graded depolarization that led to a continuous train of action potentials at suprathreshold values (Fig. 3-6). The relation of current to voltage was closely approximated by a linear function when the membrane potential was measured at the point of maximum deflection of the hyperpolarization. Figure 3C, 4C, 5C and

6C show examples of VNLL neurons with essentially linear current-voltage curves.

Some neurons (27%, 16 of 59 neurons) exhibited non-linear current-voltage relationship. Negative current injection produced a graded hyperpolarization of the cell membrane. All “linear” neurons had a gradual “sag” in the membrane potential with continued application of negative current. Passing positive current produced a depolarization which led to only one spike at the onset of the injection (shown as trace 2 in Fig. 2B). Increasing the intensity of the current injection did not lead to a continuous train of action potentials but produced only one spike (shown as trace 1 in Fig. 2B) or a few spikes (shown as Fig. 2A). The current-voltage relation was non-linear, as shown in Fig. 2C.

Firing Patterns

Each individual neuron observed in the present study had its own distinct firing pattern. According to the characteristics of the firing patterns, the neurons in the VNLL could be categorized into five types: onset, regular I, onset-pause, regular II and burst.

Onset Type In 27% of the cells examined (16 of 59 neurons), only one spike at the onset of depolarization was produced by lower positive current pulses and two or three spikes by higher positive current pulses (shown as Fig. 2A and traces 1 and 2 in Fig. 2B). The discharge threshold of neurons with this type of response was the highest (366.25 ± 18.54 pA) among the five types. These neurons typically showed a non-linear current-voltage relationship. With application of negative current, the membrane potential was hyperpolarized, always with an obvious “sag”. Usually, a depolarization, which could elicit an action potential, appeared immediately after the offset of hyperpolarizing pulses (13 of 16 neurons, 81%). The “sag” and after-depolarization probably reflect activation and deactivation, respectively, of an

inward hyperpolarization-activated current (I_h) as described for many neurons in the auditory and other systems (Maccaferri et al. 1993; Bayliss et al. 1994; Pape 1996; Fu et al. 1997). The resting potential of neurons with an onset type response pattern was -57.50 ± 1.32 mV. Their input resistance was lower (38.34 ± 7.18 M Ω) and their time constant was shorter (6.87 ± 0.76 ms) compared to the other 4 types of neurons.

Regular I Type In 19% of the cells examined (11 of 59 neurons), a continuous train of action potentials with almost regular intervals was produced by positive current injection. First spike occurred immediately following the onset of the current pulse. The train of action potentials induced by higher current strength often displayed smaller interspike intervals for the first several action potentials than for the later ones (shown as Fig. 3A and trace 1 in Fig. 3B). The discharge threshold of neurons with this type of response was 223.64 ± 23.12 pA. The number of action potentials produced by injection of positive current was proportionally related to current intensity as shown in Figure 7A. These neurons typically had a linear current-voltage relationship, but, similar to the onset type cells, they responded to negative current injection with hyperpolarization and an obvious “sag”. Regular I type neurons had relatively lower input resistance (54.06 ± 9.79 M Ω) and shorter time constant (9.95 ± 0.63 ms) compared to regular II and burst type neurons. The majority of them (9 of 11 cells, 82%) could produce after-depolarization or after-spikes following offset of hyperpolarizing pulses. The resting potential of regular I type neurons was -56.45 ± 1.89 mV. Figure 3 shows an example of the regular I type cell.

Onset-pause Type In 7% of the cells examined (4 of 59 neurons), small amount of positive current produced only one spike at the beginning of depolarization (shown as trace 4 in Fig. 4) or one spike followed by a long silent period, and then continuous firing (shown as

trace 3 in Fig. 4). First spike occurred immediately following the onset of the current pulse. A large amount of positive current produced a short train of spikes with a very short interspike interval, which was followed by a shorter pause and sustained regular firing (shown as traces 1 and 2 in Fig. 4). The pause became shorter and shorter as the strength of current injection increased. The number of action potentials produced by injection of positive current was also proportionally related to the current strength as shown in Figure 7B. The discharge threshold of neurons of the onset-pause type was 245.00 ± 33.17 pA. Similar to regular I type cells, these neurons typically had a linear current-voltage relationship, and responded to negative current injection with hyperpolarization and an obvious “sag”. Their membrane input resistance was lower (64.88 ± 16.77 M Ω) and time constant was shorter (9.88 ± 1.25 ms) compared to regular II and burst types. The resting potential of these cells was -55 ± 1.25 mV. All four onset-pause neurons could produce after-depolarization or after-spikes following hyperpolarizing pulses. Figure 4 shows an example of the onset-pause type cell.

Regular II Type In 20% of the cells examined (12 of 59 neurons), a continuous train of action potentials with fairly regular intervals was produced in response to positive current injection. In response to low intensity current injection, the discharge of the first spike occurred late (shown as trace 2 in Fig. 5B), and gradually occurred earlier when the intensity of the injected current was increased (shown as Fig. 5A and trace 1 in Fig. 5B). The train of action potentials induced by larger amounts of positive current often displayed smaller interspike intervals for a first few action potentials than for those seen during the sustained portion of the response (shown as Fig. 5A and trace 1 in Fig. 5B). The number of action potentials produced by injection of positive current was also proportionally related to current intensity as shown in Figure 7C. The discharge threshold (101.67 ± 14.19 pA) of these neurons

was lower than that of neurons in the above three types. Also, regular II type neurons showed a linear current-voltage relationship. But unlike the others, the regular II type neurons responded to negative current injection without an obvious “sag” of hyperpolarization or after-depolarization (92%, 11 of 12 cells). Furthermore, they had higher input resistance ($135.23 \pm 24.31 \text{ M}\Omega$) and longer time constant ($20.85 \pm 1.65 \text{ ms}$) compared to the above three types. The resting potential ($-58.92 \pm 2.10 \text{ mV}$) was close to that for the above types. An example of the regular II type cells is shown in Figure 5.

Burst Type In 27% of the cells examined (16 of 59 neurons), a train of action potentials was produced by positive current injection. The action potentials were a train of burst discharges arising from a depolarization “hump” that lasted for more than 100 ms at the lower current levels (shown as traces 1 and 2 in Fig. 6B). The burst discharge was followed by regular sustained firing at higher current levels (shown as Fig. 6A). The number of action potentials produced by injection of positive current was also proportionally related to current intensity as shown in Figure 7D. The discharge threshold ($85.00 \pm 7.18 \text{ pA}$) was close to that of the regular II type cells. Burst type neurons also displayed a linear current-voltage relationship. Half of them (8 of 16 cells) showed a “sag” of hyperpolarization and after-depolarization or after-spikes in response to negative current. Similar to regular II type cells, they had a higher input resistance ($124.49 \pm 14.68 \text{ M}\Omega$) and higher time constant ($25.79 \pm 3.14 \text{ ms}$). The resting potential ($-59.63 \pm 1.30 \text{ mV}$) of these cells was similar to those of the above mentioned types. An example of the burst type cells was showed in Figure 6.

The resting potential, input resistance, time constant and discharge threshold of neurons for each of the five types are summarized in Table 1. Generally, the onset type, regular I type and onset-pause type cells had lower input resistance, shorter time constant and higher

discharge threshold than the regular II type and burst type cells. Of the five types, onset type cells had the lowest input resistance, shortest time constant and highest discharge threshold.

Synaptic Responses

Electrical stimulation of the lateral lemniscus ventral to the VNLL elicited excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) in VNLL neurons for all 5 firing types. Synaptic responses were recorded from 56 VNLL neurons. Of 56 neurons, 22 had EPSPs only (39%), 10 had IPSPs only (18%) and 24 displayed both EPSPs and IPSPs (43%). For neurons with combined postsynaptic responses, the IPSP had a lower threshold than the EPSP in 63% of the neurons, the EPSP had a lower threshold than the IPSP in 29% of the cases, and the EPSP and IPSP had the same threshold in the remaining 8% of cells.

EPSP In 39% of the neurons examined, an EPSP was apparent without any evidence of an IPSP. Figures 8A, 8B and 9 show examples of the graded EPSPs produced by increasing stimulus intensity in 3 different neurons, respectively. The stepwise increase in EPSP amplitude in these cases implies a recruitment of several convergent excitatory inputs as stimulus strength was increased. The duration of the EPSP varied among neurons and was between 11.5 ms to 196 ms. The duration of the EPSPs recorded from a neuron as shown in Fig. 8A was much shorter than that of the EPSPs of other 2 neurons as shown in the Fig. 8B and 9. For these neurons, there was no apparent IPSP in the response under any stimulation condition, although this does not rule out the possibility that some inhibitory component was present but obscured by the excitatory potential. For cells with longer EPSP duration, some generated only one action potential in response to higher voltage stimulation (shown as the top

trace in Fig. 8B), whereas others could generate two to five spikes (shown as top trace in Fig. 9). The multiple spikes always arose from the long duration EPSP, while the short duration EPSP could result in only one action potential (shown as top trace in Fig. 8A).

In general, the EPSP duration of onset, regular I and onset-pause type neurons (36.50 ± 9.45 ms) were much shorter than that of regular II and burst type neurons (110.07 ± 9.13 ms) (Fig. 21D). Only one postsynaptic action potential could be elicited in all of the onset, regular I and onset-pause type neurons, half of the regular II type cells and 36% of the burst type cells tested in the present study, whereas in 50% of the regular II type and 64% of the burst type cells electrical stimulation of the lateral lemniscus could evoke multiple spikes.

IPSP In 18% of VNLL neurons examined an IPSP was apparent without any evidence of an EPSP. The duration of the IPSP was between 11 ms and 90 ms. Figure 10 shows an example of the graded IPSPs produced by increasing stimulus intensity. The stepwise increase in IPSP amplitude in this case implies a recruitment of several convergent inhibitory inputs as stimulus strength was increased. Even though no EPSP was detected in these neurons, it does not prove the absence of an excitatory component which might be obscured by the inhibitory potential.

Combination of EPSP and IPSP In 42% of neurons examined, electrical stimulation of the lateral lemniscus evoked combined postsynaptic responses. The majority (63%) had lower thresholds for EPSPs, while the others (29%) had lower thresholds for IPSPs. Figures 11A and B show examples of combined EPSPs and IPSPs recorded from two neurons. For the neurons shown in Figure 11A, near-threshold stimulation (0.6V) produced a small EPSP, and a slightly higher stimulation elicited an EPSP followed by a small IPSP. The amplitude of the

EPSP and IPSP increased substantially with greater stimulus strength (1V, 2V and 3V), but the EPSP was dominant throughout the response. A single action potential could be evoked with sufficient stimulus strength (3V). For the neurons shown in Fig. 11B, near-threshold stimulation (0.8V) produced an IPSP. Higher stimulus levels (3V, 6V, 15V and 30V) evoked a larger IPSP followed by an EPSP. The amplitude of the EPSP increased as the intensity levels were increased. However, no postsynaptic action potential was elicited in this neuron. It may imply that the IPSP was strong enough to resist depolarization from reaching threshold for the generation of an action potential. In 8% of the neurons, EPSPs and IPSPs had similar thresholds, that is, near-threshold stimulation evoked both EPSP and IPSP.

A summary of postsynaptic potentials of VNLL neurons with 5 different firing patterns was shown in Table 2. It seems that there was no obvious difference in the incidence of excitatory and inhibitory postsynaptic responses among the onset, regular I and onset-pause type cells. The EPSPs only and IPSPs only were evoked in 23% of these three type cells, respectively. The synaptic potentials with a combination of EPSPs and IPSPs were displayed in 53% of these cells. There were more excitatory postsynaptic responses in regular II and burst type neurons. The EPSPs only and IPSPs only were evoked in 58% and 11% of these two type cells, respectively. The synaptic potentials with a combination of EPSPs and IPSPs were displayed in 31% of these cells.

Morphological Properties of VNLL Neurons

49 neurons with stable physiological recordings in the VNLL were labeled with neurobiotin. They were subdivided into two broad categories on the basis of their dendritic

morphology, viz., bushy-like and stellate cells. Neurons in these two groups had some similar features, such as the shape, area and orientation of the cell body, and the number of dendritic branches. But, they had some different characteristics such as dendritic field and soma position in the VNLL.

Bushy-like Cell

23 of 49 (47%) neurons were classified as bushy-like cells. Most cell bodies of bushy-like cells (16, 70%) were oval in shape (shown as Fig. 12A, B and C). Some cell bodies (6, 26%) were round (shown as Fig. 12D) and only one (4%) was elongate. The area of the cell bodies was $181.13 \pm 12.74 \mu\text{m}^2$ (range: 78-297 μm^2). Most of bushy-like cells (19 of 23, 83%) were located in the ventral area of the VNLL. Only 3 cells (13%) were found in the dorsal area of the VNLL and one cell (4%) in the middle area. No apparent lateral-medial preference was found; 8 cells in the lateral, 9 cells in the medial and remaining 5 cells in the central region. For orientation of the cell body, 57% (13 of 23) cells were horizontal; 17% (4 of 23) cells were vertical and 26% (6 of 23) cells were non-oriented. A summary of the soma characteristics of bushy-like neurons is shown in Table 3.

Dendritic branching of the bushy-like cell was evident, with 3 to 7 primary dendrites exiting directly from the cell body. Each dendrite was further branched. The dendritic field extended over $249.38 \pm 16.66 \mu\text{m}$ mediolaterally, $278.24 \pm 24.06 \mu\text{m}$ dorsoventrally and $194.29 \pm 15.85 \mu\text{m}$ rostrocaudally (n=21). Dendrites of bushy-like cells branched profusely and compactly within a relatively restricted area (Fig. 14-16). Some dendrites were moderately spiny and some were sparsely spiny.

The axon diameter of the bushy-like cells ranged between 0.5-3.3 μm (n=15), measured

from the beginning segment. 4 bushy-like cells had axons thicker than $2.2\ \mu\text{m}$ after exiting from the cell bodies. The axons usually turned at an almost right angle, then headed dorsally and coursed along the fibers of the lateral lemniscus up to $2200\ \mu\text{m}$ as being traced (some were cut by making the slice initially), and finally ended at the IC. In 6 cases, the main axon sent a recurrent branch that spanned $55\text{-}368\ \mu\text{m}$ down to the ventral area of the VNLL. Most of axons (13 of 15) had collaterals within the VNLL, one in the IC also. The axon collaterals could be followed to their bouton terminals in the VNLL or vicinity of the original cell (shown as neuron 2 in Fig. 14, neuron 4, 5 in Fig. 15 and neuron 6, 7, 8 in Fig. 16). The thin collaterals could exit from the recurrent branch of an axon as well. Figure 13A shows the recurrent branch with the thin collaterals and terminals. Figure 13B shows the main axon with the collaterals and terminals.

Detailed data about the dendritic fields and axons of the bushy-like cells are shown in Table 5.

Stellate Cell

26 of 49 (53%) neurons were classified as stellate cells. Similar to the bushy-like cells, most of stellate cells ($n=15$, 58%) had oval somas. Some cell bodies ($n=4$, 15%) were round (shown as Fig. 12H) and some ($n=4$, 15%) were elongate (shown as Fig. 12G). However, some neurons had cell bodies that were not found in the bushy-like cells; these are referred to here as stellate cells (shown in Figure 12E, F; $n=3$, 12%). The area of stellate cell bodies was $163.54 \pm 16.42\ \mu\text{m}^2$ (range: $69\text{-}435\ \mu\text{m}^2$). In contrast to the bushy-like cells, most of the stellate cells (16 of 26, 62%) were located in the dorsal area of the VNLL. 8 cells (31%) were in the ventral area of the VNLL and 2 cells (7%) in the middle area. No apparent lateral-medial

preference was found; 8 cells in the lateral, 8 cells in the medial and remaining 10 cells in the central region. The cell body orientation of the stellate cells was not different from that of the bushy-like cells: 42% (11 of 26) cells were horizontal; 31% (8 of 26) cells were vertical; and 27% (7 of 26) cells were non-oriented. Table 4 shows a summary of soma characteristics of the stellate cells.

The stellate cells had 2 to 7 primary dendrites which exited immediately from the cell body. Each dendrite was further branched. The dendritic field ($n=22$) extended over $249.38 \pm 16.66 \mu\text{m}$ mediolaterally, $278.24 \pm 24.06 \mu\text{m}$ dorsoventrally and $194.29 \pm 15.85 \mu\text{m}$ rostrocaudally. Stellate cells displayed a significantly larger dendritic tree in mediolateral and dorsoventral directions than bushy-like cells (Fig. 20). Dendrites of stellate cells radiated away from the cell bodies and extended over a relatively large area in all directions, but branched less in comparison to the dendrites of the bushy-like cells. Some dendrites were not obviously spiny and some were apparently spiny. Figure 17-19 shows 6 stellate neurons.

The axon diameter of the stellate cells ranged between $0.8\text{-}1.7 \mu\text{m}$ ($n=13$), measured from the beginning segment. The axons also turned at an almost right angle after exiting from the cell bodies, then headed dorsally and coursed along the fibers of the lateral lemniscus up to $3551 \mu\text{m}$ as being traced (some were cut by making the slice initially), and finally ended in the IC. Only in one case, the main axon sent a recurrent branch that spanned $148 \mu\text{m}$ down to the ventral area of the VNLL. Most axons of the stellate cells (11 of 13) had collaterals within the VNLL, one in DNLL also and one in IC also. However, unlike bushy-like cells, there were not so many dense collaterals and terminals near the original cell.

The detailed data about the dendritic fields and the axons of the stellate cells are shown in Table 6.

Relation of Physiology with Cell Morphology

From the data shown above, we know that in the VNLL there are two groups of neurons with different morphological characteristics and five types of neurons with different physiological properties. This raises the question, “Is there any relation between their physiology and morphology?” The answer is undoubtedly yes.

The onset type, regular I type and onset-pause type neurons had some common physiological properties, i.e., low input resistance, short time constant, higher discharge threshold, short EPSP duration and only single postsynaptic action potential. And, we found that the morphology of these three types of neurons was typically bushy-like. For example, bushy-like morphology is shown for 3 onset neurons in Figure 14 (#1, #2 and #3), 4 regular I neurons in Figure 15 (#4 and #5) and 16 (#6 and #7) and one onset-pause neuron in Figure 16 (#8). Therefore, it is clear that the onset type, regular I type and onset-pause neurons are the bushy-like cells. Summarizing the physiological data of these bushy-like neurons, the input resistance was $47.32 \pm 5.36 \text{ M}\Omega$, time constant was $8.35 \pm 0.53 \text{ ms}$, discharge threshold was $300.00 \pm 17.57 \text{ pA}$ and EPSP duration was $36.50 \pm 9.45 \text{ ms}$.

The regular II type and burst type neurons had distinct physiological characteristics, i.e., higher input resistance, longer time constant, lower discharge threshold, longer EPSP duration and multiple postsynaptic action potentials. We found that the morphology of neurons with these physiological properties was typically stellate. For example, stellate morphology is shown for 3 regular II neurons in Figure 17 (#9 and #10) and Figure 18 (#12), and 3 burst neurons in Figure 18 (#11) and Figure 19 (#13 and #14). Therefore, the regular II type and

burst type neurons are the stellate cells. Summarizing the physiological data of stellate neurons, the input resistance was $129.09 \pm 12.64 \text{ M}\Omega$, time constant was $23.67 \pm 1.89 \text{ ms}$, discharge threshold was $92.14 \pm 7.08 \text{ pA}$ and EPSP duration was $110.07 \pm 9.13 \text{ ms}$.

Comparison of the input resistance, time constant, discharge threshold and EPSP duration between the bushy-like and stellate cells showed that the differences were all significant. Figure 21 summarizes the mean differences in physiological characteristics between bushy-like and stellate cells.

Figure 22 shows a summary of the VNLL neurons characterized by their physiological properties and morphological features.

Figure 1. Photomicrograph of a cresyl violet-stained frontal section through the midbrain and lateral lemniscus to illustrate the position of stimulating electrodes relative to the ventral nucleus of the lateral lemniscus (VNLL). S: location of the stimulating electrode. Scale bar: 400 μ m.

Figure 2. Current voltage relation for an onset type VNLL neuron. **A:** injection of 800 pA positive current for 250 ms produced two action potentials at the beginning of the injection. The second action potential was smaller than the first one. **B1, B2 and B3:** injection of 640 pA, 400 pA and 240 pA positive currents for 250 ms produced depolarization and only one action potential at the beginning of injection. The discharge threshold of this cell was 400 pA. **B4 and B5:** the effects of injecting -320 pA and -800 pA for 250 ms. The hyperpolarization tended to sag with continuous application of current in this neuron and an after-spike followed the offset of the hyperpolarization pulse. **C:** relation between current and voltage as measured by the peak shift in membrane potential.

A

B

C

Figure 3. Current voltage relation for a regular I type VNLL neuron. A, B1, B2, and B3: injection of 800 pA, 560 pA, 240 pA and 80 pA positive currents for 350 ms produced depolarization and continuous trains of action potentials. The discharge threshold of this cell was 240 pA. The number of action potentials was related to the strength of current. The interspike intervals were almost the same, but the first several intervals were shorter than the intervals during sustained firing at 560 pA and 800 pA. **B4 and B5:** the effects of injecting -400 pA and -800 pA for 350 ms. The hyperpolarization tended to sag with continuous application of current in this neuron and an after-depolarization followed the offset of the hyperpolarization pulse. **C:** relation between current and voltage as measured by the peak shift in membrane potential.

B

Figure 4. Current voltage relation for an onset-pause type VNLL neuron. A, B1, B2, B3 and B4: injection of 1200 pA, 1000 pA, 800 pA, 500 pA, and 300 pA positive currents for 350 ms produced depolarization and action potentials. The discharge threshold of this cell was 300 pA. Smaller current injections (300 pA and 500 pA) evoked one action potential at the beginning of injection period and continuous firing at the late phase of the injection. There was a silent period between early and late action potential firing. Larger current injection (800 pA, 1000 pA and 1200 pA) evoked more action potentials at both the early and the late firing periods, but the silent periods between them were gradually shorter. The interspike intervals in the early period were shorter than those in the late period. **B5 and B6:** the effects of injecting -500 pA and -1000 pA for 350 ms. The hyperpolarization tended to sag with continuous application of current in this neuron and the after-depolarization followed the offset of the hyperpolarization pulse. **C:** relation between current and voltage as measured by the peak shift in membrane potential.

Figure 5. Current voltage relation for a regular II type VNLL neuron. A, B1, B2, and B3: injection of 200 pA, 140 pA, 80 pA and 40 pA positive currents for 350 ms produced depolarization and continuous trains of action potentials. The discharge threshold of this cell was 80 pA. The number of action potentials was related to the strength of current. The interspike intervals were almost the same, but the first few intervals were shorter than the intervals during sustained firing at 140 pA and 200 pA. The discharge of the first spike occurred late when 80 pA was delivered. The discharge occurred earlier as 140 pA and 200 pA were injected. B4 and B5: the effects of injecting -60 pA and -200 pA for 350 ms. C: relation between current and voltage as measured by the peak shift in membrane potential.

B

Figure 6. Current voltage relation for a burst type VNLL neuron. A, B1, B2, and B3: injection of 360 pA, 280 pA, 120 pA and 80 pA positive currents for 350 ms produced depolarization and trains of action potentials. The discharge threshold of this cell was 120 pA. Smaller current injections (120 pA and 280 pA) evoked a train of burst discharge arising from a depolarization “hump”. The burst was followed by regular sustained firing at 360 pA level. **B4 and B5:** the effects of injecting -120 pA and -400 pA for 350 ms. **C:** relation between current and voltage as measured by the peak shift in membrane potential.

B

Figure 7. Firing rates as a function of the strength of positive current injected into the VNLL neurons with different firing patterns. A: regular I type. **B:** onset-pause type. **C:** regular II type. **D:** burst type. Each trace represents one neuron.

Figure 8. The excitatory postsynaptic responses of 2 VNLL cells (A and B) to electrical stimulation of the lateral lemniscus. Traces from bottom to top show graded EPSPs and a suprathreshold response at different intensities shown on the right side of each trace. **A:** The EPSP duration was shorter. **B:** The EPSP duration was longer. **Arrows:** time of the stimulus and resulting artifact.

A

B

Figure 9. The excitatory postsynaptic responses of a VNLL cell to electrical stimulation of the lateral lemniscus. Traces from bottom to top show graded EPSPs and 4 postsynaptic action potentials from the long duration EPSP at different intensities shown on the right side of each trace. **Arrow:** time of the stimulus and resulting artifact.

10 mV
20 ms

Figure 10. The inhibitory postsynaptic responses of a VNLL cell to electrical stimulation of the lateral lemniscus. Traces from bottom to top show graded IPSPs at different intensities shown on the right side of each trace. **Arrow:** time of the stimulus and resulting artifact.

Figure 11. The postsynaptic responses of 2 VNLL cells (A and B) to electrical stimulation of the lateral lemniscus. Traces from bottom to top show graded EPSPs and IPSPs at different intensities shown on the right side of each trace. **A:** EPSPs with IPSPs. The EPSP was dominant and had lower threshold. **B:** IPSPs with EPSPs. The IPSP was dominant and had lower threshold. **Arrows:** time of the stimulus and resulting artifact.

A

B

Figure 12. Photomicrographs of eight VNLL neurons labeled by neurobiotin. A-D: the bushy-like cells. E-H: the stellate cells. Scale bar: 50 μm .

Figure 13. Photomicrographs of an axon and its recurrent branch with collaterals from a bushy-like cell. A: the recurrent branch of the axon. **B:** the main axon. **a:** axon. **re:** recurrent branch. **Arrows:** bouton terminals. **Scale bar:** 50 μm .

Figure 14. Camera lucida drawings of 3 neurobiotin-labeled bushy-like cells and their locations in the VNLL. The physiological properties of these 3 cells were onset type. The outline of the VNLL was drawn from the section containing the labeled cell body. **a:** axon. **co:** collaterals. **D:** dorsal. **L:** lateral. **Scale bars:** 50 μm and 400 μm , apply to 3 cells and VNLL outlines, respectively.

Figure 15. Camera lucida drawings of 2 neurobiotin-labeled bushy-like cells and their locations in the VNLL. The physiological properties of these 2 cells were regular I type. The outline of the VNLL was drawn from the section containing the labeled cell body. **a:** axon. **co:** collaterals. **D:** dorsal. **L:** lateral. **Scale bars:** 50 μm and 400 μm , apply to 2 cells and VNLL outlines, respectively.

Figure 16. Camera lucida drawings of 3 neurobiotin-labeled bushy-like cells and their locations in the VNLL. The physiological properties of cell #6 and #7 were regular I type. Cell #8 was the onset-pause type neuron. The outline of the VNLL was drawn from the section containing the labeled cell body. **a:** axon. **co:** collaterals. **D:** dorsal. **L:** lateral. **Scale bars:** 50 μm and 400 μm , apply to 3 cells and VNLL outlines, respectively.

Figure 17. Camera lucida drawings of 2 neurobiotin-labeled stellate cells and their locations in the VNLL. The physiological properties of these 2 cells were regular II type. The outline of the VNLL was drawn from the section containing the labeled cell body. **a:** axon. **D:** dorsal. **L:** lateral. **Scale bars:** 50 μm and 400 μm , apply to 2 cells and VNLL outlines, respectively.

400 μ m

50 μ m

Figure 18. Camera lucida drawings of 2 neurobiotin-labeled stellate cells and their locations in the VNLL. The physiological properties of cell #11 were burst type. Cell #12 was regular II type neuron. The outline of the VNLL was drawn from the section containing the labeled cell body. **D:** dorsal. **L:** lateral. **Scale bars:** 50 μm and 400 μm , apply to 2 cells and VNLL outlines, respectively.

400 μm

50 μm

Figure 19. Camera lucida drawings of 2 neurobiotin-labeled stellate cells and their locations in the VNLL. The physiological properties of these 2 cells were burst type. The outline of the VNLL was drawn from the section containing the labeled cell body. **a:** axon. **D:** dorsal. **L:** lateral. **Scale bars:** 50 μm and 400 μm , apply to 2 cells and VNLL outlines, respectively.

Figure 20. Mean dendritic fields of the bushy-like and stellate neurons. M-L: mediolateral field. **D-V:** dorsoventral field. **R-C:** rostrocaudal field. **Bar:** SEM. *: significant difference between means, $p < 0.05$.

Figure 21. A comparison of physiological characteristics between the bushy-like and stellate neurons. **A:** mean input resistance of the bushy-like and stellate neurons. The input resistance of the stellate cells (n=28) was significantly higher than that of the bushy-like cells (n=31). **B:** mean time constant of the bushy-like and stellate neurons. The time constant of the stellate cells (n=28) was obviously longer than that of the bushy-like cells (n=31). **C:** mean discharge threshold of the bushy-like and stellate neurons. The discharge threshold of the stellate cells (n=28) was significantly lower than that of the bushy-like cells (n=31). **D:** mean EPSP duration of the bushy-like and stellate neurons. The EPSP duration of the stellate cells (n=15) was much longer than that of the bushy-like cells (n=7). **Bar:** SEM. *: significant difference between means, $p < 0.05$.

A **B** **C** **D**

Figure 22. A summary of the VNLL neurons characterized by their physiological properties and morphological features.

Bushy-like Cell

Onset

Regular I

Onset-pause

Stellate Cell

Regular II

Burst

Table 1. A Summary of Physiological Characteristics for Five Types of the Neurons

	V _m (mV)	Input Resistance (M Ω)	Time Constant (ms)	Discharge Threshold (pA)
Onset (n=16)	-57.50 $\pm$ 1.32	38.34 $\pm$ 7.18	6.87 $\pm$ 0.76	366.25 $\pm$ 18.54
Regular I (n=11)	-56.45 $\pm$ 1.89	54.06 $\pm$ 9.79	9.95 $\pm$ 0.63	223.64 $\pm$ 23.12
Onset-pause (n=4)	-55.00 $\pm$ 1.25	64.88 $\pm$ 16.77	9.88 $\pm$ 1.25	245.00 $\pm$ 33.17
Regular II (n=12)	-58.92 $\pm$ 2.10	135.23 $\pm$ 24.31	20.85 $\pm$ 1.65	101.67 $\pm$ 14.19
Burst (n=16)	-59.63 $\pm$ 1.30	124.49 $\pm$ 14.68	25.79 $\pm$ 3.14	85.00 $\pm$ 7.18

**Table 2. A Summary of Postsynaptic Potentials for
5 Types of the VNLL Neurons (n=56)**

	Onset		Regular I		Onset-pause		Regular II		Burst	
	Cells	%	Cells	%	Cells	%	Cells	%	Cells	%
EPSP Only	5	33	2	18	0	0	5	45.5	10	67
IPSP Only	3	20	2	18	2	50	1	9	2	13
Both	7	47	7	64	2	50	5	45.5	3	20

Table 3. Somatic Morphology of Bushy-like Cells

Neuron Number	Shape	Area (μm^2)	Orientation ($^\circ$)	Position in VNLL
1	Round	154	None	Mid & C
2	Oval	142	76(Hor.)	V & M
3	Round	129	None	V & L
4	Oval	277	36(Ver.)	V & L
5	Oval	297	69(Hor.)	V & C
6	Elongate	172	90(Hor.)	V & M
7	Oval	249	26(Ver.)	V & L
8	Oval	235	78(Hor.)	V & M
9	Round	172	None	V & M
10	Oval	106	90(Hor.)	V & L
11	Oval	78	60(Hor.)	V & M
12	Oval	103	62(Hor.)	V & C
13	Oval	193	34(Ver.)	V & M
14	Oval	132	90(Hor.)	V & L
15	Oval	201	28(Ver.)	V & L
16	Oval	155	48(Hor.)	V & M
17	Round	123	None	D & L
18	Oval	144	75(Hor.)	V & M
19	Oval	230	90(Hor.)	V & L
20	Round	161	None	D & C
21	Round	272	None	V & L
22	Oval	189	74(Hor.)	D & C
23	Oval	252	58(Hor.)	V & M

Hor.: horizontal. **Ver.:** vertical. **D:** dorsal. **V:** ventral. **Mid.:** middle between D and V. **L:** lateral. **M:** medial. **C:** central between M and L.

Table 4. Somatic Morphology of Stellate Cells

Neuron Number	Shape	Area (μm^2)	Orientation ($^\circ$)	Position in VNLL
1	Elongate	86	2(Ver.)	D & M
2	Round	137	None	D & C
3	Oval	168	54(Hor.)	Mid & C
4	Oval	75	20(Ver.)	V & M
5	Stellate	346	None	D & M
6	Oval	156	77(Hor.)	D & C
7	Oval	137	35(Ver.)	D & L
8	Oval	142	27(Ver.)	D & L
9	Elongate	146	85(Hor.)	V & L
10	Oval	72	82(Hor.)	D & C
11	Round	69	None	D & M
12	Round	110	None	D & L
13	Elongate	86	62(Hor.)	V & L
14	Oval	202	76(Hor.)	V & L
15	Oval	176	4(Ver.)	D & C
16	Oval	221	90(Hor.)	V & L
17	Elongate	163	74(Hor.)	D & M
18	Oval	140	22(Ver.)	V & M
19	Oval	142	14(Ver.)	V & M
20	Oval	78	74(Hor.)	D & M
21	Oval	107	52(Hor.)	D & C
22	Oval	435	50(Hor.)	V & C
23	Stellate	176	None	D & C
24	Oval	221	34(Ver.)	D & C
25	Round	229	None	Mid & C
26	Stellate	222	None	D & L

Hor.: horizontal. **Ver.:** vertical. **D:** dorsal. **V:** ventral. **Mid.:** middle between D and V. **L:** lateral. **M:** medial. **C:** central between M and L.

Table 5. Dendritic Field and Axonal Properties of Bushy-like Cells

Neuron Number	Dendritic Field			Axon		
	M-L (μm)	D-V (μm)	R-C (μm)	Length (μm)	Diameter (μm)	Collateral Position
1	208	385	240			
2	315	467	360	111	1.6	VNLL
3	220	426	120	465	2.2	VNLL
4	247	471	300	1158 -247	1.6	VNLL
5	422	457	300			
6	293	232	120	508	0.8	VNLL
7	325	153	120	246	0.5	VNLL
8	174	253	180	365	1.1	VNLL
9	261	102	240	685 -99	1.1	VNLL
10	285	187	120	10 -212	0.8	VNLL
11	167	223	180	2200 -368	1.7	VNLL, IC
12	187	193	180			
13	265	334	180	-55	1.1	
14	228	290	210	670	2.5	VNLL
15	333	238	210	-92	0.8	
16	223	204	120			
17	359	337	240	130	2.2	VNLL
18	127	188	180	640	1.1	VNLL
19	158	193	240			
20	153	197	60	1082	3.3	VNLL
21	287	313	180			

M-L: mediolateral field. **D-V:** dorsoventral field. **R-C:** rostrocaudal field.

“-” represents recurrent axon branch back to the ventral area of the VNLL.

Table 6. Dendritic Field and Axonal Properties of Stellate Cells

Neuron Number	Dendritic Field			Axon		
	M-L (μm)	D-V (μm)	R-C (μm)	Length (μm)	Diameter (μm)	Collateral Position
1	338	450	240			
2	346	489	240			
3				392	0.8	
4	397	252	120			
5	318	595	180	491	1.6	VNLL
6	620	554	210			
7	322	255	180	602	1.7	
8	418	387	120	317 -148	0.8	VNLL
9	338	230	120			
10	383	292	210	222	1.7	VNLL
11	583	202	150			
12	255	321	180			
13	218	472	270	733	0.8	VNLL
14	305	391	120	810	1.6	VNLL
15	283	275	150	443	1.1	VNLL
16	192	372	150			
17	308	350	150	3551	0.8	VNLL, IC
18	300	243	270	618	0.8	VNLL
19	390	250	180			
20	280	430	180	1260	1.6	VNLL & DNLL
21	187	317	120	1460	1.6	VNLL
22	357	207	180	1143	1.6	VNLL
23	417	337	240			

M-L: mediolateral field. **D-V:** dorsoventral field. **R-C:** rostrocaudal field.

“-” represents recurrent axon branch back to the ventral area of the VNLL.

Discussion

The main purpose of the present study was to study intrinsic membrane characteristics and synaptic physiology as well as morphological features of VNLL neurons. With intracellular and whole-cell patch-clamp recordings the intrinsic membrane properties of VNLL neurons were investigated by intracellular injection of current. The synaptic potentials were studied by recording responses to electrical stimulation of the lateral lemniscus. The morphological features of individual VNLL neurons from which the whole-cell patch-clamp recording was made were examined by intracellular labeling of neurobiotin. Two distinct morphological groups of VNLL neurons, bushy-like and stellate, could be recognized. The bushy-like cell group contained neurons with three physiological response patterns, i.e. onset, regular I and onset-pause in response to intracellular current injection; the stellate cell group contained neurons with two types of response patterns, i.e. regular II and burst. Onset, regular I and onset-pause cells had physiological properties in common, whereas regular II and burst cells had similar physiological properties. Both bushy-like and stellate cells responded to stimulation of the lateral lemniscus with excitatory and inhibitory synaptic potentials.

Morphology of the VNLL Neurons

A variety of neuronal classes has been recognized in the VNLL on the basis of somatic and dendritic morphology in previous anatomical studies (Adams 1979; Willard and Ryugo 1983; Covey and Casseday 1986; Huffman and Covey 1995; Merchán and Berbel 1996; Schofield and Cant 1997; Vater et al. 1997). Generally, small round cells and multipolar cells can be identified in Nissl-stained material. Other classes of cells found in VNLL are oval,

giant, elongate and vertical cells. Merchán and Berbel (1996) indicated that the rat's VNLL contained only flat stellate neurons, but in their Golgi preparations detailed dendritic morphology of VNLL neurons could not be displayed. Willard and Ryugo (1983) illustrated the distribution of different morphological types of neurons in the VNLL and the orientation of dendritic trees of VNLL neurons with respect to the fibers of the lateral lemniscus. The lateral part of the mouse's VNLL contains small rounded cell bodies which have long, thin dendrites oriented parallel to the trajectory of the lemniscal fibers; the medial part contains large multipolar cells with long, relatively unbranched dendrites that radiate away from the cell body, orthogonal to the trajectory of the lemniscal fibers (Willard and Ryugo 1983).

Neurons labeled by intracellular injections of neurobiotin in brain slice preparations of the rat's VNLL in the present study were classified as bushy-like and stellate cells, mainly based on their dendritic morphology. The shape, size and orientation of the cell body varied within the bushy-like cell group or the stellate cell group. The bushy-like cells could be oval (70%), round (26%) or elongate (4%). The area of the bushy-like cell bodies ranged from $78 \mu\text{m}^2$ to $297 \mu\text{m}^2$. Except for the round cells, whose soma have no preferential orientation, most of the bushy-like cell bodies were horizontal (perpendicular to the fibers of the lateral lemniscus) and some were vertical (parallel to the fibers of the lateral lemniscus) in coronal sections. The stellate cells could be oval (58%), round (15%), elongate (15%) and stellate (12%). The area of the stellate cell bodies ranged from $69 \mu\text{m}^2$ to $435 \mu\text{m}^2$. Most of the stellate cell bodies were also horizontal, some were vertical and some were non-oriented. There was a tendency for the two groups to be differentially distributed within the VNLL. Bushy-like cells tended to be localized in the ventral area of the VNLL whereas stellate cells tended to be localized in dorsal areas. These tendencies are consistent with observations by other

investigators. The bushy-like cells probably correspond to the neurons in the ventral zone of the VNLL of cat (Adams 1979) and in the VLLv of guinea pig (Schofield and Cant 1997). The stellate cells probably correspond to the neurons in the dorsal and middle zones of the VNLL of cat (Adams 1979), and in the VLLd of guinea pig (Schofield and Cant 1997).

The dendritic morphology of neurons is an important attribute for recognizing neuronal types. In the present study, most labeled VNLL neurons had three to seven primary dendrites. Only one stellate neuron had two primary dendrites. This neuron can not be considered a bipolar cell, because some dendrites might have been cut off when the brain slice was made. But at least we can say that most of the VNLL neurons are multipolar cells. This conclusion is consistent with the observations of Merchán and Berbel (1996). However, when the detailed dendritic morphology is considered, it becomes apparent that the VNLL is not a homogenous group of neurons as they suggested. In the present study the two classes of VNLL neurons differed in their dendritic morphology. The dendritic trees of the bushy-like cell were smaller and more compact than those of the stellate cell. Primary dendrites of the bushy-like cell usually branched profusely and densely within a relatively restricted area, whereas dendrites of the stellate cell radiated away from the cell body and extended over a relatively large area in all directions. In comparison with Golgi preparations made by other investigators, the dendritic features of the stellate cell almost certainly correspond to those of the multipolar neurons in the medial part of the mouse's VNLL (Willard and Ryugo 1983) and in the bat's VNLLm (Covey and Casseday 1991). The dendritic morphology of the bushy-like cell is somewhat similar to that of the bat's VNLLc neuron which also has a short and compact dendritic tree, but is not like that of the mouse's VNLL round cell which has relatively long and thin dendrites.

The axons of both bushy-like and stellate cells labeled in the present study all arose from the cell body. After running for a distance from the cell body the axon usually turned at an almost right angle, headed dorsally and coursed along the fibers of the lateral lemniscus, and finally ended in the IC. The main axons of some neurons sent recurrent branches down to the ventral area of the VNLL. Both main axons and recurrent branches could give rise to collaterals, which terminated within the VNLL, DNLL or IC. These results suggest that VNLL neurons innervate the IC, as well as the DNLL and even the VNLL itself via axon collaterals. Although we could not follow the recurrent branches out of the VNLL in the present study, the recurrent collaterals within the VNLL may have had a more ventral projection corresponding to the descending projections from the VNLL to the SOC and CN described by other authors (Whitley and Henkel 1984). Recurrent collaterals with bouton terminals could be found in the vicinity of the original cell within the VNLL. These terminals suggest that many VNLL neurons, including bushy-like and stellate cells, project locally as well as to the auditory midbrain. As most of VNLL neurons are immunoreactive to glycine and/or GABA antibodies, it is likely that these projections serve an inhibitory function.

Bushy-like cells in the VNLL in some ways resemble bushy cells in the VCN, T stellate cells and octopus cells in the PVCN, principal cells in the MNTB and MSO. For example, all these cells have compact or tufted dendrites in a spatially restricted area, although the number of primary dendrites, the size of dendritic field and the branching of dendrites vary in the different nuclei. Also, these neurons have similar intrinsic physiological properties (discussed below).

Response of the Cell Membrane to Hyperpolarization

All VNLL neurons examined in the present study responded to intracellular injection of negative current with graded hyperpolarization. The magnitude of the initial hyperpolarization was an approximately linear function of the current strength. However, with sustained current injection, more than half of neurons showed an initial hyperpolarization followed by a gradual sag of the membrane potential towards the level of the resting potential. The sag was often accompanied by an after-depolarization, which sometimes produced spikes, following the offset of the hyperpolarizing pulses. This type of depolarizing sag resembles the inward rectification associated with activation of a mixed cation current (I_h) by hyperpolarization in many central neurons, including auditory neurons, e.g., neurons in the VCN, DCN, MNTB, DNLL and the auditory thalamus (Oertel 1983; Manis 1990; Banks et al. 1993; Ströhmann et al. 1994; Hu 1995; Pape 1996; Fu et al. 1997). The after-depolarization and after-spikes may be accounted for by deactivation of I_h (Dekin 1993; Pape 1996).

I_h current is a slowly developing, time and voltage dependent inward current evoked by hyperpolarization of the membrane (Pape 1996). In some neurons I_h seems to stabilize the resting potential (Maccaferri et al. 1993; Bayliss et al. 1994), whereas in others it has been shown to be a “pacemaker” involved in the generation of rhythmic oscillatory activity (McCormick and Pape 1990). I_h may also interact with excitatory synaptic events to enhance the influence of excitatory inputs (Fu et al. 1997). In the present study the onset, regular I and onset-pause neurons all exhibited a very obvious depolarizing sag that was characteristic of I_h . This result implies that these three types of neurons can be distinguished from other two types of neurons, regular II and burst, by the presence of I_h or other ion channels on the membrane. Although the exact role of I_h in these neurons could be explored only after further study of the properties of this current with voltage clamp and pharmacological methods, generally, the

depolarizing sag associated with inward rectification would prevent these cells from becoming too hyperpolarized in response to inhibitory inputs, which may promote rapid transmission of information to the higher level of the auditory system.

Intrinsic Membrane Properties

The VNLL neurons have distinct intrinsic membrane properties in response to intracellularly injected current. The patterns observed in the present study could be categorized into five types: onset, regular I, onset-pause, regular II and burst. An onset type is characterized by “non-linear” current-voltage relationships, only one or a few action potentials in response to injection of positive current, higher discharge threshold, an obvious “sag” of hyperpolarization in response to injection of negative current, lower input resistance and a shorter time constant. A regular I type is characterized by “linear” current-voltage relationships, a continuous train of action potentials with almost regular intervals in response to injection of positive current, higher discharge threshold, an obvious “sag” of hyperpolarization in response to injection of negative current, lower input resistance and a shorter time constant. An onset-pause type is characterized by “linear” current-voltage relationships, a train of action potentials with a silent period in response to injection of positive current, higher discharge threshold, an obvious “sag” of hyperpolarization in response to injection of negative current, lower input resistance and a shorter time constant. A regular II type is characterized by “linear” current-voltage relationships, a train of action potentials with regular intervals in response to injection of positive current, lower discharge threshold, hyperpolarization without an obvious “sag” in response to injection of negative current, higher input resistance and a longer time constant. A burst type is characterized by “linear” current-

voltage relationships, a burst discharge arising from a depolarization “hump” in response to injection of positive current, lower discharge threshold, higher input resistance and a longer time constant. The five distinct types of membrane properties observed in the VNLL neurons may reflect different expression of ion channels on the cell membrane. A number of studies about ion channels responsible for distinct membrane properties that are similar to these seen in VNLL neurons have been conducted on neurons in various auditory nuclei by voltage clamp experiments (discussed below). Certainly further studies are necessary for revealing different ion channels or different combination of ion channels expressed in VNLL neurons with different membrane properties.

The five different firing patterns observed in the VNLL brain slice by injection of intracellular current may correspond to different post-stimulus time histograms (PSTHs) recorded from VNLL neurons in response to acoustic stimulation *in vivo*. For example, in the bat, neurons in the VNLLc and VNLLm divisions respond to tone and noise bursts differently (Covey and Casseday 1991). In the VNLLc the majority of neurons respond to tone bursts with one spike at short and constant latencies. This “onset” type of neuron is also present in the VNLL of other mammals but appears to be intermingled with other cell types (Vater et al. 1997). The response pattern of the onset type in the present study resembles the phasic type in the VNLLc of the big brown bat (Covey and Casseday 1991), the VNLL of horseshoe bat (Metzner and Radtke-Schuller 1987), the “onset” cell type of cat VNLL (Adams 1997; Guinan et al. 1972a), and the onset neurons of rabbit VNLL (Batra 1998). The onset response observed in *in vivo* studies could be attributed to the onset type intrinsic membrane characteristics, although the neurons must be activated by synaptic inputs from excitatory afferent neurons in response to acoustical stimulation. Onset VNLL neurons in the rat, perhaps

like VNLLc neurons in the bat (Casseday and Covey 1995), might transmit information about stimulus onset. Batra (1998) proposed that there were transient type phasic neurons in the rabbit VNLL. The transient neurons respond to tone burst with a burst of four or more action potentials without pronounced chopping pattern. This type of neuron probably corresponds to the burst type neurons in the present study.

In the previous *in vivo* physiological studies of cat VNLL, most neurons were found having 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 an investigation of Covey and Casseday (1991), 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 (as defined by Pfeiffer, 1966). Similar discharge types, i.e., tonic and phasic patterns were found in VNLL neurons in horseshoe bat (Metzner and Radtke-Schuller 1987). Neurons with regular I or II, and onset-pause type demonstrated in the present study probably correspond to neurons with chopper or tonic and pauser types, respectively. Therefore, the intrinsic properties of the cell membrane may contribute to the distinct discharge patterns in response to acoustical stimulation.

Few intracellular investigations have been made from VNLL neurons. Wu (1998) did intracellular recordings from rat's VNLL neurons in brain slices and found that there were type I and type II neurons in the rat's VNLL as defined by intrinsic membrane properties. Type I neurons have a linear current voltage relation and display three different firing patterns: regular, onset-pause and adaptive. Type II neurons have a non-linear current voltage relation and respond to positive current with only one or a few action potentials. In many ways, the present results are consistent with her results. For example, neurons with onset, regular I or II,

and onset-pause type in the present study may correspond respectively to type II, regular and onset-pause neurons in her study. It appears that the burst type of response in the present study is not the adaptive type reported by Wu (1998). In her study she did not find a burst type, whereas in my study I didn't find an adaptive type. Whether or not the missing type of response in each study resulted from different recording methods (intracellular recording for her study and whole-cell patch-clamp recording for my study) is not clear. In general, these results support the idea that intrinsic membrane properties are involved in the generation of different firing characteristics in auditory neurons (Manis 1990). Different types of neuron likely encode different kinds of auditory information. The results from the present study also support the suggestion that VNLL neurons with onset response may encode the onset of a sound, and VNLL neurons with sustained discharges may process information about the intensity and duration of a sound (Covey 1993).

Comparison of VNLL Neurons with Neurons in Other Auditory Nuclei

Apparently, there are similarities in cell morphology and physiology among bushy cells in the AVCN (Wu and Oertel 1984; Oertel 1991), octopus cells in the PVCN (Oertel 1991; Golding et al. 1995), principal cells in the MNTB and MSO (Wu and Kelly 1991; Banks and Smith 1992; Smith 1995), and onset type of bushy-like cells in the VNLL (the present study). These neurons all have non-linear current-voltage relationships and respond to injection of positive current with a reduction in input resistance, and generation of one or only a few action potentials. The input resistance of the cell membrane for these neurons is also similar. It is 30-50 M Ω for bushy cells (Oertel 1991), 42.5 ± 12.6 M Ω for type II VNLL neurons by intracellular recording (Wu 1998) and 38.34 ± 7.18 M Ω for onset type VNLL neurons by

whole-cell patch-clamp recording (present study). However, when the cell is excited the input resistance of these neurons becomes lower. The consequence of the reduced input resistance is a limitation in the generation of action potentials and a faster repolarization in response to excitatory inputs.

Oertel (1983) first proposed that the membrane properties of bushy cells in the VCN made them well suited to maintain and convey the precise timing of synaptic inputs. When these cells are depolarized by synaptic input, the input resistance of the membrane drops, resulting in rapid repolarization of the cell membrane, which makes excitatory input brief and prevents summing in time. Therefore, the temporal pattern of excitation of these cells can reflect precisely the temporal firing pattern of inputs from the afferents. These properties are very important for precisely encoding auditory temporal information (Oertel 1991, 1997). The population of onset type neurons in the rat's VNLL may precisely signal the time of onset of a sound, like VNLLc neurons in the big brown bat (Covey 1993).

Voltage clamp investigations have shown that a low-threshold (about -70 mV) K^+ conductance is responsible for the highly rectifying current-voltage relationship of bushy cells in the AVCN, and principal cells in the MNTB (Manis and Marx 1991; Forsythe and Barnes-Davies 1993; Brew and Forsythe 1995). Whether or not onset type VNLL cells have K^+ channels similar to those in bushy cells in the AVCN and principal cells in the MNTB requires further investigation.

Surprisingly, the morphology of these auditory brainstem neurons is also very similar: cells of each type have round to oval cell bodies, profuse dendritic trees and tufted dendritic branching. All these cells provide afferent input to the VNLL (Warr, 1972, 1982; Glendenning et al., 1981; Fraiuf and Ostwald, 1988). Another similarity among these neurons is that they

receive large terminals from afferent fibers. Bushy cells are covered by end bulbs of Held that arise from the auditory nerve fibers (Lenn and Reese 1966; Lorente de Nó 1976, 1981; Schwartz and Gulley 1978; Ryugo and Sento 1991). Principal cells of the MNTB are covered by calyces of Held that come from the axons of the globular bushy cells in the contralateral AVCN (Morest 1968a, b; Friauf and Ostwald 1988). Although most octopus cells receive terminal boutons, sometimes they receive modified end bulbs on their soma (Roman and Avan 1997). Some VNLL neurons in cat, rat 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; Willard and Ryugo 1983; Zook and Casseday 1985; Covey and Casseday 1986; Vater and Feng 1990; Covey 1993; Wu 1998). These large synaptic terminals are expected to provide a large synaptic current when action potentials arrive at the terminals. Thus, the postsynaptic neuron is still be able to reach the level of discharge threshold even though the input membrane resistance is low immediately after excitatory synaptic input.

Intracellular labeling studies have shown that cells with linear current-voltage relationships are also found in stellate cells of the VCN (Wu and Oertel 1984; Oertel et al. 1990) and principal cells of the LSO (Wu and Kelly 1991; Wu and Fu 1998). These neurons all display a repetitive firing pattern to depolarizing current pulses. For example, T-stellate cells in the VCN fire at regular intervals when depolarized with positive current (Wu and Oertel 1984; Oertel et al. 1990). LSO cells generate multiple action potentials that increase in number in proportion to the strength of stimulation (Wu and Kelly 1991). Non-principal cells in the MSO are depolarized and also fire repetitively to injection of positive current at the level close to spike threshold (Smith 1995). Although all these cells exhibit linear current voltage relationship, by looking at their firing patterns closely we suggest that regular I neurons in this

study are similar to T-stellate cells in the VCN and non-principal cells in the MSO. These neurons not only are capable of firing repetitively and regularly when they are depolarized above the threshold for generating an action potential, but also they fire immediately following the onset of the current pulse. The frequency of the spikes becomes higher as the amount of current injected increases. Morphologically, regular I and T-stellate cells are also similar. They have relatively shorter and tufted dendrites in a confined area. An *in vivo* study by Bank and Sachs (1991) showed that the timing of the first action potential of T-stellate cells in the AVCN reflected the onset of the sound, while the timing of later action potentials depended on stimulus intensity and intrinsic membrane properties. Our regular I type cells may also encode the onset and intensity of the sound.

Our regular type II cells are similar to D-stellate cells in the PVCN (Oertel et al 1990). The D-stellate cells also have linear current voltage relationships and fire regularly in response to injection of positive current. Morphologically, regular II VNLL neurons and D-stellate cells in the PVCN are similar. Both types of cells have widely spread smooth dendrites extended long in all directions. We speculate that the regular II VNLL neurons may not encode precise timing of the onset of a sound as the first spike occurs with some delay following the onset of the current pulse. These cells might encode intensity and duration of a sound.

Onset-pause firing pattern is also observed in pyramidal cells in the DCN of guinea pig (Manis 1990). The behavior of these cells is similar to that of the onset-pause cells in rat VNLL (Wu 1998). The onset-pause firing pattern will become regular or long first spike latency pattern as the resting potential of the cell is depolarized or hyperpolarized. Recently Kanold and Manis (1998) found that a type of K^+ conductance, the transient A-current, may be responsible for the behavior of onset-pause firing. Such non-linearity of the temporal firing

pattern should be evident for dynamic acoustic stimuli, depending on the intensity of the tone and the cell resting potential (Manis 1990). Exact role of these cells in the auditory pathway is unknown.

There are several reports about burst discharges in auditory brainstem neurons. Hirsch and Oertel (1988) described that some neurons in the DCN of the mouse generated bursts of smaller graded spikes superimposed on a slow depolarization. The graded spikes could be reversibly blocked by tetrodotoxin (TTX), a specific blocker for voltage-gated Na^+ channels. The slow depolarization could be removed by eliminating Ca^{2+} from the perfusate. These results suggests that the action potentials are Na^+ -dependent and the slow depolarization is Ca^{2+} -dependent. Later, these bursting neurons were identified as cartwheel cells in the DCN (Zhang and Oertel 1993). Neurons of the auditory thalamus also exhibit burst firing patterns (Jahnsen and Llinás 1984; Steriade et al. 1993; Tennigkeit et al. 1996, 1998). Investigations on the dorsal thalamic nuclei have identified the bursting action potentials as fast transient potentials, Na^+ -dependent, triggered by a low-threshold Ca^{2+} -spike (LTS) (Jahnsen and Llinás 1984; Steriade et al. 1993). Tennigkeit et al. (1998) suggested that a subthreshold (persistent) Na^+ -conductance amplified the effect of depolarizing inputs, enhancing membrane excitability in the tonic firing mode and amplifying the LTS in the burst firing mode. We can generally speculate that there are at least two components mediated by different ionic channels in the burst type response observed in the present study: fast burst spikes and a slow "hump" of depolarization. Whether or not burst firing of VNLL neurons is also attributed to Ca^{2+} and persistent Na^+ conductance requires further investigation. Burst firing in the auditory thalamus may change the quality of the different signals transmitted in the thalamocortical system

during wakefulness, sleep or pathological states (Steriade et al. 1993; Tnnigkeit et al. 1998). The functional significance of burst firing of VNLL neurons is totally unknown.

Ascending Synaptic Inputs

In the present study stimulation of the lateral lemniscus ventral to the VNLL could elicit either EPSPs or IPSPs or both in both bushy-like cells and stellate cells. The lateral lemniscus is the main route of ascending fibers to the VNLL from the trapezoid body and lower brainstem (Glendenning et al. 1981; Helfert and Aschoff 1997). Therefore, stimulation of the lateral lemniscus immediately ventral to the VNLL in the brain slice is most likely to activate afferent inputs that make synapses onto VNLL neurons.

Most of the afferents to the VNLL originate in the contralateral VCN, with a small projection coming from the ipsilateral VCN (Browner and Webster 1975; Adams and Warr 1976; Glendenning et al. 1981; Warr 1982; Spangler et al. 1985; Zook and Casseday 1985; Covey and Casseday 1986; Friauf and Ostwald 1988; Helfert et al. 1991; Huffman and Covey 1995; Schofield and Cant 1997). Bushy, multipolar and octopus cells in the VCN are the main sources of the afferents to the VNLL (Warr 1982; Friauf and Ostwald 1988; Schofield and Cant 1997; Thompson 1998). Large terminals, the calyces of Held, found in the VNLL probably come from thick axons of octopus cells in the PVCN (Vater and Feng 1990; Adams 1997; Schofield and Cant 1997; Thompson 1998). Calyceal endings are known to be excitatory, so 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 (Rhode et al. 1983; Adams 1997; Smith et al. 1998). Vater et al. (1997) observed the synaptic organization of VNLLc neurons in the big brown bat at the electron microscopic

level and proposed that VNLLc neurons received excitatory inputs that may arise from large calyces derived from neurons, probably octopus cells or large multipolar neurons, in the PVCN. Some of the excitatory synaptic responses recorded from the VNLL neurons in the present study may originate from octopus cells in the PVCN.

In contrast to the calyceal endings from thick axons of octopus cells, the medium-sized bead-like boutons probably from collaterals of thinner axons derived from bushy and/or stellate (multipolar) cells in the AVCN and PVCN, which pass through the VNLL towards the IC (Iwahori 1986; Friauf and Ostwald 1988; Schwartz 1992; Covey 1993). These collaterals terminate as small boutons in the VNLL (Friauf and Ostwald 1988; Schofield and Cant 1997; Wu 1998). The electron microscopy study about projections to the IC from the AVCN has shown that axonal endings of CN 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. As VNLL receives axon collaterals of AVCN neurons whose main axons ultimately terminate in the IC, it is very likely that the AVCN provides excitatory inputs to the VNLL as well. Neurochemical studies of Suneja et al. (1995a, b) who measured uptake and release of D-[³H] aspartate in the VNLL, further suggest that glutamate or aspartate may be an excitatory transmitter for the CN-VNLL synapses. Some of the excitatory synaptic responses recorded from the VNLL neurons in the present study may originate from bushy and stellate cells in the VCN. But due to a limitation of our patch-clamp amplifier (Axonpatch 200A, Axon Instrument Inc.), we could not accurately measure the latency, rising phase and time course of the synaptic events. Therefore, the EPSPs originating from the octopus cell or from the bushy or multipolar cells could not be differentiated.

In addition, the VNLL also receives inhibitory inputs. We know that there are minor inputs coming from the ipsilateral MNTB and periolivary nuclei (Elverland 1978; Glendenning et al. 1981; Spangler et al. 1985; Vater and Feng 1990; Huffman and Covey 1995; Warr and Beck 1996). MNTB principal neurons are immunoreactive for glycine (Helfert et al. 1989). Axons of MNTB principal neurons, which project to the LSO, give rise to collaterals to the VNLL, so the projection of MNTB-VNLL is very likely inhibitory and glycinergic as well. Neurons in the periolivary region, especially in the VNTB and LNTB, have been shown by immunocytochemistry to be GABAergic (Moore and Moore 1987; Roberts and Ribak 1987; Helfert et al. 1989; Adams and Mugnaini 1990; Winer et al. 1995; González-Hernández et al. 1996). These neurons are another possible source of inhibition to the VNLL. Evidence that there are glycine- and GABA-immunoreactive puncta and flattened synaptic vesicles associated with neurons in the VNLLc of big brown bat (Vater et al. 1997), and glycine- and GABA-immunoreactive perisomatic puncta in the VNLL of cat (Saint Marie et al. 1997) further supports the idea that VNLL neurons receive both glycinergic and GABAergic inhibitory inputs. The IPSPs recorded from VNLL neurons in this study may originate from the MNTB and VNTB or LNTB. These IPSPs may be glycinergic or GABAergic.

In the present study, each neuron responded to stimulation of the lateral lemniscus with either EPSP or IPSP or both. More than 40% of VNLL neurons recorded in the present study responded to stimulation of the lateral lemniscus with a combination of EPSPs and IPSPs. These results suggest that many VNLL neurons receive and integrate excitatory and inhibitory afferent inputs. The output of synaptic integration from one neuron may depend on relative strengths of excitatory and inhibitory inputs that impinge on it (Fig.11).

More specifically, in bushy-like cells the synaptic potentials displayed 53% combination of EPSPs and IPSPs, 23% EPSPs only and 23% IPSPs only. In stellate cells the synaptic events displayed 31% combination of EPSPs and IPSPs, 58% EPSPs only and 11% IPSPs only. These results suggest that there may be distribution differences in synaptic inputs between bushy-like cells and stellate cells, even though there is so far no anatomical evidence available about what kind of synaptic inputs projecting to different types of neurons. Bushy-like cells may more likely receive both excitatory and inhibitory synaptic inputs, whereas stellate cells may more likely receive excitatory inputs only.

In addition to excitatory and inhibitory inputs from the lower auditory brainstem, local circuits may also contribute to the synaptic responses of VNLL neurons. As most VNLL neurons are glycinergic or GABAergic, their axon collaterals or recurrent branches can make local inhibitory connection with many neurons near the original neuron or even with the original neuron itself. If it is the case, the IPSPs which followed the EPSPs (Fig. 11A) may be evoked by collaterals of interneurons or the neuron from which the recording was made. Bushy-like cells have much denser axon collaterals in the vicinity of the neuron than stellate cells (the present study), which may account for higher incidence of a combination of EPSPs and IPSPs in bushy-like cells than in stellate cells.

In comparison with the excitatory postsynaptic responses between bushy-like and stellate cells, the EPSP duration of bushy-like cells was shorter and only one suprathreshold postsynaptic action potential could be elicited, while the EPSP duration of stellate cells was much longer and multiple spikes could be often evoked. There are two factors, which can determine the time course of the EPSP: the time constant of the cell membrane, and influence of the synaptic connection. The shorter the time constant is, the shorter the EPSP is. The fact

that bushy-like cells have shorter time constants than stellate cells (the present study) makes bushy-like cells faster in response to synaptic inputs. Secondly, the morphological data from the present study showed that bushy-like cells had denser axon collaterals terminating near the original cell than stellate cells. These collaterals could make local inhibitory synapses impinging on the original cell. Therefore the shorter EPSP in the bushy-like cell may be also due to an influence of the IPSP that was elicited by the local collateral, which would then truncate the duration of the EPSP. The stellate cells had longer duration EPSPs, which is consistent with the longer time constant of the cell membrane. The collaterals of stellate cells were not as dense as those of bushy-like cells, which would make it less likely that they would form local inhibitory circuits on the original cell. Therefore the duration of the EPSP of stellate cells would be longer.

The relation of morphology and physiology of VNLL neurons

Besides our data, the only information about the relation of morphology and physiology of VNLL neurons is for the bat (Covey and Casseday 1986, 1991). Therefore, we would like to closely compare the results in the two species. In the bat's VNLL the specific physiological response pattern correlates neurons with distinct morphology, and neurons with distinguishable physiology and morphology are located in separate areas (Covey and Casseday 1986, 1991). For example, in the dorsal part of the bat's VNLL, named VNLLc, neurons are more broadly tuned, have no spontaneous activity, always respond to tone burst with only one spike. The latency is precisely locked to the stimulus onset. Morphologically, all neurons in the VNLLc are bushy cells. They are round or oval in shape and have one large, thick dendrite that branches profusely. These cells are tightly packed in a columnar arrangement between

bundles of fibers. Covey and Casseday (1986, 1991) suggest that VNLLc neurons are specialized to encode very precisely the onset of sound. In the ventral area of the bat's VNLL, named VNLLm, neurons exhibit various response patterns, such as tonic, chopping and phasic. VNLLm neurons do not have single-spike constant-latency responses. Most VNLLm neurons are multipolar in shape. They are larger than VNLLc neurons and have several sparsely branching thick dendrites. The cell bodies and dendrites of neurons in VNLLm have no consistent orientation relative to the fibers of the lateral lemniscus. These neurons may encode other properties of a sound.

From the present study, we found that several different physiological response patterns share one class of morphology in the rat's VNLL. Onset, regular I and onset-pause cells have a bushy-like morphology; regular II and burst cells have a stellate morphology. Bushy-like and stellate cells are not located in completely separate areas. Certainly, the relation of the morphology and physiology of VNLL neurons in the rat is different from that in the bat. The monaural nuclei of the lateral lemniscus, including INLL and VNLL, are extraordinarily large and are exquisitely differentiated into morphologically distinct regions in the bat (Covey and Casseday 1995). The bat is an echo-locating animal. The highly specialized neuronal groups in the VNLL and segregation of bushy cells from multipolar cells is likely important for echolocation. It seems that neurons in the rat's VNLL are not as highly specialized as those in the bat. Physiologically, five response patterns can be clearly differentiated. Morphologically only two broad types can be discerned. But the correlations of onset, regular I and onset-pause with bushy-like, and regular II and burst with stellate cells are unambiguous. Bushy-like cells (onset, regular I and onset-pause response patterns) have some similar physiological characteristics, such as lower input resistance, shorter time constant, depolarizing sag, and the

first spike immediately following the current pulse. All these properties make these cells respond rapidly to an acoustic stimulus. Among them, bushy-like onset cells may encode precisely the onset of a sound, and bushy-like regular I and onset-pause may encode the onset as well as the intensity and duration of a sound. In contrast, stellate cells have higher input resistance, longer time constant, without obvious depolarizing sag, and the first spike following the current pulse with some delay. All these properties make these cells respond relatively slowly and may enhance their ability to encode ongoing temporal information.

Conclusions

On the basis of physiological and morphological characteristics, VNLL neurons in the rat could be classified into two distinct groups: bushy-like cells and stellate cells. Bushy-like cells were mainly located in the ventral portion of the VNLL. They had relatively dense and small dendritic trees, with profuse and compact branches within a relatively restricted area. In response to depolarizing current pulses these cells started to fire at the onset of the current pulse, but with a higher discharge threshold. They responded to a hyperpolarizing current pulse with an obvious “sag”. These cells had lower membrane input resistance and shorter time constants. Bushy-like cells contained neurons with three types of intrinsic firing patterns: onset, regular I and onset-pause. An onset pattern was characterized by only one or a few action potentials in response to a depolarizing pulse, and “non-linear” current-voltage relationship. A regular I pattern was characterized by a regular train of action potentials in response to depolarizing pulse and “linear” current-voltage relationship. An onset-pause pattern was characterized by a train of action potentials with a silent period in response to a depolarizing pulse and a “linear” current-voltage relationship.

Stellate cells were located mainly in the dorsal portion of the VNLL. They had long and less branched dendrites that radiated away from cell body, and a relatively large dendritic field. In response to depolarizing current pulses these cells started to fire following the onset of the current pulse with some delay, but with a lower discharge threshold. Most of stellate cells responded to hyperpolarizing pulses without an obvious “sag”. They had a higher membrane input resistance, a longer time constant and a “linear” current-voltage relationship. Stellate cells contained neurons with two types of intrinsic firing patterns: regular II type, responding

to depolarizing pulses with a regular firing pattern; and burst type, characterized by a burst of discharges arising from a depolarization “hump” in response to a depolarizing pulse.

Both bushy-like cells and stellate cells could respond to ascending inputs of the lateral lemniscus with excitatory or inhibitory responses or both. Stellate cells may more likely receive excitatory inputs than bushy-like cells. The EPSP duration of the stellate cells was longer than that of bushy-like cells. Multiple postsynaptic action potentials could more often be evoked in stellate cells than in bushy-like cells.

The results suggest that different VNLL neurons may play different roles in processing information of a sound, and the different responses of VNLL neurons may associate with different ion channels and synaptic receptors on the cell membrane. Further researches are necessary to reveal mechanism of ion channels, transmitters and receptors that are responsible for different behaviors of VNLL neurons.

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IMAGE EVALUATION TEST TARGET (QA-3)

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Fax: 716/288-5989

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