**Significance of the Ventral Nucleus of the Lateral Lemniscus in Auditory Processing:
Responses to Single and Repetitive Synaptic Stimulation in Physiologically Identified
Neurons in Rat's Brain Slice**

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**A thesis submitted to the Faculty of Graduate Studies and Research in partial
fulfillment of the requirements for the degree of**

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**Department of Psychology and Institute of Neuroscience
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submitted by

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Abstract

VNLL neuronal responses to intracellular depolarizing current injection and stimulation from shocks to the lateral lemniscus were recorded. VNLL neurons displayed five types of firing patterns in response to depolarizing current injection: regular, adaptation, pauser, onset and burst. In response to single pulse stimulation of the lateral lemniscus, VNLL neurons displayed both EPSPs and IPSPs. Pharmacological manipulations revealed that VNLL neurons receive both NMDA and AMPA receptor mediated excitation as well as glycine and GABA receptor mediated inhibition from the lower auditory brainstem. These results suggest that the VNLL may be viewed as an integrative nucleus for the convergence of excitatory and inhibitory inputs from the lower auditory structures. In response to repetitive electrical stimulation of the lateral lemniscus, VNLL neurons can follow repetitive stimulation at low repetition rates (10-20 Hz). At these repetition rates, the jitter is minimal. At higher repetition rates, the firing probability decreases and the jitter increases. Thus, at low repetition rates, inhibitory inputs from the VNLL to the IC may result in the precise temporal processing in the IC. At higher repetition rates, the VNLL provides a sustained inhibition due to temporal summation in the IC, which in turn may be integrated within a wide range of time with other incoming information to the IC.

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List of Abbreviations

ACSF:	artificial cerebrospinal fluid
AMPA:	alpha-amino-3-hydroxy-5-methylisoxazole-4 propionic acid
4-AP:	4-aminopyridine
APV:	2-amino-5-phosphono-valerate
AVCN:	anteroventral cochlear nucleus
CN:	cochlear nucleus
CNQX:	6-cyano-7-nitroquinoxaline-2,3-dione
CPP:	($\pm$)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid
DCN:	dorsal cochlear nucleus
DNLL:	dorsal nucleus of the lateral lemniscus
EPSC:	excitatory postsynaptic current
EPSP:	excitatory postsynaptic potential
FG:	fluorogold
FM:	frequency modulation
GABA:	gamma-aminobutyric acid
HEPES:	3 <i>N</i> -2-hydroxyethylpiperazine- <i>N'</i> -2-ethanesulfonic acid
I _A :	rapidly activating and inactivating potassium current
IC:	inferior colliculus
ICC:	central nucleus of the inferior colliculus
I _h :	inward hyperpolarization-activated current
I _{K(Ca)} :	calcium-dependent potassium current

INLL:	intermediate nucleus of the lateral lemniscus
IPSP:	inhibitory postsynaptic potential
ISI:	interspike interval
I-V:	current-voltage
LL:	lateral lemniscus
LNTB:	lateral nucleus of the trapezoid body
MNTB:	medial nucleus of the trapezoid body
NLL:	nuclei of the lateral lemniscus
nMAG:	nucleus magnocellularis
NBQX:	1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[f]quinoxaline-7 sulfonamide
NMDA:	<i>N</i> -methyl-D-aspartic acid
PVCN:	posteroventral cochlear nucleus
R_{input} :	input resistance
SAM:	sinusoidal amplitude modulation
sl-sustained:	short-latency sustained
SOC:	superior olivary complex
τ :	membrane time constant
VNLL:	ventral nucleus of the lateral lemniscus
VNLLa:	anterior region of the ventral nucleus of the lateral lemniscus
VNLLc:	columnar region of the ventral nucleus of the lateral nucleus

VNLLd:	dorsal region of the ventral nucleus of the lateral lemniscus
VNLLl:	lateral limb of the ventral nucleus of the lateral lemniscus
VNLLm:	multipolar region of the ventral nucleus of the lateral lemniscus
VNLLv:	ventral region of the ventral nucleus of the lateral lemniscus
VNTB:	ventral nucleus of the trapezoid body
VPO:	ventral periolivary nucleus

1. INTRODUCTION

1.1 Introductory Remarks

This paper discusses auditory processing in the ventral nucleus of the lateral lemniscus (VNLL) of the central auditory brainstem. However, a brief description of peripheral auditory processing is introduced here before proceeding with a discussion of the central auditory system.

The peripheral auditory system consists of the external ear, middle ear and inner ear. Sound waves first enter the pinna and external auditory canal of the external ear. These waves then enter the middle ear. The middle ear consists of the tympanic membrane (eardrum) and the three middle ear ossicles (malleus, incus and stapes). The primary function of the middle ear is to ensure efficient transmission of sound from air into the fluid of the inner ear. One way in which this is accomplished is through the decrease in area from the eardrum to the stapes. The area of the eardrum is approximately 35 times larger than the area of the stapes, which results in a corresponding increase in pressure when sound is transmitted from the eardrum to the stapes. From the middle ear, sound waves enter the inner ear. The inner ear contains a coil-shaped sensory organ, the cochlea. Within the cochlea is the organ of Corti which rests on the basilar membrane and is covered by the tectorial membrane. The organ of Corti contains two types of receptor hair cells, the inner and outer hair cells. A tonotopic organization exists at this level, where high sound frequencies cause maximal motion at the base of the cochlea and lower frequencies cause more motion at the apex of the cochlea. This tonotopic organization is preserved throughout most of the central auditory system.

Movement of the basilar and tectorial membrane, induced by sound vibrations, causes a bending of the stereocilia. The inner hair cells are contacted by type 1 afferent neurons and the outer hair cells are contacted by type 2 afferent neurons (Brown, 1987; Simmons and Liberman, 1988). The central axons of these neurons form the auditory nerve. Auditory information is then sent to the brain from the auditory nerve for further processing (Brown, 1999).

I will now discuss the central auditory system relevant to the VNLL.

The lateral lemniscus is a major fiber pathway connecting the lower auditory brainstem with the inferior colliculus (IC) and the medial geniculate nucleus (Helfert and Aschoff, 1997). The lateral lemniscus begins rostral to the superior olivary complex (SOC) where its axons join with those from the cochlear nucleus (CN) to ascend rostrally first, then dorsally, as a single tract of fibers to the IC (Helfert and Aschoff, 1997).

Within the lateral lemniscus (LL) there are the neurons that compose its nuclei (NLL). The NLL comprise three morphologically distinct nuclei closely apposed to one another forming a continuous chain between the SOC and IC. They have been named, according to their position in the LL, ventral nucleus of the LL (VNLL), intermediate nucleus of the LL (INLL), and the dorsal nucleus of the LL (DNLL) (Helfert and Aschoff, 1997). It should be noted that some authors consider the VNLL to comprise all clusters of neurons ventral to the DNLL, but distinguish a dorsal division termed VNLLd (Adams, 1979, 1997; Whitley and Henkel, 1984). However, most authors consider VNLLd to be a distinct nucleus, the intermediate nucleus of the lateral lemniscus (Covey

and Casseday, 1991; Glendenning et al., 1981; Saint Marie et al., 1997, Schofield and Cant, 1997; Vater et al., 1997; Winer et al., 1995).

In vertebrates, the central auditory system, above the CN, consists of a complex network of parallel pathways, each with its own role in signal processing. These parallel pathways can be grouped into two classes: a monaural system that receives input from the contralateral ear only and a binaural system that receives input from both ears (Huffman and Covey, 1995).

The monaural system includes direct pathways from the CN to the midbrain as well as indirect pathways via the VNLL and INLL (Huffman and Covey, 1995). The VNLL and INLL are innervated primarily by contralaterally driven monaural structures such as the CN. This suggests that the VNLL and INLL are involved in monaural processing (Saint Marie et al., 1997). Research has shown that most VNLL units are monaural since they are only excited by contralateral stimulation and not influenced by ipsilateral stimulation (Covey and Casseday, 1991; Covey, 1993).

The binaural system includes the lateral and medial superior olive, where input from the two ears first converges (Huffman and Covey, 1995). The DNLL is involved in binaural processing since it receives most of its input from binaural nuclei in the SOC (Saint Marie et al., 1997).

The VNLL plays a role in the processing of temporal information of sound. Only two papers have addressed electrophysiological in vitro VNLL research (Wu, 1999; Zhao and Wu, 2001) and a small number of electrophysiological in vivo studies have been conducted. Most of the in vivo research has focused on the properties of VNLL neurons

in the bat (Covey and Casseday, 1991; Vater et al., 1997; Huffman and Covey, 1995), however there have also been some studies conducted in the cat (Aitkin et al., 1970) and rabbit (Batra and Fitzpatrick, 1999, 2002). In the following sections, I will review several aspects of this structure such as anatomical pathways, morphology, physiology, significance for temporal processing and relevance to repetitive synaptic stimulation. I will also review research about coding temporal information in other auditory brainstem nuclei. My research has focused on the synaptic physiology and pharmacology of VNLL neurons, and relations between parameters such as neuronal discharge patterns and synaptic responses to single and repetitive stimulation of synaptic inputs to the VNLL.

1.2 VNLL Anatomical Pathways

1.2.1 Projections to the VNLL

Primary input to the VNLL comes from the contralateral CN (see Fig. 1) (Glendenning et al., 1981; Zook and Casseday, 1985; Covey and Casseday, 1986). Suneja et al. (1995) found that unilateral CN ablation in the guinea pig resulted in a dense preterminal degeneration of fibers in the contralateral VNLL. The findings suggest that CN efferents project heavily to the contralateral VNLL. Within the cochlear nucleus, the major projection to the VNLL is from the contralateral anteroventral cochlear nucleus (AVCN) and contralateral posteroventral cochlear nucleus (PVCN) (Vater et al., 1997).

Bushy, multipolar, and octopus cells in the contralateral VCN have been identified as major cell types that project to the VNLL (Friauf and Ostwald, 1988; Schofield and Cant, 1997; Thompson, 1998; Warr, 1982). Schofield and Cant (1997)

found that in the guinea pig, octopus, multipolar, spherical bushy and globular bushy cells in the VCN are sources of projections to the VNLL (Schofield and Cant, 1997). Research focusing on the octopus cells of the PVCN in the cat has found that they send thick axons into the intermediate acoustic stria that terminate as large Calyces of Held in the VNLL (van Noort, 1969; Warr, 1969, 1982; Adams and Warr, 1976, Adams, 1983). These findings are consistent with reports in other species (Glendenning et al., 1981; Huffman and Covey, 1995). Spherical bushy cells and multipolar cells in the VCN send thin axons that terminate as boutons in the VNLL (Schofield and Cant, 1997; Schwartz, 1992; Covey, 1993). This conclusion is consistent with the results from anterograde degeneration and intracellular labeling studies (Warr, 1982; Friauf and Ostwald, 1988).

The ipsilateral projection from the VCN to the VNLL is less than the contralateral projection (Glendenning et al., 1981). Small projections from the ipsilateral cochlear nucleus to the VNLL have been described in the cat (Glendenning et al., 1981), the rhesus monkey (Strominger and Strominger, 1971) and the guinea pig (Suneja et al., 1995). Also, in the big brown bat, labeled cells were present in the ipsilateral cochlear nucleus in only 2 of 7 cases involving transport from injections restricted to the VNLL, and in those two cases, in only a few cells (Huffman and Covey, 1995).

There is also a small projection from the ipsilateral SOC and a very slight projection from the contralateral dorsal cochlear nucleus (DCN) via the dorsal acoustic stria to the VNLL (Glendenning et al., 1981; Zook and Casseday, 1985; Covey and Casseday, 1986). For example, in a variety of species, such as the cat and the bat, the VNLL receives minor inputs from the ipsilateral MNTB and periolivary nuclei (lateral

nucleus of the trapezoid body, LNTB, ventral nucleus of the trapezoid body, VNTB, and ventral periolivary nucleus, VPO) (Elverland, 1978; Glendenning et al., 1981; Huffman and Covey, 1995; Spangler et al., 1985; Vater and Feng, 1990; Warr and Beck, 1996). In the big brown bat, the LNTB, which receives input mainly from the ipsilateral cochlear nucleus sends a robust projection to the VNLL ipsilaterally (Covey et al., 1984; Cant, 1991). This is also the case in the mustached bat (Zook and Casseday, 1987), but much less so in the cat (Glendenning et al., 1981).

1.2.2 Projections from the VNLL

The VNLL projects to the ipsilateral INLL (Covey and Casseday, 1986), DNLL and IC (Saint Marie et al., 1997; Irvine, 1986; Schwartz, 1992; Helfert and Aschoff, 1997). The principal target of the VNLL is the ipsilateral IC. In the cat, for example, a group of neurons in the VNLL send large diameter axons to the ipsilateral IC. The fibers course in the lateral lemniscus, pass through the intermediate and dorsal nuclei of the lateral lemniscus, and terminate primarily in the ipsilateral ventrolateral central nucleus of the inferior colliculus (Whitley and Henkel, 1984). These fibers also terminate ipsilaterally in the external nucleus of the IC (Adams and Warr, 1976; Kane and Finn, 1977; Kudo, 1981; Whitley and Henkel, 1984).

There have also been some reports suggesting that a small number of VNLL cells in the cat, gerbil, mouse and guinea pig project to the contralateral IC (Whitley and Henkel, 1984; Moore, 1988; Nordeen et al., 1983).

1.3 VNLL Morphology

The VNLL varies in its morphology from one species to another. Although essentially the same cell types are present in all mammals, they are segregated in some species and intermingled in others (Glendenning et al., 1981; Zook and Casseday, 1982; Covey and Casseday, 1986; 1991; Vater and Feng, 1990).

1.3.1 Echolocating Bats

Echolocating bats, a species placing special demands on its auditory system (Schwartz, 1992), are ideal for researchers to study the structure and function of the VNLL because the nucleus is especially large, well differentiated, and there is nearly complete segregation of specific cell types (Zook and Casseday, 1982; Covey and Casseday, 1991; Vater and Feng, 1990; Covey, 1993).

In most echolocating bats, for example, the big brown bat, the mustached bat, and the horseshoe bat, Golgi staining of the VNLL reveals two distinct subdivisions which are termed the columnar area (VNLLc) and the multipolar area (VNLLm) (Covey and Casseday, 1986; 1991; Huffman and Covey, 1995; Vater et al., 1997). The most conspicuous is the columnar division (Zook and Casseday, 1982; Covey and Casseday, 1995).

The VNLLc contains a single cell type (Covey and Casseday, 1991; Vater et al., 1997). These cells are spherical cells that are round to oval in shape and have one large, thick dendrite that branches profusely some distance away from the cell body. At least some of these branches appear to extend parallel to the ascending fibers. The cells are

tightly packed in a columnar arrangement between bundles of fibers, hence the name columnar nucleus (Covey and Casseday, 1986). These cells are contacted by large calyx-like synaptic terminals, possibly arising from octopus cells in the PVCN, in addition to small bouton terminals (Vater et al., 1997).

In the VNLLm, there is a more heterogeneous group of cells. The VNLLm derives its name from the observation that most neurons in this region 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 the VNLLm have no consistent orientation relative to the fibers of the lateral lemniscus (Covey and Casseday, 1991). These cells receive punctate terminals on the cell body and dendrites as opposed to the large calyceal endings seen in the more homogenous group of spherical cells in the VNLLc (Covey and Casseday, 1986).

1.3.2 Nonecholocating Mammals

In nonecholocating species such as the cat and rodent, the VNLL is normally recognized as a single group of cells of mixed character (Schwartz, 1992) as opposed to the clear segregation of cell types observed in echolocating bats. In cats, for example, Nissl staining of the VNLL shows that the dorsal and middle region contains a larger number of multipolar and horizontally oriented elongated neurons, and the ventral region contains mostly oval-shaped cells (Adams, 1979). In the rat VNLL, Zhao and Wu (2001) found two broad types of neurons: bushy and stellate, labeled by neurobiotin. Stellate cells could be further classified as stellate type 1, stellate type 2 and elongate.

The distribution of rat VNLL cells was similar to that in other mammals in that different types of neurons were intermingled in the VNLL. However, there was a tendency for certain types of neurons to be located in certain regions of the VNLL. For example, bushy cells were primarily located in the ventral area of the VNLL. All three types of stellate cells were located throughout the VNLL, although the stellate type 1 cells tended to be more prevalent in the ventral region and stellate type 2 cells in the dorsal region.

Bushy Cell Morphology of the Rat VNLL:

Bushy cells were characterized by round to oval cell bodies and highly branched dendrites. The dendritic tree of the bushy cell was smaller, more compact and highly branched than that of the stellate cell. Primary dendrites of the bushy cell usually branched profusely within a relatively restricted area. The dendritic morphology of the rat's VNLL bushy cell resembles that of the spherical cells in the VNLLc of the bat, i.e., both have short and compact dendritic trees (Covey and Casseday, 1991). However, the bushy cells in the rat have 3-6 primary dendrites and in the VNLLc of the bat have one or two thick primary dendrites.

It is not known if the bushy cells in the rat VNLL are contacted by large axonal terminals. One should note that large Calyces of Held were labeled by biocytin in the rat VNLL (Wu, 1999). Also, since previous research has found calyces cover spherical shaped cells in the VNLL of the bat (Vater et al., 1997) and cat (Adams, 1979), it is likely

that bushy cells in the rat VNLL may also receive afferent inputs originating from thick axons of octopus cells in the PVCN (Zhao and Wu, 2001).

In contrast to the calyceal endings, medium-sized bead-like boutons were also found in the rat VNLL (Friauf and Ostwald, 1988; Schofield and Cant, 1997). They may originate from collaterals of thinner axons derived from bushy and/or stellate (multipolar) cells in the AVCN and PVCN which pass through the VNLL toward the IC (Schwartz, 1992; Covey, 1993).

Stellate Cell Morphology of the Rat VNLL:

Stellate cells had fewer dendritic branches than bushy cells. The dendrites of the stellate cells radiated away from the cell body and extended over a relatively large area. The dendritic tree of stellate type 1 cells had no directional preference. Stellate type 1 and elongate cells had similar numbers of dendritic branches. Stellate type 2 cells had longer, but fewer dendritic branches than the other cell types. The dendritic features of the stellate type 2 cells are very similar to those of the multipolar neurons in the medial part of the bat's VNLLm (Covey and Casseday, 1991). Elongate cells were characterized by elongated cell bodies and dendritic arbors that ran along the longitudinal axis of the cell body. Other studies of rat VNLL morphology using Nissl staining have shown the presence of stellate neurons throughout the VNLL (Merchan and Berbel, 1996).

In the guinea pig, three subdivisions of the VNLL, the ventral (VNLLv), dorsal (VNLLd) and anterior (VNLLa) regions were distinguished by Nissl staining (Schofield

and Cant, 1997). The VNLLv is the largest and most morphologically complex region of the VNLL. Features that characterize it are primarily the presence of globular cells, giant cells and alternating bands of densely and loosely packed cells. These alternating bands are located in the caudal part of the VNLLv. Globular cells are most numerous in the rostral portion of the VNLLv and giant cells are most common in the posterior and medial part of the VNLLv. The VNLLd in guinea pigs is populated primarily by multipolar cells. The VNLLa contains both elongate and multipolar cell types, where the elongate cells are more common.

Two groups of axons were distinguished in the guinea pig VNLL on the basis of morphology and terminal distribution. These two groups - thick axons and thin axons - appear to arise from different cell types. Thick axons were labeled in the VNLL only when cells in the octopus cell area in the VCN were labeled by the injection of anterograde tracers. Conversely, octopus cells were labeled by retrograde tracers only when the injection included the ventral subdivision of the VNLL, where thick axons terminate. The anterograde studies showed thick axons only on the side contralateral to an injection in the VCN. This provides evidence that in the guinea pig, octopus cells are the source of the thick axons in the contralateral VNLL. This conclusion is consistent with results found in the cat and bat (Warr, 1982; Adams, 1979; Zook and Casseday, 1985). In contrast to the thick axons, the thin axons arose from more than one cell type in the VCN. Thin axons were observed in the VNLL following injection of tracers into any part of the VCN. Multipolar cells, which have a widespread distribution in the VCN, were labeled in the retrograde tracing experiments. Thus, it is likely that these cells give

rise to at least some of the thin axons. Another cell type that was labeled was the spherical bushy cell. Injections of anterograde tracers into the spherical cell area of the CN which contains both spherical bushy cells and multipolar cells (Moore, 1986; Hackney et al., 1990) labeled only thin axons in the VNLL. That the axons of spherical bushy cells were labeled in these cases was confirmed by identifying labeled boutons in the medial superior olivary nucleus, which receives inputs from spherical bushy cells but not from multipolar cells (Schwartz, 1992). It was concluded that both spherical bushy cells and multipolar cells in the VCN give rise to thin axons in the VNLL.

1.3.3 Comparison Across Species

Although there are differences in morphological distribution with regards to the extent of segregation, it is interesting to note some similarities in the general morphological characteristics across species. For example, in the cat and the guinea pig, oval and globular cells, which likely correspond to the bushy cells in the rat, are all largely found in the ventral zone, and multipolar cells likely corresponding to the stellate type 2 cells in the rat (Zhao and Wu, 2001), are all mainly located in the dorsal and middle zones (Adams, 1979; Schofield and Cant, 1997). In addition, the VNLLc division of echolocating bats contains spherical cells which are similar to the bushy cells of the rat, and globular and oval cells of the guinea pig and cat. Also, there are similarities in input pathways across species. In the rat, for example, it is likely that bushy cells are contacted by calyx endings possibly arising from the thick axons of octopus cells in the PVCN (Zhao and Wu, 2001). In the guinea pig, there is also evidence of thick axons

arising from octopus cells terminating in the ventral region of the VNLL primarily containing globular cells (Schofield and Cant, 1997). In the VNLLc of the bat, spherical cells may also be contacted by thick axons of octopus cells in the PVCN. Also, multipolar cells in the VCN may be one of the sources of thinner axons in the VNLL in both the rat and the guinea pig.

1.4 VNLL Physiology

1.4.1 Neuronal Discharge Patterns

1.4.1.1 In Vivo Studies:

1.4.1.1.1 Echolocating Bats

Many physiological studies have been conducted on echolocating bats. In most cases, it was possible to apply the same response classes that have been used to describe neurons in the cochlear nucleus (Pfeiffer, 1966). Covey and Casseday (1991), for example, found that neurons in the VNLL of echolocating bats exhibited a variety of discharge patterns which were evoked by pure tone bursts presented contralaterally at the units BF (best frequency), 20 dB above threshold.

Phasic Discharge Pattern:

Phasic neurons fired only one or a few spikes at the onset of the stimulus and remained inactive thereafter. Phasic responses could be subdivided into two types, constant-latency and variable-latency. Constant-latency neurons seldom fired more than one spike per stimulus, and this spike was tightly locked to the stimulus onset. Phasic

variable-latency neurons fired one to three spikes at the onset of a stimulus, but the latency of the first spike varied by as much as several milliseconds from one stimulus presentation to another.

Phasic neurons were found throughout the VNLL but there was a clear segregation of the constant-latency and variable-latency types. In the columnar nucleus, VNLLc, phasic constant-latency units were the only type found. Phasic variable-latency neurons were found only in the VNLLm.

Chopper Discharge Pattern:

Choppers responded throughout the duration of the stimulus with spikes occurring at regular interspike intervals (ISIs) and tight locking of the first spike to the time of stimulus onset. The units that were classified as choppers mostly had ISIs of less than 2.5 msec and therefore were fast choppers (Tsuchitani, 1988a,b). They were found in VNLLm but not in VNLLc.

Tonic Discharge Pattern:

Tonic neurons discharged at a constant high rate throughout the duration of the stimulus, with no adaptation even when the stimulus duration was long (50 msec or more). They differed from choppers in that the first spike was poorly locked to the time of stimulus onset. Although the ISI for the majority of tonic units was irregular, some fired with an ISI as regular as that of a chopper even though the first spike was not synchronized to the onset of the sound. For a few of these tonic neurons, the first spike

became synchronized to onset at high sound levels, and they responded in a chopper pattern. Tonic neurons were only found in VNLLm.

Primary-like Discharge Pattern:

These cells responded throughout the stimulus duration, but their firing rate diminished after an initial transient “on” response. A very small population of units in the divisions of the lateral lemniscus responded in a primary-like pattern.

Pauser Discharge Pattern:

Pausers responded with an initial spike followed by an inactive period and then resumed sporadic firing at a diminished rate for the remainder of the stimulus duration. Pausers were only found in the VNLLm.

Thus, in echolocating bats, the VNLLm comprises mixed response types. However, in the VNLLc, a cytoarchitecturally and physiologically unique region, responses are of a single type, phasic constant-latency (Covey and Casseday, 1991).

1.4.1.1.2 Nonecholocating Mammals

One study has also focused on the neuronal firing pattern in the rabbit VNLL (Batra and Fitzpatrick, 1999). The authors found that neurons in the VNLL of the unanesthetized rabbit responded to pure tone bursts at the contralateral ear with a variety of discharge patterns. Most exhibited sustained or phasic patterns. Sustained neurons

included ordinary chopper neurons and unusual chopper neurons. Most sustained neurons were classified as short-latency sustained (sl-sustained), but a few were of long latency. Some sl-sustained neurons exhibited a chopper discharge pattern, whereas other sl-sustained neurons did not exhibit this pattern.

Ordinary Chopper Neurons:

In ordinary chopper neurons, the multiple peaks corresponded to the evenly spaced action potentials of a regular discharge.

Unusual Chopper Neurons:

In unusual chopper neurons, the action potential associated with a particular peak could fail to occur during any one presentation of the stimulus. Unusual chopper neurons had a relatively irregular discharge.

Phasic Neurons:

Phasic neurons were of two types: onset and transient. Onset neurons typically responded with a single action potential at the onset of the tone. Transient phasic neurons responded to tones with a burst of four or more action potentials without a pronounced chopping pattern.

Localization of Neurons with Different Response Types:

Different response types were partially segregated within the VNLL. Sustained neurons predominated in the lateral limb of the VNLL (VNLLl), whereas onset neurons predominated in the medial limb of the VNLL (VNLLm) (Batra and Fitzpatrick, 1999).

Physiological studies of the cat have also found that VNLL neurons exhibit various discharge patterns to pure tones. These patterns include single onset spikes, sustained firing and sustained firing interrupted with a pause during the discharge (Aitkin et al., 1970).

1.4.1.1.3 Comparison of Response Across Species

In the bat VNLLc, the majority of neurons respond with one spike at short and constant latencies, i.e., the phasic constant-latency neurons (Covey and Casseday, 1991). This “onset” type of neuron is also present in the VNLL of other mammals (Vater et al., 1997). The response pattern of the onset VNLL cells in the cat and phasic onset neurons in the rabbit resemble the phasic constant-latency type in the VNLLc of the big brown bat (Batra and Fitzpatrick, 1999; Covey and Casseday, 1991; Guinan et al., 1972; Adams, 1997).

It may be worth noting, however, that contrary to findings in the bat VNLL, the VNLL of the rabbit did not exhibit distinct populations of constant-latency and variable-latency neurons. Instead there appeared to be a continuum such that neurons with less variable latencies responded sooner than those with more variable latencies. This

relationship appears to be at least partially inherited from the auditory nerve (Heil and Irvine, 1997).

With respect to other discharge patterns, there are also across species similarities. For example, the choppers of the echolocating bat are similar to the ordinary choppers of the rabbit. Also, in some physiological studies of the cat, most VNLL neurons were found to have chopper, pauser and primary-like patterns (Aitkin et al., 1970; Guinan et al., 1972) similar to what is found in the bat.

1.4.1.1.4 Inheritance of Discharge Patterns

Some studies have examined the relations between the characteristics of discharge patterns in the VNLL and the discharge characteristics of cells providing input to the VNLL. Since the primary input to the VNLL is from the contralateral VCN, it is interesting to study to what degree responses in the VNLL reflect these inputs.

For example, Batra and Fitzpatrick (1999) found that the different subtypes of sustained discharge patterns that are present in the VNLL are also observed in the VCN (Rhode and Greenberg, 1992). The chopper neurons in the VNLL appear similar to those in the VCN. Similarly, the nonchopper neurons of the VNLL produced discharge patterns that were similar to the primary-like pattern responses seen in the VCN. Also, onset neurons are present in both the VCN and the VNLL. There is evidence that some of the onset responses in the VNLL are inherited via inputs from the VCN octopus cells, which appear to project to the VNLL and terminate there in calyceal endings (Adams, 1997; Schofield and Cant, 1997). Such endings would serve to faithfully reproduce the

onset discharge pattern of octopus cells in the neurons of the VNLL. Thus, these neurons could well have received their input from octopus cells of the VCN (Batra and Fitzpatrick, 1999).

However, despite these similarities, one should also note the differences. For example, in the rabbit, the dynamic range of sl-sustained neurons in the VNLL was considerably larger than that of most neurons in the VCN. For sl-sustained neurons, the average dynamic range was almost 50 dB, however, most neurons in the VCN have a dynamic range of about 25 dB (Rhode and Smith, 1986). Thus, it is likely that the wide dynamic range of neurons in the VNLL is a result of neural processing in the VNLL itself and is a consequence of the convergence of inputs from the VCN that encode different ranges of intensity. In addition, there were onset neurons in the rabbit that did not appear to have inherited their discharge pattern from octopus cells. These onset neurons were of low best frequency and were located primarily in the VNLLm. At low frequencies, octopus cells do not produce an onset response but instead a sustained, phase-locked discharge. Furthermore, the calyceal endings that are believed to come from octopus cells have been reported only in the VNLLl (Batra and Fitzpatrick, 1999).

1.4.1.2 In Vitro Studies:

There have only been two in vitro studies conducted focusing on the physiology of VNLL neurons. Wu (1999) investigated the membrane properties of VNLL neurons in the rat between the ages of 17 and 37 days, by recording intracellular potentials in response to current injection into the cell. Zhao and Wu (2001) conducted whole-cell

patch clamp recordings in the rat VNLL between the ages of 12 and 16 days. In both studies, the current-voltage relationship and temporal firing pattern were used to characterize and distinguish cell types in the VNLL. There were two broad types of VNLL neurons found in both studies, onset and sustained, that exhibited different current-voltage relationships.

1.4.1.2.1 Sustained Neurons

In these cells, for both intracellular and patch clamp recording, intracellular injection of negative current led to a hyperpolarization of the cell membrane that was proportional to the amount of current injected, thus indicating a linear I-V curve. Wu (1999) found that with continued application of current, the hyperpolarization was followed by a sag of the membrane potential toward the resting level. Following the end of the hyperpolarization, there was a depolarization that elicited two to three action potentials. The sag and after-depolarization may reflect activation and deactivation, respectively, of an inward hyperpolarization-activated current (I_h).

In response to positive current, sustained cells in both studies produced a graded depolarization which led to a train of action potentials at suprathreshold levels, where the relation of current to voltage near the resting potential was linear. These sustained cells were referred to as type 1 cells in the Wu (1999) study. In the Zhao and Wu (2001) study, they were termed the linear group on the basis of their current-voltage curves. Both studies found that in response to positive current, sustained neurons showed three different firing patterns: Regular, Onset-pause and Adaptation.

Regular Firing Pattern:

In this pattern, Wu (1999) found that intracellular injection of positive current produced a depolarization and action potentials. Smaller current produced a few spikes at the onset of injection. As the current was increased, a regular train of action potentials was produced throughout the entire period of current injection. The criteria for identifying this pattern of cell firing for this study was that the ratio of the last ISI to the initial ISI should be less than or equal to 1.2. Zhao and Wu (2001) also found this regular firing pattern where a continuous train of action potentials with almost regular interspike intervals was produced by positive current injection.

Onset-pause Firing Pattern:

With this firing pattern, both studies found that intracellular positive current injection produced a graded depolarization and a train of action potentials, the number of which increased with current strength. Wu (1999) found that cells with this onset-pause firing pattern usually had one to three action potentials at the onset of current injection following a silent period before a train of action potentials. As current strength increased, the pause became progressively shorter, ultimately leading to sustained firing without any pause. Zhao and Wu (2001) found that small positive current produced only one spike at the beginning of depolarization. However as current strength increased, more spikes were generated at the onset of current injection, followed by a pause after spike(s) onset, and then sustained firing.

Adaptation Firing Pattern:

With the adaptation firing pattern, both studies found that action potentials were more frequent during the onset of current injection but progressively diminished during the remaining current injection interval. Wu (1999) identified the adaptation pattern when the ratio of the last ISI to the initial or subsequent ISI was larger than 1.2. Zhao and Wu (2001) found that in response to low intensity current, there was a delay between the onset of current injection and the discharge of the first spike. However, when the current intensity was increased, the first spike appeared earlier. They found that the firing showed adaptation at lower current levels and displayed regular interspike intervals at higher current levels. This is consistent with the Wu (1999) study.

Burst Firing Pattern:

This pattern of firing was only found in the whole-cell patch clamp recording study by Zhao and Wu (2001). Burst cells were characterized by a short burst of discharges on a slow depolarization. They fired sustained spikes when the intensity of current was high, however several burst spikes always appeared at the early period of current injection.

Both recording procedures, intracellular and whole-cell patch clamp, found that sustained neurons discharged continuously for the duration of the stimulus in response to more intense current injection. The firing rates usually increased as the amount of current increased.

1.4.1.2.2 Onset Neurons

Both studies found that onset neurons responded to intracellular injection of negative current with a graded hyperpolarization of the cell membrane and that their current-voltage relation was nonlinear. Wu (1999) referred to these cells as type 2 cells and Zhao and Wu (2001) referred to these cells as belonging to a nonlinear group, based on their nonlinear current-voltage relations.

Wu (1999) found that intracellular injection of positive current elicited one action potential at the onset of the injection. As current strength increased, the cells continued to respond with only one action potential (2-3 spikes in some cells). Following the spike(s), the membrane potential of the cell remained near rest and quickly returned to the resting potential after the end of the current pulse. Zhao and Wu (2001) also found that intracellular injection of positive current produced only one action potential at the onset of the current injection and a small depolarization.

Wu (1999) found that the mean membrane resting potential and input resistance of the sustained cells was not significantly different from onset cells. Also, there was no significant difference in resting membrane potential between the sustained and onset group of cells in the Zhao and Wu (2001) study.

1.4.1.2.3 Localization of Different Cell Types

There was a tendency for certain cell types to be located in certain regions. For example, onset neurons were located primarily in the ventral area of the VNLL.

Although regular and onset-pause neurons were found throughout the VNLL, they were more prevalent in the ventral region. Adaptation neurons were more prevalent in the dorsal region and bursting neurons were found throughout the VNLL (Zhao and Wu, 2001).

1.4.1.3 Correlation of Morphology with Discharge Pattern:

In the rat VNLL, bushy neurons had membrane properties that showed an onset firing pattern and a non-linear current-voltage relationship (Zhao and Wu, 2001).

Stellate type 1 cells show linear current-voltage relationships and two firing patterns, either regular or onset-pause, which have some features in common. For example, the first spike often appears immediately after the onset of depolarization and they both fire action potentials at similar frequencies in response to positive current injection.

Stellate type 2 cells have an adaptation firing pattern and a linear current-voltage relationship. In response to a lower intensity of current, stellate type 2 cells fire action potentials with obvious adaptation. The first spike often appears with some delay following the onset of current injection. Elongate cells exhibit burst firing and a linear current-voltage relationship (Zhao and Wu, 2001).

1.4.2 Synaptic Physiology and Neurotransmission

1.4.2.1 Synaptic Potentials:

With reference to the two in vitro physiological studies in the rat, both intracellular (Wu, 1999) and whole-cell patch clamp recording (Zhao and Wu, 2001) studies found that electrical stimulation of the lateral lemniscus ventral to the VNLL could elicit EPSPs and/or IPSPs in all cell types (Wu, 1999; Zhao and Wu, 2001).

Zhao and Wu (2001) found that there were a similar proportion of cells exhibiting EPSPs only and a mixture of both EPSPs and IPSPs. However, proportions of cells displaying only IPSPs were less. Also it is interesting to note that the durations of EPSPs differed across neurons. For example, the durations of EPSPs for onset, regular, and onset-pause neurons were much shorter than those for adapting and bursting neurons. Although the short duration of EPSPs may be due to membrane properties, such as low input resistance, it is possible that IPSPs truncate the EPSPs.

Wu (1999) found that there were similar proportions of sustained and onset cells that responded to electrical stimulation of the lateral lemniscus with EPSPs or IPSPs or both. The latencies of most EPSPs and IPSPs were short. Some onset cells had EPSPs with a shorter latency, larger amplitude and shorter duration, which resembled the characteristics of EPSPs observed in bushy and octopus cells in the cochlear nucleus (Golding et al., 1995). In sustained neurons, there was the same number of cells exhibiting only IPSPs and a mixture of EPSPs and IPSPs. Sustained cells showing only EPSPs were fewer in number. With reference to onset cells, there were similar numbers

of cells exhibiting only EPSPs and a mixture of both EPSPs and IPSPs. Onset cells showing only IPSPs were few.

With respect to the synaptic responses of neuronal subtypes, Wu (1999) found that the incidence of both excitatory and inhibitory synaptic responses recorded from neurons in the onset-pause group was higher than in the adaptation and regular groups. This result suggests that neurons with an onset-pause firing pattern may be more likely to receive mixed excitatory and inhibitory inputs from the lower auditory brainstem. However, additional information needs to be available about what kinds of synaptic inputs and neurotransmitters are correlated with the different firing patterns (Zhao and Wu, 2001).

Thus, both whole-cell patch clamp and intracellular recording studies found that many VNLL neurons may receive and integrate excitatory and inhibitory afferent inputs from the lower auditory brainstem.

1.4.2.2 Synaptic Transmission in the VNLL:

As noted previously, the PVCN sends thick axons to the VNLL that terminate as large Calyces of Held (van Noort, 1969; Warr, 1969,1982; Adams and Warr, 1976, Adams, 1983; Glendenning et al., 1981; Huffman and Covey, 1995). Calyceal endings are known to be excitatory since neurons that receive calyceal terminals, in the VCN, medial nucleus of the trapezoid body (MNTB) and VNLL, are excited by acoustical stimulation (Adams, 1997; Smith et al., 1998). Also, in the big brown bat, an electron

microscopic (EM) study about the synaptic organization of VNLL neurons suggests that these neurons receive excitatory inputs that may arise from large calyces of octopus cells in the PVCN (Vater et al., 1997). In addition, the spherical bushy and multipolar cells which send thin axons from the VCN and terminate as boutons in the VNLL (Schofield and Cant, 1997; Schwartz, 1992; Covey, 1993) may in fact receive axon collaterals from AVCN neurons whose main axons provide excitatory synaptic input to the IC (Oliver, 1987). Thus, it is likely that projections from the AVCN to the VNLL are also excitatory.

Other research has also supported the role of excitatory neurotransmission from the CN to the VNLL. For example, Suneja et al. (1995) studied if projections ascending from the guinea pig cochlear nucleus could be glutamatergic and/or aspartatergic. They reasoned that if there are glutamatergic synaptic endings in these nuclei provided by CN efferents, then destruction of the CN should induce degeneration of these efferents and depress the calcium-dependent release and high-affinity uptake of glutamate at these sites. To test this hypothesis, they measured the release and high-affinity uptake of D-[³H]aspartate in the VNLL after unilateral ablation of the CN. They found that CN ablation depressed evoked calcium-dependent release and high-affinity uptake of D-[³H]aspartate in the VNLL contralaterally. These findings imply that CN efferents that project to the contralateral VNLL may use excitatory amino acids as a transmitter.

The VNLL also receives minor inputs from the ipsilateral MNTB and periolivary nuclei (Elverland, 1978; Glendenning et al., 1981; Huffman and Covey, 1995; Spangler

et al., 1985; Vater and Feng, 1990; Warr and Beck, 1996). MNTB principal neurons are known to be immunoreactive for glycine (Helfert et al., 1989) indicating that the projection of MNTB to VNLL is likely glycinergic. Neurons in the periolivary region, e.g., in the VNTB and LNTB, have been shown to be GABAergic (Moore and Moore, 1987; Helfert et al., 1989; Vater et al., 1992). These neurons are another possible source of inhibition to the VNLL. Also, it has been suggested that in the rat, VNLL neurons receive inhibitory inputs from the MNTB, VNTB or LNTB (Zhao and Wu, 2001).

1.4.2.3 Synthesis of Neurotransmitters in the VNLL:

Some immunostaining studies have examined the types of neurotransmitters found in VNLL neurons. For example, Riquelme et al. (2001) studied the expression of GABA and glycine in cell bodies of the VNLL in the rat. They found that the rat VNLL contains two main populations of neurons. Approximately two-thirds of neurons were double-labeled for GABA and glycine. These inhibitory neurons which colocalized GABA and glycine were located mainly ventrally in the VNLL. The remaining one-third did not stain and were presumed to be excitatory. These neurons were located mainly in the dorsal region of the VNLL. Thus, it was concluded there are two populations of VNLL cells in terms of neurochemical profile: one population that lacks the capacity for GABA and glycine synthesis and that predominates at dorsal levels of the nucleus, and one population that is capable for co-synthesis and predominates more ventrally. Saint

Marie et al. (1997) also found that many VNLL neurons of the cat were doubly-immunoreactive for GABA and glycine.

The significance of having both transmitter substances in the same neuron is not completely understood. One or both transmitters may act presynaptically to modulate the uptake and /or release of the other (Deisz and Lux, 1985; Thomson and Gahwiler, 1989; Raiteri et al., 1989). Alternatively, both may act postsynaptically, one as the primary transmitter and the other as a modulator. For example, it has been shown that GABA can modulate the postsynaptic effect of glycine at some synapses (Werman, 1980). Thus, the two transmitters found in the VNLL may act together at synapses, such as in the IC, to produce a more refined postsynaptic response than would be possible with either transmitter alone (Saint Marie et al., 1997).

Based on the absolute numbers of neurons in its projection, the VNLL is probably the single largest source of inhibition in the IC. The prominence of the VNLL as a source of glycinergic input to the IC was demonstrated by Saint Marie and Baker (1990). They found that when tritiated glycine was used as a retrograde tracer to mark glycinergic inputs to the IC in the cat, approximately two thirds of the labeled cells were located in the ipsilateral VNLL, suggesting that the VNLL is a major source of inhibitory input to the IC.

Also, Adams and Wenthold (1987) have reported that, in the cat and gerbil, a group of glycine-immunoreactive neurons in the VNLL send large diameter axons to the IC. This same projection has been seen in the mustached bat (Winer et al., 1995) and the big brown bat (Vater et al., 1997). In all of these species, the VNLL contains a

population of glycine-positive neurons that receive large calyx-like endings. This suggests that the VNLL is expected to transform the rapid robust excitatory input derived from the contralateral ear into inhibitory output that influences information processing in the auditory midbrain (Vater et al., 1997).

In addition to being the largest single source of glycine in the IC, the VNLL could also represent a large extrinsic source of GABAergic inhibition in the IC. Zhang et al. (1998) conducted a study utilizing fluorogold (FG) tracing with GABA immunocytochemistry in the rat. They found that approximately 30-40% of fluorogold retrogradely labeled neurons in the ipsilateral VNLL were labeled with GABA antibody indicating that many neurons in VNLL send GABAergic projections to the IC ipsilaterally. Specifically, they found that numerous small to medium sized round cells in the ipsilateral VNLL were double labeled with GABA antibody and FG.

These findings suggest that projections from the VNLL could play a significant role in inhibitory processing in the IC (Schofield and Cant, 1997). The VNLL may provide feed-forward, GABAergic and/or glycinergic, contralaterally driven, monaural inhibition to the IC (Saint Marie et al., 1997).

It should be noted, however, that while the majority of VNLL cells appear to use inhibitory transmitters, there is evidence that some VNLL cells that project to the IC use glutamate (Saint Marie, 1994). These projections would thus be expected to excite their postsynaptic target(s) (Schofield and Cant, 1997).

1.5 Significance of the VNLL in Temporal Processing

The physiological properties encountered in *in vitro* studies of the rat suggest the significance of the VNLL in temporal processing. For example, bushy cells had membrane properties that showed an onset firing pattern and a non-linear current-voltage relationship, which reflects a lower membrane input resistance while the cell is excited (Zhao and Wu, 2001). The low input resistance of the membrane when the cell is depolarized (involving outward potassium conductance) (Manis and Marx, 1991) results in rapid repolarization of the cell membrane, which shortens the excitation and prevents summation in time. Therefore, the temporal pattern of excitation in these cells can reflect precisely the temporal firing pattern of inputs from the afferents. Such properties are essential for these neurons to precisely encode auditory temporal information and the onset of sound (Oertel, 1991; 1997).

In echolocating bats and dolphins, the VNLL is orderly in structure and greatly expanded in size (Zook and Casseday, 1982; Zook et al., 1988). The high degree of development and differentiation in two different echolocating species suggests that the VNLL may play an important role in echolocation. It has been suggested that the function of the VNLL may be to analyze and encode information about the temporal structure of sequential auditory signals such as pulse-echo pairs (Covey and Casseday, 1986).

The majority of research studying temporal processing in the VNLL *in vivo* has focused on the echolocating bat. For example, Covey and Casseday (1991) used single-unit recording methods to describe the response to sound of cells in the VNLL in the big brown bat. Responses of single units recorded in the VNLLc and VNLLm of the big

brown bat indicated that they share certain characteristics. First, they are both broadly tuned to frequency. Second, most units have little or no spontaneous activity. Third, all have short integration times, responding robustly to stimuli less than 5 msec in duration. These findings indicate that the VNLLc and VNLLm can be considered as a unitary system of monaural pathways to the midbrain and are well suited for the accurate encoding of temporal information of sounds.

However, there are also differences in response properties among the two divisions. For example, contrary to VNLLm cells, VNLLc cells have a relatively high excitatory threshold. This threshold may be created through intrinsic membrane properties or through inhibitory inputs. Also, neurons in VNLLc are more broadly tuned to frequency than those in the VNLLm, have no spontaneous activity, and always respond with one spike per stimulus. The latency of the spike in the VNLLc is precisely locked to the stimulus onset, with very little variability from trial to trial. In addition, the latency remains constant over large variations in frequency or intensity. However, in VNLLm, response patterns are about equally distributed between tonic, chopping, and phasic. There are no single-spike constant-latency responses of the type seen in VNLLc, although some choppers and pausers do respond with constant first-spike latency.

These observations indicate that VNLLc may be specialized to encode very precisely the onset of sound, whereas VNLLm may encode ongoing properties of a sound (Covey and Casseday, 1991).

1.5.1 Reflection of Input

In the big brown bat, researchers maintain that given the homogeneous response properties found in VNLLc, it may seem reasonable to predict that this cell group receives input from a more restricted number of sources than does VNLLm, where response properties are heterogeneous. Knowing the sources of input to each nucleus should provide insight into the type of signal transformations that occur. For example, if VNLLc were found to receive input primarily from one population of cells with similar response properties (e.g., octopus cells in the posteroventral cochlear nucleus), this would indicate that it serves mainly as a relay nucleus with a minor contribution to signal processing. If, on the other hand, VNLLc was found to receive input from other sources, this would indicate that integration and modification of the incoming signal takes place (Huffman and Covey, 1995).

1.5.1.1 Anatomical Observations Regarding Ascending Inputs:

Huffman and Covey (1995) studied the input to the divisions of the VNLL in the big brown bat to determine if different response properties between VNLLc and VNLLm could be attributed to differential ascending input. The results of this study indicate that the unique response type found in VNLLc is not explained by inputs that are more limited to specific nuclei or cell types than are those to VNLLm. On the contrary, both divisions, including VNLLc, receive projections from the AVCN, PVCN and MNTB.

It should be noted, however, that although the divisions of the VNLL receive projections from the same set of brainstem nuclei, there is a systematic change in the

relative proportions of input to each division from AVCN, PVCN and MNTB. The distribution of inputs is such that the VNLLm receives input mostly from the AVCN, and VNLLc receives an intermediate balance of input from MNTB, AVCN and PVCN. The projection to the VNLL in the cat (Glendenning et al., 1981) and the mustached bat (Zook and Casseday, 1987) show evidence of a similar organization.

The observation that each division of the VNLL receives a unique ratio of afferents from different sources suggests that signal processing is different in each division, but it is not clear how these ratios of input contribute to the observed response properties associated with the different divisions. What is clear is that the different response types must result from an extensive integration of these multiple inputs (Covey and Casseday, 1986).

Further anatomical and physiological studies will be necessary to determine how connections, synaptic morphology, and membrane properties of different cell types act together to shape response properties in the VNLL (Huffman and Covey, 1995).

1.5.2 Implication of Physiological Response in Auditory Function

So far, most research has focused on the auditory function in bats. The finding that the VNLLc in the bat is composed almost exclusively of constant-latency responders has significance for echolocation. VNLLc neurons appear to function as a set of partially over-lapping broad-band filters, each of which gives an all or none signal that is perfectly correlated with the moment when a stimulus enters its range of sensitivity. The big brown bat uses an echolocation call that sweeps from high to low frequencies. The

duration and frequency parameters of the call vary depending on the bat's behaviour. For example, when the bat is actively pursuing an insect, the most intense behaviour, the first harmonic sweeps from about 60 kHz to 25 kHz in 3-5 msec (Simmons, 1989). One can predict the neural response to echolocation sounds based on testing of constant-latency neurons in the VNLLc using FM sweeps. Cells in any given projection sheet in VNLLc should fire at the moment the sweep enters the upper range of their tuning curves. Thus, there would be a successive ventral to dorsal activation of the sheets during the vocalized call. There would be a second wave of activation, in the same sequence, during the returning echo. The time delay between the response to the pulse and the response to the echo is proportional to the target distance. In this way, VNLLc may represent the timing input for the neural comparators for measuring target range (Covey and Casseday, 1991).

All chopper and most tonic neurons in the VNLLm possess characteristics that provide good temporal resolution: low spontaneous activity, broad frequency tuning, and short integration time. In addition, choppers are also precise markers of the onset of a sound. The fact that different neurons have different chopping rates raises the possibility that there are different channels, each with its own clock rate. It remains to be seen whether the rate of chopping is synchronized with some aspect of the stimulus. One possibility is amplitude and frequency modulations that are present in echoes from textured objects (Covey and Casseday, 1991).

Research has also been conducted in other species, although the function is not clear. For example, Batra and Fitzpatrick (2002) proposed that in the rabbit, the VNLL may be involved in binaural processing.

1.6 Synaptic Mechanisms for Coding Temporal Information in the Auditory Brainstem

To understand the significance of the VNLL in the temporal processing of auditory information, one should be familiar with the synaptic mechanisms involved in coding temporal information in other auditory brainstem nuclei. A number of studies have been conducted on the CN and MNTB of mammals and the nucleus magnocellularis (nMAG) of the chick and barn owl. This section will be a brief summary of these studies.

Neurons in the cochlear ganglion and auditory brainstem nuclei preserve the relative timing of action potentials passed through sequential synaptic levels. The precise timing of the response is essential to transmit the onset and frequency of an acoustic stimulus. As signals pass from synapse to synapse, conduction and synaptic delays accumulate. The processing of temporal information, requires that these delays be highly uniform (Trussell, 1999). Uniform latencies are achieved in part by the large size of the EPSP, which ensures that the variability in the timing or jitter in the timing is kept short (Zhang and Trussell, 1994). The shape of the EPSP is also fundamental to auditory timing circuits, since narrow EPSPs help minimize temporal summation and ensure a brief refractory period. EPSPs in auditory neurons must be brief in order to accommodate rapid transmission and minimize temporal distortion through synaptic networks (Trussell, 1999). Brief EPSPs can be achieved only by having short EPSCs, since the duration of the EPSC is the starting point for controlling the time course of EPSPs (Trussell, 1997).

There are various factors which contribute to the brevity and rapidity of EPSCs in auditory neurons. The electrical properties of bushy cells in the VCN, for example, are well-suited to carry precise timing information. Specifically, Oertel (1991) found that bushy cells have a short membrane time constant in the physiological range which allows for rapid synaptic responses and preserves the timing of suprathreshold inputs.

Potassium currents may also contribute to the brevity of EPSCs involved in synaptic responses of auditory neurons. Potassium currents seem to play a prominent role in the ability of auditory neurons to relay spike timing (Trussell, 1999). Outward rectifying currents are observed from several cell types in the cochlear nucleus and superior olivary complex. In these cells, the outward rectification is so strong that prolonged depolarizing current steps generate only a single, short-latency action potential (Golding et al., 1995; Smith, 1995). Outward rectification near the resting potential shortens the membrane time constant so that synaptic potentials are brief (provided that the synaptic currents are also brief), and temporal summation of subthreshold, converging inputs is minimized (Oertel, 1983). After EPSPs reach threshold, a short membrane time constant allows the membrane to return quickly to a ready state for the next stimulus (Trussell, 1997). Thus, when the cell is driven by brief current pulses, it can fire well-timed spikes at a high rate (Trussell, 1999).

There are also interactions among various ionic conductances in the bushy cells of the VCN which make responses to synaptic current brief. Specifically, bushy cells in the VCN have a low input resistance in the physiological voltage range (Oertel, 1991). One contributor to the low input resistance was found by Manis and Marx (1991) to be a

specific potassium conductance, a 4-aminopyridine (4-AP) sensitive low-threshold potassium conductance that is strongly and quickly activated by depolarization from the resting potential. This conductance causes voltage changes by current pulses to be smaller and more rapid in the depolarizing than in the hyperpolarizing voltage range (Trussell, 1997) and allows synaptic currents to produce rapid voltage changes. Responses to current pulses also revealed the presence of a conductance that is activated by hyperpolarization, a mixed-cationic conductance, I_h . This inward rectifier results in the membrane potential returning to rest (Manis and Marx, 1991). The interaction between the low threshold potassium conductance and the inward rectifier contributes to setting the resting potential and thus to the input resistance and to the temporal acuity of bushy cells (Oertel, 1997).

In addition, the low input resistance in the physiological voltage range which enables these cells to respond rapidly requires excitatory synaptic inputs of bushy cells to have large synaptic currents in order to be effective. Bushy cells receive their inputs from the auditory nerve through the large end bulbs of Held (Oertel, 1991). These endings are ideal for generating large synaptic currents (Ryugo and Sento, 1991).

1.6.1 Responses of Auditory Neurons to Repetitive Synaptic Stimulation

Research has shown that the precision of temporal processing in the auditory brainstem is reflected in part by the ability of various auditory neurons to respond faithfully to synaptic stimulation. Thus, it is worthwhile to address the research of neuronal responses to synaptic repetitive stimulation in the auditory brainstem.

In auditory nerves, the timing of sound is encoded primarily through phase-locking, whereby axonal firing is precisely timed to a particular phase of a sound stimulus (Trussell, 1997). For example, nMAG neurons, the avian homolog of the mammalian VCN, are specialized to phase-lock their firing with stimulus tones of up to 2000 Hz in chicks (Warchol and Dallos, 1990) and 9000 Hz in barn owls (Sullivan and Konishi, 1984) in order to preserve the timing of sound input from auditory nerve fibers (Zhang and Trussell, 1994). The ability to fire an action potential so precisely correlated with synaptic input implies that these neurons have specialized synaptic mechanisms (Raman and Trussell, 1992). At the calyceal synapse formed by auditory nerve fibers on nMAG neurons, glutamate activates postsynaptic AMPA receptors, a subtype of ionotropic glutamate receptors which are involved in mediating fast excitatory neurotransmission (Dingledine and McBain, 1999), generating a large and brief EPSP (Zhang and Trussell, 1994). Thus, at the calyceal synapse of the nMAG, the postsynaptic cell can reach spike threshold with minimal delay from trial to trial, preserving the timing of signals from the auditory nerve (Zhang and Trussell, 1994).

Responses to repetitive stimulation have also been studied, *in vitro*, in other auditory nuclei, such as the MNTB. Wu and Kelly (1993) found that neurons in the mouse MNTB were capable of following very high rates of repetitive stimulation. In their study, with current pulses delivered to the contralateral trapezoid body, MNTB cells responded 90% of the time to stimulation at rates of 667 Hz. Similar results were also obtained by repeated electrical stimulation of MNTB neurons in the anesthetized cat. For

example, Guinan and Li (1990) found that electrical stimulation of the contralateral trapezoid body evoked reliable responding in MNTB neurons at rates up to 500 Hz.

The membrane characteristics of neurons in the MNTB are well-suited for preserving the timing of afferent impulses from the cochlear nucleus. In both rat and mouse, MNTB neurons have non-linear current-voltage curves near resting potential (Wu and Kelly, 1991) reflecting a low input resistance near rest resulting in the maintenance of timing precision of incoming auditory inputs (Oertel, 1983). These cells also show a decrease in the membrane time constant (Wu and Kelly, 1993). Consequently, MNTB cells are resistant to temporal summation and are capable of following presynaptic events up to very high rates of stimulation (Wu and Kelly, 1993). The morphology of the synapse also contributes to the rapid responding of cells in the MNTB. The MNTB principal cells receive their major projection from bushy cells in the contralateral ventral cochlear nucleus and are contacted by the excitatory terminals of the Calyces of Held (Friauf and Ostwald, 1988; Jean-Baptiste and Morest, 1975) providing rapid and efficient synaptic transmission to the MNTB.

These results suggest that MNTB neurons are specialized for preserving the timing of stimulus events.

1.6.2 Synaptic Depression of Synaptic Responses to Repetitive Stimulation in Auditory Neurons

In response to stimuli delivered at rates of several hertz or higher, EPSCs in bushy cells and in cells of nMAG exhibit pronounced synaptic depression (Zhang and Trussell, 1994; Isaacson and Walmsley, 1996). Synaptic depression is a progressive decrease in

the amount of transmitter released during a train of action potentials. This phenomenon is often encountered when a synapse is stimulated at a high rate and may result from the depletion of neurotransmitter from the terminal (Levitan and Kaczmarek, 1997). Zhang and Trussell (1994) studied the activation of current-clamped neurons in the chick nMAG by eighth nerve stimulation in the brain slice preparation using patch electrodes. Synaptic depression was evident during trains of 200 Hz and above, such that EPSPs often fell below suprathreshold levels. Thus, during trains of stimuli at these frequencies, the rate of rise of suprathreshold EPSPs decreased during the train, indicating a reduction in the size of the synaptic currents. These results suggest that in the nMAG, at frequencies of 200 Hz or higher, synaptic depression of the EPSPs causes the spike to be initiated slightly later and with a lower probability.

One mechanism involved in synaptic depression in nMAG neurons appears to be the desensitization of AMPA receptors. Rapid synaptic currents are due to conformational changes in postsynaptic receptors that result directly from the energy of binding of the neurotransmitter. In the presence of an agonist, the conformation associated with the open, conducting state is not stable. Rather, the receptor undergoes a further change to a nonconducting or desensitized state (Trussell and Fischbach, 1989). The AMPA receptor desensitization may be due to delayed glutamate clearance from the synaptic cleft (Trussell, 1997). Due to the broad dimensions of the synaptic cleft at calyceal synapses of nMAG neurons, and the large number of vesicles released, clearance of glutamate from the synapse is delayed, resulting in persistent levels of glutamate in the synaptic cleft, requiring several milliseconds to be removed. A consequence of this

delayed clearance is receptor desensitization (Otis et al., 1996) which accounts for a significant amount of the depression observed during repetitive stimulation (Trussell, 1997). For example, Trussell and Fischbach (1989) studied the physiological role of desensitization in glutamate receptors by measuring responses of these receptors to rapidly applied glutamate in outside-out membrane patches from chick spinal neurons. Results showed that at concentrations of 1 mM glutamate, there was a rapid phase of desensitization which accounted for the initial decline of excitatory synaptic currents. The time constant for this rapid state was identical to the time constant of decay of synaptic currents, suggesting that glutamatergic synaptic currents are terminated or curtailed, in part, by glutamate receptor desensitization (Trussell, 1999).

Since nMAG and other auditory neurons are probably activated at a relatively high frequency *in vivo*, such depression must occur routinely. However, it seems paradoxical to desensitize postsynaptic receptors and depress the EPSP in a region that apparently performs a precise function. A possible adaptable function of desensitization may be to prevent cell death. For example, in the chick nMAG, the rapid kinetics of AMPA receptors is paralleled by a high calcium permeability through their associated ion channel, indicating that auditory activity results in calcium loading of postsynaptic cells (Otis et al., 1995). In this regard, desensitization after transmitter release may serve to limit postsynaptic calcium influx during chronic auditory activity, preventing cell death associated with excessive calcium loading of nMAG neurons (Zirpel et al., 1995).

1.6.3 Alternative Mechanisms Involved in Synaptic Depression

It should be noted that depression at the calyceal synapse may also have a presynaptic component, in that the number of quanta released per action potential falls during repetitive stimulation (Zhang and Trussell, 1994). This reduction in release appears to have several sources. As the probability of release is high at calyceal synapses (Barnes-Davies and Forsythe, 1995; Borst and Sakmann, 1996; Trussell et al., 1993), the number of quanta available for release may be rapidly depleted (Trussell, 1997). Also, glutamate released from synapses, such as the MNTB synapse during high-frequency stimuli, may feed back onto the nerve terminal and inhibit calcium channels and transmitter release through metabotropic glutamate receptors (Barnes-Davies and Forsythe, 1995; Takahashi et al., 1996). An additional source of depression may arise from the activation of GABAergic inputs. For example, activation of GABA_B receptors inhibit glutamate release in the nMAG (Otis and Trussell, 1996). For example, Otis and Trussell (1996) found that application of 100- μ M baclofen or 50- μ M GABA in the presence of a GABA_A receptor antagonist produced an 85% reduction of EPSCs, consistent with the activation of presynaptic GABA_B receptors. The reduction in EPSC amplitude by GABA or baclofen was accompanied by a 20% reduction in the exponential time constant of decay of the EPSC.

1.6.4 Role of NMDA Receptors in Temporal Processing During Repetitive Stimulation

In addition to the AMPA receptor, the NMDA receptor, another type of ionotropic glutamate receptor, may also mediate the time course of synaptic responses in the

auditory brainstem. For example, Ma et al. (2002) examined the synaptic mechanisms underlying excitation in the rat's ICC by making whole-cell patch clamp recordings in brain slice preparations of the auditory midbrain. Responses were elicited by single current pulse stimulation of the lateral lemniscus and recordings were made from the ICC. They found that excitatory postsynaptic responses consisted of two distinct components, an early brief component and a late slow component. Pharmacological manipulations revealed that only the early brief component was blocked by AMPA antagonists such as CNQX and only the slow late component was blocked by NMDA antagonists such as APV. Measurement of parameters such as rise time, decay time, time to peak and half-width showed that the time course and kinetics were much slower for NMDA than for AMPA receptor-mediated responses. These results indicate that excitatory responses in the ICC are mediated by two different receptor types: AMPA which mediates fast, brief synaptic responses and NMDA which mediates slower and longer responses.

With respect to repetitive synaptic stimulation in the auditory brainstem, NMDA receptors may determine how well a cell is able to follow repetitive stimulation. For example, Ma et al. (2001) studied postsynaptic responses of ICC neurons evoked by repetitive stimulation of the lateral lemniscus. Individual responses to repetitive pulses at rates less than 50 Hz could be observed, but were smaller for later pulses. Also, there was a large, long depolarization which emerged throughout the period of the response, obscuring individual responses, particularly at high rates of stimulation. However, blockade of NMDA receptors resulted in a smaller depolarization and more discernible

individual responses. These findings suggest that NMDA receptor mediated temporal summation may limit the ability of the cell to accurately follow repetitive stimulation.

Also, when Zhang and Trussell (1994) studied the activation of chick nMAG neurons by eighth nerve stimulation, they found that stimulation of presynaptic fibers at rates of 100-500 Hz for brief periods produced a series of stimulus-locked orthodromic spikes where there was a plateau formed by the overlap of successive slow EPSPs. Application of APV increased the decay rate following the train and slightly reduced the amplitude of the plateau. Thus, NMDA receptors generated a gradual increase in the plateau during the train.

Therefore, it can be suggested that since NMDA receptors mediate synaptic responses with a slow time course and kinetics, a successive overlap or summation of these slow potentials during repetitive stimulation would impair the ability of the cell to precisely follow repetitive synaptic stimulation.

1.6.5 Latency Variability or “jittering” in Repetitive Stimulation

An interesting phenomenon observed in repetitive stimulation is the variation in action potential latency, i.e., jitter. Zhang and Trussell (1994) examined the precision of timing of orthodromic spikes at different frequencies of stimulation in nMAG neurons of the chick. The variation in the timing, or jitter of the spike, was assessed by measuring the standard deviation of the response latency, from the beginning of the stimulus artifact to the peak of the spike. They found that at low frequencies (for example, 1 Hz in their study) the orthodromic spikes for successive stimuli were consistent in their timing. At

higher frequencies (e.g. 10 Hz), the spikes had more variability. At frequencies of 33.3 and 50 Hz, an increase in jitter was accompanied by an increase in response latency. At even higher levels, (e.g. 100 Hz and 200 Hz), the latency and the jitter increased markedly just before spikes began to fail. From these results, the authors concluded that in nMAG neurons, the highest precision in synaptic transmission by single auditory nerve fibers only appeared to be maintained for low-frequency stimuli (1 Hz). As described above, at higher stimulus rates there is a gradual increase in jitter, response latency, and number of spikes which fail to present. The shift in latency and failure of spike presentation is consistent with a progressive synaptic depression, possibly due to AMPA receptor desensitization, during the course of the stimulus train.

2. PURPOSE OF PRESENT STUDY

2.1 Purpose 1: Synaptic Pharmacology of VNLL Neurons

In order to understand how the VNLL integrates and processes incoming information, we need to know the types of synaptic receptors which mediate synaptic excitation and inhibition. Thus, one purpose of the present study was to examine first the synaptic pharmacology of VNLL neurons by applying both excitatory and inhibitory synaptic receptor antagonists and observe their influence on synaptic responses. The correlation between the type of firing pattern, and the synaptic response pattern and synaptic receptor type was also studied. Specifically, pharmacological manipulations involved the application of inhibitory synaptic receptor antagonists, such as strychnine (a glycine receptor blocker) and bicuculline (a gamma-aminobutyric acid (GABA) receptor

blocker) as well as excitatory glutamate receptor antagonists such as 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) (an α -amino-3-hydroxy-5-methylisoxazole-4 propionic acid (AMPA) receptor blocker) and 2-amino-5-phosphono-valerate (APV) (an N-methyl-D-aspartic acid (NMDA) receptor blocker). These pharmacological manipulations may provide some indication as to what types of synaptic receptors are involved in the synaptic transmission of VNLL neurons. By dissecting different components of the synaptic response, the convergence of synaptic inputs and the interaction between them can also be demonstrated. The results will give us an insight into the mechanisms of synaptic transmission and integration in the VNLL.

2.2 Purpose 2: Limits of Responses in VNLL Neurons to Follow Repetitive Stimulation

Some neurons in the auditory brainstem preserve incoming temporal information through precise and accurate timing mechanisms. Several studies of auditory nerve fibers in the cat and monkey (Kiang et al., 1965; Rose et al., 1967) have shown that auditory nerve fibers are synchronized to the stimulation period of pure tone in a very accurate fashion for frequency of up to 2 kHz. Many neurons in the cochlear nucleus also lock their discharges to stimulus periodicity (Joris et al., 1994). This feature allows the timing of low-frequency sounds to be precisely encoded (Trussell, 1997). In addition, auditory neurons need to display precise temporal mechanisms in order to effectively locate the source of sound (Trussell, 1999). For a better understanding of how VNLL neurons preserve timing information, we need to know what their ability to follow repetitive stimulation is. We examined the limits of postsynaptic responses to repetitive stimulation

and how these responses followed repetitive stimulation precisely. The relationship of neuronal discharge pattern and the responses to repetitive stimulation was also studied. We attempted to apply excitatory and inhibitory synaptic receptor antagonists to study how different excitatory and inhibitory synaptic receptors shaped responses to repetitive stimulation.

2.3 Purpose 3: “Jittering” in the VNLL

Although consistent timing is necessary for auditory neurons to transmit the onset and frequency of acoustic information (Trussell, 1999), variabilities in response latency, i.e., jitter introduced by transmission across multiple synapses, also occur (Trussell, 1999). Jittering has been observed in auditory neurons in response to repetitive stimulation. Zhang and Trussell (1994) found that in chick nMAG neurons, there was an increase in response latency variability of spike presentation at higher frequencies. In order to better understand how VNLL neurons process temporal information we also need to know about jittering in VNLL neurons. Thus, the third purpose involved studying the extent of action potential latency variability of VNLL neurons in response to repetitive synaptic stimulation. The relation between the extent of jittering and the neuronal discharge pattern was also studied. Pharmacological manipulations in these experiments were conducted to investigate contributions of various synaptic receptors to jittering.

3. MATERIALS AND METHODS

3.1 Subject and Slice Preparation

Brain slices were obtained from young albino rats (Wistar strain, Charles River Co., Quebec, Canada) between the ages of two to three weeks. The rats were first anesthetized by inhaling halothane gas in an isolated delivery chamber for approximately 60 - 90 seconds, and then euthanized by decapitation. The brain was then removed and submerged in a blocking dish containing warm (approximately 32°C) oxygenated normal artificial cerebrospinal fluid (ACSF). The ACSF consisted of (in mM): 129 NaCl, 3 KCl, 1.2 KH₂PO₄, 2.4 CaCl₂, 1.3 MgSO₄, 20 NaHCO₃, 3 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 10 glucose in distilled water, saturated with 95% O₂ - 5% CO₂. The pH was maintained at about 7.4 after complete saturation with O₂-CO₂. The auditory midbrain was blocked at an angle of approximately 30 ° in the frontal plane and mounted on a stage of a tissue slicer using cyanoacrylate adhesive.

Brain slices of 400 µm were taken in the frontal plane through the VNLL using the tissue slicer. A slice containing the IC, DNLL, INLL and VNLL was selected on the basis of structural clarity of the VNLL (see Fig. 2). This slice was placed in a recording chamber, held securely in place by two pieces of nylon mesh, and perfused by oxygenated ACSF. The remaining slices were placed in a beaker with oxygenated ACSF and submerged in a water bath, where the temperature was maintained at approximately 30°C. These slices were kept as a precautionary measure should the slice selected for recording need to be replaced.

With regards to the slice placed in the recording chamber, the ACSF circulated

continuously through the recording chamber and around the brain slice during the entire course of the experiment. The volume of the chamber was kept at approximately 0.6 ml and the flow rate was maintained at 8 to 10 ml/min. The temperature of the ACSF in the chamber was kept at about 34°C by a heating element. The slice was perfused in the recording chamber for approximately one hour before any recordings were made.

3.2 Experimental Protocol

3.2.1 Intracellular Recording

The brain slice was illuminated from below the recording chamber by light passing through a darkfield condenser. The IC, DNLL, INLL and VNLL were visible with a Leitz dissecting microscope. Intracellular recordings were made with glass micropipettes. The pipettes were made from 1.0 mm outer diameter (OD) and 0.5 mm inner diameter (ID) standard-walled capillaries with microfilament (Borosilicate with filament, Sutter Instruments Co.). These micropipettes were pulled with a Flaming/Brown micropipette puller (P-80/PC) and filled with 4 M potassium acetate. The electrode impedance was kept between 130 and 180 M Ω . Once the electrode was positioned over the recording area, it was inserted into the VNLL under direct visual control. The pipette then advanced in 1- μ m steps through the brain slice with a Burleigh piezoelectric driver (Inchworm). Intracellular penetration was facilitated by an oscillating current pulse through the recording pipette. Recordings were made with an Axon Instruments Axo-probe 1-A amplifier.

Intracellular current injection was used to examine the cell's intrinsic membrane

properties such as cell membrane input resistance, time constant and neuronal firing pattern through programs designed to produce current-voltage relations. Data acquisition and current injection protocols were under computer control using Pclamp6 software. The protocol consisted of collecting responses to a series of current pulses of 100 ms (short I-V curve) and 400 ms (long I-V curve) between -1 and 1 nA in 0.1 nA steps. The cells with a sustained firing pattern were also injected by prehyperpolarizing current (-0.6 nA) of 300 ms followed by depolarizing current of 300 ms between 0.1 nA and 1.0 nA in 0.1 nA steps to determine the pauser firing pattern.

For the recording of synaptic responses from VNLL neurons, a bipolar stimulating electrode made of insulated tungsten wires with a tip separation of approximately 50 μm , was placed on the lateral lemniscus just ventral or medial to the VNLL and in close proximity to the recording electrode.

Synaptic responses were evoked by 0.1 ms square wave pulses, obtained from a Grass S-8800 stimulator and delivered through an isolation unit at a repetition rate of one stimulus per second. The data was stored with Pclamp6 acquisition system and analyzed with Pclamp8 software. When a synaptic response was encountered, the stimulus intensity was gradually increased. Graded postsynaptic potentials and suprathreshold responses were recorded.

With repetitive stimulation of the lateral lemniscus, suprathreshold responses were recorded for 100 or 200 pulses ranging from repetition rates of 10 Hz to 250 Hz. Frequency of stimulation was controlled by manipulating the pulse interval. Following the recording of neuronal responses to repetitive stimulation at a particular repetition rate, the single pulse

response was then recorded at the same stimulus intensity to ensure that the response threshold did not change during the course of repetitive stimulation.

3.2.2 Pharmacology

Following the recording of intrinsic membrane properties and responses to single pulse and repetitive stimulation in normal ACSF, pharmacological manipulations were conducted. The effects of excitatory and inhibitory synaptic receptor antagonists on responses to single pulse and, if possible, on repetitive stimulation were studied. With regards to responses to single pulse synaptic stimulation, if excitatory synaptic responses were observed, the excitatory synaptic receptor antagonists, APV (an NMDA synaptic receptor antagonist) and CNQX (an AMPA synaptic receptor antagonist) were applied by bath application. APV (50 μ M - 100 μ M) was perfused onto the slice first to investigate the contribution of NMDA receptor mediated excitation to synaptic responses in the VNLL. CNQX (10 μ M-20 μ M) was then added to APV to study any AMPA receptor mediated excitation. The rationale for this particular order was justified by the notion that the NMDA receptor is voltage-dependent (Levitan and Kaczmarek, 1997). Therefore, if the AMPA receptor were blocked first, this may prevent the full expression of the NMDA receptor-mediated response. Thus, APV was applied before CNQX. If inhibitory synaptic responses were observed, 1 μ M strychnine (a glycine synaptic receptor antagonist) and/or 10 μ M bicuculline (a GABA_A synaptic receptor antagonist) was perfused onto the slice preparation to study the contribution of any glycinergic and/or GABAergic mediated inhibition. If the cell

was robust after pharmacological manipulations to single pulse synaptic stimulation, the effects of pharmacology on responses to repetitive synaptic stimulation were also studied.

Blocking effects by CNQX, APV, strychnine and bicuculline usually took place a few minutes after drug application in a recording chamber and with a perfusing rate similar to what were used in the previous studies (Wu and Kelly, 1992, 1996). In this study, to ensure a reliable drug effect, there was a significant time lapse after drug application. For strychnine, a 1 μM concentration was used and a time lapse of approximately 5-10 min was observed. For bicuculline, a 10 – 20 μM concentration was perfused onto the slice, and a time lapse of approximately 5-10 min was used. For APV, a 50 – 100 μM concentration was used, and a time lapse of approximately 10-20 min, possibly longer, occurred. For CNQX, a 10 – 20 μM concentration was used and a time lapse of approximately 10-20 min was observed.

3.3 Data Analysis

The analyses were performed using pClamp8 software.

The input resistance (R_{input}) of the cell membrane, measured in megaohms ($\text{M}\Omega$), was obtained by calculating the slope, from a range of values that were approximately linear, of the current strength and change in membrane potential elicited by intracellular injection of negative current. The change in membrane potential was obtained by measuring the baseline membrane potential and the peak of hyperpolarization. The time constant (τ) of the cell membrane from these neurons was obtained by measuring the time it took the membrane potential to move 63 % of the way towards its final value in response to a hyperpolarizing

current pulse. Group data were expressed as means and standard errors.

The neuronal discharge pattern of each cell was determined by responses to injection of depolarizing and hyperpolarizing current. The criteria for identification of onset, regular, adapting, pauser and bursting patterns were the same as those set by Wu (1999) and Zhao and Wu (2001).

With reference to synaptic pharmacology, there were two indicators used to study the drug effects on excitatory responses: *total block* and *no effect*. A total block of a synaptic response by an excitatory receptor antagonist was defined as the elimination of the response at the same stimulus intensity used before drug application. In order to ensure that the response to the drug was not due to changes in threshold levels, pharmacological responses at slightly higher stimulus intensities were also observed. No effect was observed when the response remained unchanged after drug application. To be certain that a drug had no effect, this conclusion was arrived at after a time lapse of at least 15-20 minutes following excitatory synaptic receptor antagonist application. For studying the effects of pharmacology on inhibitory responses, a *partial block* indicator was used in addition to that of total block and no effect. A partial block of the synaptic response by an inhibitory receptor antagonist was defined as the reduction in the amplitude of the response at the same stimulus intensity used before drug application. The criterion for total block was the same as that used for studying the excitatory responses. To be certain that a drug had no effect or a partial blocking effect, this conclusion was arrived at after a time lapse of at least 15-20 minutes following inhibitory receptor antagonist application.

The suprathreshold response to repetitive synaptic stimulation to 100 or 200 stimulus pulses for a particular repetition rate was illustrated in a scatterplot. In the scatterplot, the ordinate represented the latency of the spike, which was obtained by measuring the time between the beginning of the stimulus artifact to the peak of the spike, and the abscissa represented the order of the stimulus pulse in the pulse train. Thus, each point on the scatterplot represented the occurrence time of a spike. Three main parameters of the suprathreshold response to repetitive stimulation were studied: firing probability, shift in response latency and jittering.

The firing probability of the neuron in response to 100 or 200 stimulus pulses at different repetition rates was studied. The firing probability was defined as the ratio between the number of spikes and the number of pulses in the pulse train. A 50% cut-off repetition rate (i.e., the repetition rate at which a neuron showed a firing probability of 50%) was used to describe a neuron's ability to follow high repetition rates.

The shift in response latency over the train of 100 or 200 stimulus pulses was also studied and the slope of the linear regression line for the spike latency scatterplot was used to describe the trend of latency shift.

Since some neurons showed a more apparent change in their response latency for the initial spikes than for the entire stimulus train, the slope of the regression line based on the responses to the first 30 pulses was also calculated. A comparison of these slopes were also made across different repetition rates.

Jitter was examined by comparing the spike latency of responses to 100 or 200 pulses.

Jitter of spike latency of responses over the entire train of pulses was quantitatively represented by the standard deviations of the latencies of spikes elicited by every 10 consecutive pulses.

4. RESULTS

4.1 Intrinsic Membrane Properties of VNLL Neurons

Intracellular current injection through the recording electrode was used to investigate the cell's intrinsic membrane properties such as cell membrane input resistance, time constant and neuronal firing pattern. A total of 79 neurons were studied, where each cell had a resting membrane potential of at least -50 mV (mean = -59.6 ± 0.8 mV). The mean of the input resistance (R_{input}) was -37.2 ± 2.8 M Ω and the mean of the membrane time constant (τ) was 4.4 ± 0.4 ms.

4.1.1 Neuronal Discharge Patterns

Intracellular depolarizing current was injected through the recording electrode into VNLL neurons in order to study the neuronal temporal discharge patterns reflecting the intrinsic membrane properties of the cells. In response to positive intracellular current injection, five types of firing patterns were observed: Pauser, Adaptation, Regular, Burst and Onset.

Pauser Firing Pattern:

Fig. 3 shows an example of a pauser discharge pattern. This type of pattern was observed in 21/79 (26.5%) cells. In response to depolarizing current pulses, multiple action potentials were observed (Fig. 3A, 1-2). The number of action potentials increased with increasing current strength. Injection of negative current led to a hyperpolarization of the cell membrane (Fig. 3A, 3-4). More hyperpolarization was produced by more negative current injection. A prehyperpolarizing current pulse following a depolarizing

current injection was used to identify the pauser pattern (Sivaramakrishnan and Oliver, 2001) (Fig. 3B, 1-3). The pause was observed at the onset of the depolarizing current pulse for low current (Fig. 3B (3)). As the current strength increased, an action potential was observed at the onset of current injection followed by a pause which became progressively shorter with increasing current strength (Fig. 3B, 1-2). The pause was followed by sustained firing (Fig. 3B, 1-3).

Adaptation Firing Pattern:

Fig. 4 is an example of an adaptation firing pattern. There were 36/79 (45.6%) cells which displayed adaptation. This pattern showed action potentials being more frequent during the onset of the depolarizing current injection, but progressively diminishing during the remaining current injection. The number of action potentials increased with increasing current strength (Fig. 4A, 1-2). This discharge pattern was identified when the ratio of the last interspike interval (ISI) to the initial or subsequent ISI was larger than 1.2 (Wu, 1999). Injection of negative current showed hyperpolarization of the cell membrane (Fig. 4A, 3-4). Larger negative current injection produced more hyperpolarization. Since the pauser cell may display a pattern similar to adaptation in response to depolarizing current pulses, the present cell's response to depolarization following prehyperpolarization was observed. With this manipulation, the cell showed a firing pattern different from the pauser discharge pattern (Fig. 4B, 1-3). Specifically, there was no pause observed at the onset of current injection for low current (Fig. 4B (3)). Also, higher depolarizing current injection did not elicit the pattern of a single action

potential being followed by a pause (Fig. 4B, 1-2). Thus, this cell was identified as being different from a pauser.

Regular Firing Pattern:

Fig. 5 shows a neuron with a regular firing pattern. This pattern was observed in 10/79 (12.6%) cells. The regular neuron showed a train of action potentials with consistent interspike intervals produced throughout the entire period of depolarizing current injection. The regular neuron also showed increasing firing frequency with increasing current (Fig. 5A, 1-2). The cell showed hyperpolarization of the cell membrane in response to negative current injection (Fig. 5A, 3-4). More hyperpolarization was produced by higher negative current injection. As was the case for adaptation neurons, pauser cells may also show regular firing in response to depolarizing current injection. Fig. 5B, 1-3 is the response of this cell to depolarization of the cell membrane following a prehyperpolarizing current pulse. This cell type also displayed a pattern different from the pauser. Sustained firing, without any pause, was observed for all levels of depolarizing current injection (Fig. 5B, 1-3) distinguishing it from the pauser pattern.

Burst Firing Pattern:

Fig. 6 illustrates a neuron with a burst firing pattern. This pattern was rare in the VNLL since it was observed in only 4/79 (5.1%) cells. Burst cells were characterized by a short burst of action potentials overriding a slow depolarization at the onset of a

depolarizing current injection (Fig. 6, 1-2). Inset 1 and 2 show a more detailed view of the burst of action potentials on the slow depolarization. Fig. 6, 3-4 shows the response of the cell to negative current injection where more negative current produced a greater hyperpolarization.

Onset Firing Pattern:

Fig. 7 shows a neuron with an onset firing pattern. Only 8/79 (10.1%) cells showed this pattern. Onset cells typically displayed a single action potential at the onset of depolarizing current injection (Fig. 7, 1-2). Injection of negative current led to hyperpolarization of the cell membrane where a greater hyperpolarization was produced by more negative current (Fig. 7, 3-4). In addition, with the onset cell, an asymmetrical pattern was observed in the change of membrane potential between depolarizing and hyperpolarizing currents for the same absolute current strength. For example, Fig. 7 (1) shows the response to depolarizing current injection of 0.6 nA and Fig. 7 (4) shows the response to hyperpolarizing current injection of -0.6 nA. An asymmetry was observed between the change of membrane potential from baseline to the plateau of depolarization (Fig. 7 (1)) and the change of membrane potential from baseline to the peak of hyperpolarization (Fig. 7 (4)), respectively. The same observation was noted for responses to depolarizing and hyperpolarizing current of 0.4 nA and -0.4 nA respectively (Fig. 7, 2-3).

4.1.2 Summary

There were five different firing patterns observed in response to depolarizing intracellular current injection in VNLL neurons: Pauser, Adaptation, Regular, Burst and Onset. These findings were consistent with results from previous studies with intracellular and whole-cell patch clamp recordings (Wu, 1999; Zhao and Wu, 2001). All cells except the burst and onset types showed a sustained firing pattern in response to depolarizing current injection. With respect to the sustained firing neurons, the pauser cell was identified by the pause pattern in response to a depolarizing current pulse following prehyperpolarization. The regular and adaptation cells did not display this pattern in response to depolarization following prehyperpolarization.

4.2 Synaptic Physiology of VNLL Neurons

Synaptic responses of physiologically identified VNLL neurons, i.e., pauser, adaptation, regular, burst and onset cells, were evoked by electrically stimulating the lateral lemniscal fibers just ventral or medial to the VNLL. Table 1 shows the distribution of excitatory and inhibitory synaptic responses for each cell type in the VNLL.

Pauser Cell Type

Pauser cells displayed a higher proportion of excitatory post-synaptic potentials (EPSPs) only (15/21) as compared to inhibitory post-synaptic potentials (IPSPs) only (2/21) or both EPSPs and IPSPs (4/21) (Table 1). Fig. 8 shows a pauser cell displaying a graded excitatory synaptic response from lower to higher stimulus intensities. The cell

showed a small EPSP at low intensity (Fig. 8D). At higher intensity, a double-peaked EPSP with an early component followed by a later long duration component was elicited (Fig. 8C). A suprathreshold response, i.e., action potential, was also obtained at the same intensity (Fig. 8B). This suprathreshold response showed a shift in latency as the stimulus level was increased (Fig. 8A). Fig. 9 also shows a graded excitatory synaptic response of a second pauser neuron from subthreshold (Fig. 9B) to suprathreshold (Fig. 9A) as stimulus intensity is increased. This neuron showed only one short duration component in the EPSP. Fig. 10 is an example of a third pauser neuron showing both an EPSP and IPSP. At low intensity, the cell showed a predominant IPSP preceded by a small EPSP (Fig. 10C). As stimulus intensity increased, the EPSP became more apparent (Fig. 10A, 10B). However, the EPSP was truncated by the IPSP, and therefore became very short (less than 5 ms).

Adaptation Cell Type

Neurons of adaptation type showed a greater proportion of EPSPs only (26/36) than IPSPs only (5/36) and both EPSPs and IPSPs (5/36) (Table 1). Fig. 11 shows a graded excitatory synaptic response from subthreshold to suprathreshold as stimulus intensity was increased (Fig. 11A-11C). Fig. 12 is an example of a second adaptation neuron showing a graded inhibitory synaptic response from low to high stimulus levels (Fig. 12A – 12C). Fig. 13 shows a third adaptation neuron displaying both excitatory and inhibitory synaptic responses. At low stimulus intensity, the cell showed a small EPSP (Fig. 13D). This response became suprathreshold as the intensity increased (Fig. 13C).

Further increases in stimulus levels revealed a predominant IPSP preceded by a small EPSP, where the IPSP became larger with higher intensity (Fig. 13A, 13B). Thus, the suprathreshold response was suppressed since an underlying IPSP became more dominant with increasing intensity (Fig. 13B).

Regular Cell Type

The regular cell types showed a higher proportion of EPSPs only (8/10) than IPSPs only (1/10) and both EPSPs and IPSPs (1/10) (Table 1). Fig. 14 is an example of a regular neuron displaying excitatory responses. At low stimulus levels, a long duration EPSP was observed (Fig. 14C, 14D). As intensity was increased, a double peaked EPSP displaying an early fast component followed by a late slow component occurred (Fig. 14B). Further increases in stimulus intensity produced an action potential from the late component (Fig. 14A). Fig. 15 shows a second regular cell with an inhibitory response. The amplitude of the IPSPs increased with increasing voltage intensity (Fig. 15A – 15C). Fig. 16 is an example of a third regular cell showing both inhibitory and excitatory responses. A small IPSP occurred at low intensity (Fig. 16E). Increasing intensity revealed a graded excitatory component only (Fig. 16C, 16D). A higher stimulus level showed an EPSP with an early fast component followed by a late slow component (Fig. 16B). Suprathreshold responses were elicited from both early and late components as the intensity was increased (Fig. 16A).

Burst Cell Type

This cell type displayed only EPSPs (4/4) (Table 1). There was no evidence of IPSPs in this cell type. Fig. 17 shows graded EPSPs (Fig. 17C, 17D) ultimately resulting in double action potentials overriding on a slow depolarization as stimulus intensity increases (Fig. 17A, 17B). Fig. 18 displays a similar pattern from another burst cell.

Onset Cell Type

There was a higher proportion of onset cells showing IPSPs only (5/8) than both EPSPs and IPSPs (3/8) (Table 1). There was no evidence of EPSPs only in this cell type. Fig. 19 shows a graded inhibitory response of an onset neuron from low to high stimulus intensity (Fig. 19A – 19D). At all stimulus levels tested, a very small EPSP occurred following a predominant IPSP (Fig. 19A-19D). Fig. 20 shows graded inhibitory synaptic responses of another onset neuron (Fig. 20A – 20C).

4.2.1 Summary

A summary of the distribution of excitatory and inhibitory synaptic responses for each cell type in the VNLL is shown in Table 1. With the exception of the burst cell type, all cell types responded to stimulation of the lateral lemniscus with excitatory and inhibitory synaptic potentials. However, regular, adaptation, and pauser cells displayed a higher proportion of EPSPs only than IPSPs only or both EPSPs and IPSPs. Onset cells showed a higher proportion of IPSPs only than both EPSPs and IPSPs. It should be noted that the failure to show one kind of postsynaptic potential may be due to the difference in

threshold for EPSPs and IPSPs. In other words, a cell showing an EPSP only or IPSP only in normal saline may also have underlying IPSPs or EPSPs which may not be observed. Also, these synaptic responses were observed in normal saline without any pharmacological manipulations.

It should be noted that there were a number of neurons which revealed alternate excitatory or inhibitory responses following pharmacological application. For onset cells, one neuron which showed an IPSP only in normal saline, displayed an EPSP after the IPSP was blocked with drug application, resulting in a total of 4 neurons displaying both EPSPs and IPSPs. For regular cells, there was one neuron which showed an EPSP only in normal saline, however may have had an underlying IPSP, since this neuron showed a decrease in the threshold for action potential initiation after inhibitory receptor antagonist application. For adaptation cells, there were 3 neurons that showed EPSPs only in normal saline, but revealed IPSPs after the excitation was pharmacologically blocked. In addition, there were 4 adaptation neurons in which IPSPs only were observed in normal saline, but displayed excitatory responses after application of inhibitory antagonists. Thus, there were a total of 12 adaptation neurons which displayed both excitatory and inhibitory responses. In pauser neurons, there were 3 cells which showed EPSPs only in normal saline, however showed IPSPs after the EPSPs were pharmacologically blocked, and there was one neuron which had originally displayed an IPSP only before pharmacology, but revealed an EPSP after the inhibition was blocked. Therefore, there were a total of 8 pauser neurons which displayed both EPSPs and IPSPs.

Since both EPSPs and IPSPs could be elicited by lateral lemniscal stimulation in most cell types, synaptic responses in the VNLL essentially reflected a combination of synaptic responses through the probable convergence of various synaptic effects onto single VNLL neurons.

In order to dissect excitatory and inhibitory postsynaptic potentials, and the different components of the synaptic response, pharmacological manipulations were necessary. The next section will focus on the synaptic pharmacology of VNLL neurons. Various synaptic receptor antagonists were applied to most of the neurons from which stable recordings were made in an attempt to isolate the synaptic response that was mediated by a particular type of synaptic receptor. The data from neurons with pauser, adaptation, regular and onset firing patterns were presented together as these cell types all display both EPSPs and IPSPs.

4.3 Synaptic Pharmacology of VNLL Neurons

4.3.1 Introductory Comments

For excitatory responses, two components were studied by pharmacological manipulations. An early component of the EPSP was usually represented by a time course of 20 ms or less. A late component of the EPSP was usually represented by a time course of 40 ms or more. It must be noted, however, that this duration criteria sometimes varied with the cell. Although in most instances, the aforementioned criteria could apply, in some cells the late component showed a duration much shorter than 40 ms and the

early component was shorter than 10 ms. The NMDA and AMPA receptor antagonists APV and CNQX were applied to distinguish these two components.

The presentation of results in figures preserves the sequence in which data were obtained from individual neurons. In most cases, APV was applied before CNQX, however in one instance, CNQX was applied before APV. In other instances, data was obtained from the application of only one antagonist, since this antagonist completely blocked the response. When IPSPs were observed, the inhibitory synaptic receptor antagonists, bicuculline and strychnine were applied. The order of drug application for the inhibitory synaptic receptor antagonists was not always constant. In some instances, inhibitory receptor antagonists were applied when EPSPs were observed in order to study the threshold of the EPSP. This manipulation was used to show an effect of the antagonist on the excitatory response through the inhibitory response.

4.3.1.1 Synaptic Excitation:

Most cells sampled in this study showed both early and late EPSP components. These two components were elicited usually at the same voltage intensity, indicating similar thresholds. In other cells, one component was elicited at a different stimulus intensity than the other, reflecting different thresholds. Table 2 shows the pharmacological effects of CNQX and APV on the early and late EPSP components in all VNLL cell types. It should be noted that the values represented in Table 2 are not mutually exclusive. For example, there is an overlap between the values represented by “no effect on the early EPSP by APV” and “total block on the late EPSP by APV”. The

results showed that CNQX totally blocked the early EPSP component in 14/15 cells tested. Only one neuron showed no effect of CNQX on the early EPSP. APV totally blocked the late EPSP component in 9/13 cells tested. Only one neuron showed no effect of APV on the late EPSP component. There were 10/13 cells, where APV showed no effect on the early EPSP component. Since APV was applied before CNQX in all but one case, the effect of CNQX on the late component could not be tested. In the one neuron where CNQX was applied before APV, there was no effect of CNQX on the late EPSP component. In 2/13 neurons, APV blocked the early EPSP component. However, in these two cells, CNQX was not applied and thus it cannot be concluded that the AMPA receptor did not contribute to the EPSP. Also, in one of these cells, an IPSP was observed possibly suppressing a long component.

Fig. 21 is an example of a neuron where the early EPSP component was first blocked by CNQX and the late EPSP component was then blocked by APV. Fig. 21A shows an early short EPSP in normal saline. After CNQX application, the EPSP was blocked (Fig. 21B). As the stimulus intensity was increased, a large IPSP was observed (Fig. 21C). When strychnine was applied, the IPSP was blocked and a late long EPSP was revealed (Fig. 21D). Application of APV totally blocked the long EPSP (Fig. 21E).

Fig. 22 shows an example of a neuron where the late component was first blocked by APV and the early component was then blocked by CNQX. Fig. 22A illustrates the cell with an early and late EPSP component in normal saline. After APV was applied, the late component was greatly suppressed (Fig. 22B) leaving the early component. Application of CNQX then totally blocked the early component (Fig. 22C).

Fig. 23(A,B) shows an example of a neuron with a short EPSP showing no blocking effect of APV application. Fig. 24 shows the pharmacological effects of APV on the late EPSP. A double-peaked suprathreshold response overriding an early and late component was elicited with the brain slice perfused in bicuculline and strychnine solution (Fig. 24A). Application of APV eliminated the late suprathreshold response, but had no effect on the early response (Fig. 24B). Fig. 25 is an example of a cell showing complete block of the early EPSP after CNQX application. The cell showed an early suprathreshold action potential in normal saline (Fig. 25A). After CNQX was applied, the excitatory response was totally blocked (Fig. 25B).

There were 10/18 neurons which showed both early and late components. In 9/10 of these neurons, the early EPSP could be blocked by the AMPA receptor antagonist, CNQX, and the late EPSP could be blocked by the NMDA receptor antagonist, APV. Also, in these cells, APV did not have any effect on the early EPSP component.

In addition to the neurons showing both early and late EPSP components, some other neurons showed only an early short duration EPSP (8/18). All of these cells in which CNQX was applied (5/8) were completely blocked by CNQX. In 4/5 of these cells showing an early EPSP block by CNQX, an IPSP was also observed, thus possibly obscuring a long EPSP component. Therefore, these additional 4 neurons may also have had both early and late components.

In three other cells, which showed an early short duration EPSP, APV only was applied and blocked the short EPSP in two cases, but had no effect in one case. In one of the two instances where APV blocked the short EPSP, an IPSP interacting with the EPSP

was also observed. Therefore, it is possible that this short EPSP was actually a longer duration excitatory response.

4.3.1.1.1 Comparison Among Cell Types for Synaptic Excitation

A comparison among Pauser, Adaptation, Regular and Onset cells with regards to excitatory neurotransmission could be made only after pharmacology since the different components of the synaptic response were isolated after application of specific antagonists. Pauser and Adaptation cells comprised the two major groups for comparison, since these cell types had a sufficient number of neurons on which pharmacological manipulations were performed. Pharmacology was performed on only one regular and one onset neuron. There were 10 neurons which showed an early and late EPSP component. From these neurons, 3 pauser neurons, 5 adaptation neurons and one onset neuron showed a block of both early and late components by CNQX and APV respectively. There was no blocking effect of CNQX and APV on either early or late components for the regular neuron. There were 8 additional neurons which showed only an early EPSP. From this group, there were 3 pauser cells which showed a total block by CNQX and 1 pauser cell which was blocked by APV. With respect to the adaptation cell type, there were 2 short duration neurons which were blocked by CNQX, 1 which was blocked by APV and 1 which showed no effect after APV application. Thus, there were no major differences found among the cell groups with regards to excitatory neurotransmission.

4.3.1.2 Synaptic Inhibition:

Table 3 shows the effects of the inhibitory synaptic receptor antagonists, strychnine and bicuculline on the IPSPs. Strychnine was applied in isolation for 9 neurons. In these cells, 7 neurons showed a complete block of their IPSPs. Two cells showed a partial block of the IPSP since there was only a reduction in the amplitude of the IPSP. Bicuculline was applied in isolation in 4 neurons. In three of these cells, the IPSPs were completely blocked and one cell showed a partial block. A combination of strychnine and bicuculline was used on 3 neurons, where all of these cells showed a complete block of the IPSP.

Fig. 26 shows the pharmacological effects of strychnine on an IPSP. It should be noted that prior to CNQX application, the cell showed an EPSP in normal saline. CNQX application revealed a large IPSP (Fig. 26A). The cell showed a complete block of the IPSP after strychnine application (Fig. 26B). Fig. 27 is an example of a cell being blocked after bicuculline application. This cell displayed an IPSP in normal saline (Fig. 27A). After application of bicuculline, the IPSP was completely blocked and a small EPSP was revealed (Fig. 27B). The IPSP returned after postwash (Fig. 27C). Fig. 28 is an example of a cell showing partial block by one of the antagonists. In normal saline, a large IPSP was observed (Fig. 28A). After strychnine application, the IPSP was partially blocked, and a small EPSP preceding the IPSP was revealed (Fig. 28B). The IPSP was then completely blocked after bicuculline was added to strychnine, revealing an apparent EPSP (Fig. 28C). Fig. 29 is an example of a neuron showing complete block by either bicuculline or strychnine. In normal saline, the cell showed an IPSP (Fig. 29A). With

bicuculline application, the IPSP was blocked and a suprathreshold action potential was observed (Fig. 29B). Postwash of bicuculline again revealed the IPSP (Fig. 29C). Application of strychnine also resulted in a complete block of the IPSP and a suprathreshold response was revealed (Fig. 29D).

Some cells also showed an effect of strychnine on the threshold of their excitatory response. For example, in one cell, the threshold for eliciting a suprathreshold response in normal saline was 20 v, but after strychnine application, this threshold was reduced to 8 v.

4.3.1.2.1 Comparison Among Cell Types for Synaptic Inhibition

The Pauser and Adaptation cell types had the largest number of cells from which pharmacology data were obtained. Within the Pauser group, there were 4 neurons for which pharmacology studying synaptic inhibition could be performed. There were 2 pauser cells which showed a complete block with strychnine only and one cell was completely blocked with bicuculline only. One neuron showed a partial block by bicuculline only but was also totally blocked by both strychnine and bicuculline. Within the Adaptation group, there were 7 neurons for which pharmacology could be performed. In this group, there were 5 cells which showed a complete block by strychnine only and one cell which was partially blocked by strychnine only. Two cells showed a complete block by bicuculline only, where one of these cells was also completely blocked by strychnine. One neuron was completely blocked by a combination of strychnine and bicuculline, where this cell also showed a partial block by strychnine only.

Pharmacology was performed on only 1 onset neuron and 1 regular neuron. The onset neuron showed a partial block by strychnine only and then was completely blocked by a combination of strychnine and bicuculline. The regular neuron showed a reduction in its threshold for eliciting a suprathreshold response after strychnine application.

These data suggest there were no major differences between the Pauser and Adaptation groups with regards to inhibitory neurotransmission.

4.3.2 Summary

Most cells in the VNLL showed two components of the EPSP, an early short duration component which was blocked by CNQX and a late long duration component which was blocked by APV. With respect to inhibitory responses, most cells in which strychnine was applied showed a total block of the IPSP. A small number showed a partial block. Most cells in which bicuculline was applied, showed a total block of the IPSP. All neurons in which a combination of strychnine and bicuculline was used, showed a complete block of their IPSP.

4.4 Responses of VNLL Neurons to Repetitive Stimulation

4.4.1 Introductory Comments

A total of 27 neurons were studied with respect to their suprathreshold responses to repetitive synaptic stimulation. From these neurons, four different firing patterns were found in response to intracellular depolarizing current injection. Eleven cells showed a pauser firing pattern, 9 cells displayed an adaptation pattern, 6 cells showed a regular

pattern and 1 cell showed an onset pattern. Out of the 27 neurons, 4 regular cells and 2 pauser cells showed spontaneous firing without stimulation. The onset neuron and one adaptation neuron did not show any excitatory response in normal saline.

To study the excitatory synaptic receptor mediated responses to repetitive stimulation, the AMPA receptor antagonist, CNQX, was applied in 2 neurons. The inhibitory synaptic receptor antagonist strychnine was applied in 3 neurons and bicuculline was applied in one neuron. A combination of strychnine and bicuculline was simultaneously applied in two neurons and a combination of strychnine, bicuculline and APV was simultaneously applied in one neuron.

The firing probability and latency of spikes in response to a train of 100 or 200 electric pulses were studied in all neurons except for the 6 neurons which displayed spontaneous firing.

4.4.2 Responses of VNLL Neurons to Repetitive Stimulation in Normal Saline

VNLL neurons responded to a train of pulses with a train of spikes. Fig. 30A shows the occurrence of spikes for a typical neuron with a pauser firing pattern. At low repetition rates (10 Hz), the neuron responded with a single spike to every pulse in a pulse train (upper left panel of Fig. 30A). Therefore, the firing probability was 100% (Fig. 30B). As the repetition rate increased from 10 Hz to 200 Hz, the firing probability decreased. At 100 Hz, the firing probability fell below 50%. At 200 Hz, the neuron responded to only about 10% of the pulses. The relationship between the firing probability and the pulse repetition rate is shown in Fig. 30B.

When the repetition rate was low (e.g., 10 Hz), this neuron showed fairly constant latency in response to each of the pulses throughout the pulse train (upper left panel of Fig. 30A). At higher repetition rates, however, the spike latency was no longer constant. The spikes elicited by late pulses generally had longer latencies than those elicited by early pulses (upper right and lower panels of Fig. 30A). In other words, there was a trend of increase in spike latency in the response to a train of pulses. The trend of increase in spike latency could fit with a linear regression line (solid line in Fig. 30A). The slope of the regression line was then plotted as a function of the repetition rate (Fig. 30C), showing that the increase in spike latency was more apparent at a higher repetition rate than at a low repetition rate.

In addition to an increase in spike latency in response to a train of pulses, the spike latencies also displayed random variation from the trend line (i.e., jitter) (Fig. 30A). The jitter was more apparent at higher repetition rates (lower panels of Fig. 30A). To examine whether the extent of jitter in spike latency varied over a train of pulses, standard deviations were calculated from spike latencies for every 10 consecutive pulses (dotted line in top panels, and bottom left panel of Fig. 30A). For this neuron, the extent of jitter was fairly constant over the entire stimulus train at 10 Hz (0 - .007) and 50 Hz (.003 - 0.13). At 100 Hz, a fluctuation in standard deviation was noted over the entire train of 200 pulses (0 - 0.4). However, no systematic increase or decrease of the peak value was observed.

Fig. 31 shows responses of an adaptation neuron to repetitive stimulation. The neuron responded with a high firing probability to a train of pulses at low repetition rate

(upper panels of Fig. 31A, Fig. 31B). The firing probability was 100% at 10 Hz and almost 100% at 33 Hz. The firing probability decreased to less than 50% at 200 Hz, and further at 250 Hz.

For this neuron, even at low repetition rates, a trend of increase in spike latency was observed (upper panels of Fig. 31A). At higher repetition rates, the trend of increase in latency became even more evident (lower panels of Fig. 31A). At low repetition rates, the trend of latency increase fit well with a linear regression line (solid line, upper panels of Fig. 31A). At higher repetition rates, however, the increase in spike latency was more prominent for the early spikes than for the later spikes (lower panels of Fig. 31A). Thus, it was not appropriate to use a regression line derived from the entire spike train to depict the trend of increase in spike latency. A second regression line derived from the latencies of the responses to the first 30 pulses was used (bottom panels, Fig. 31A, dashed line). At higher repetition rates (200 Hz, 250 Hz), the slope of the line based on the first 30 pulses was larger than that based on pulses of the entire train, indicating a more apparent increase in spike latency for the initial spikes (bottom panels of Fig. 31A, dashed line). The slope of the regression line based on the responses to the first 30 pulses (dashed line) and the slope of the regression line based on the responses to the entire sequence of pulse train (solid line) were then plotted as a function of the repetition rate, respectively (Fig. 31C). The result showed that the latency increase over the first 30 pulses and the entire pulse train was more apparent at high repetition rates than at low repetition rates.

For the neuron shown in Fig. 31, at a low repetition rate (10 Hz), the cell showed very little jitter in spike latency, since there was little deviation of the latency from the

trend line (upper left panel of Fig. 31A). With higher repetition rates, however, the jitter in spike latency increased considerably (upper right and bottom panels of Fig. 31A).

4.4.2.1 Summary of Neural Responses to Repetitive Stimulation:

Most of the neurons sampled in this study could follow repetitive stimulation well when the repetition rate was low (10 Hz). However, the firing probability was reduced when the repetition rate was increased. The firing probability curves from three cell types (i.e., pauser, adapting and regular) were pooled into three groups respectively (Fig. 32). The adaptation and pauser neurons are two large groups which contained the most neurons tested. Differences in the firing probability curve were noted in these two groups of neurons. At 10 Hz, only 2 out of 9 adaptation neurons showed a firing probability higher than 80%, however 8 out of 9 pauser neurons showed a firing probability higher than 80% (Fig. 32A, 32C). A 50% cut-off repetition rate (i.e., the repetition rate at which a neuron showed a firing probability of 50%) was used to describe a neuron's ability to follow high repetition rates. For adapting neurons, all but two cells, showed a 50% cut-off repetition rate lower than 20 Hz. However, all but three pauser neurons showed a 50% cut-off repetition rate of greater than 20 Hz. Based on this result, it seems that the pauser neurons have a better ability to follow repetitive stimulation. For the two regular neurons, both had a firing probability greater than 80% at 10 Hz, and their 50% cut-off repetition rate was greater than 20 Hz.

As to the trend of increase in spike latency of responses over a train of pulses, for some neurons, there was a difference in the extent of latency increase of early spikes and

the rest of the spikes in a pulse train. For other neurons, this difference was not apparent. Thus, it was decided to calculate the slope based on spikes elicited by the first 30 pulses for all the neurons. Fig. 33 shows the slopes of the spike latency for the first 30 pulses for three types of neurons. Some neurons had a slope near zero at different repetition rates indicating that their latencies in response to repetitive synaptic stimulation were relatively constant. For other neurons, the slope was above zero and increased with the repetition rate. These results indicated an overall trend of increase in spike latency in response to a train of pulses and that the trend became more apparent when the repetition rate increased. More pauser neurons showed a trend of increase in spike latency in response to repetitive stimulation compared to adaptation neurons. With respect to the regular neurons, one neuron showed a higher trend of increase compared to the other.

With respect to jittering in response latency, at low repetition rate, most neurons showed very little random variation in their spike latencies from the trend line in response to a train of pulses. However, at higher repetition rates, the jitter in spike latency became more obvious. In some neurons, the extent of jitter was higher at the later part of the pulse train compared to the earlier part of the train for a particular repetition rate. However, in other neurons, the extent of jitter remained relatively constant throughout the pulse train for a particular repetition rate.

4.4.3 Spontaneous Firing of VNLL Neurons

There were 4 regular and 2 pauser neurons showing spontaneous firing. These neurons generated spikes without stimulation and the spikes were fairly evenly

distributed over time. However, the even distribution of spikes was altered by repetitive stimulation. The spike distribution of such a neuron is shown in Fig. 34. The spikes occurring over the entire duration of a pulse train was folded into one single period equal to the inter-pulse interval. At 10 Hz, the neuron showed initial spiking after the stimulus pulses. It then ceased firing, as indicated by a silent gap, for approximately 8 ms before resuming continuous spiking. The pattern of spike distribution obtained at 10 Hz was similar to that at other repetition rates, but the number of initial spikes and the length of silent gap varied at different repetition rates. The number of initial spikes was 11 at 10 Hz and ranged from 2 to 20 at other repetition rates. These numbers were far lower than the number of spikes that would be evoked by 10 Hz in neurons without spontaneous activity. It must also be noted that the duration of the initial spikes and the silent gap was close to the duration of an IPSP.

4.4.4 Neural Responses to Repetitive Stimulation During Pharmacological Manipulation

In order to study the role of specific excitatory and inhibitory synaptic receptors in mediating neuronal responses to repetitive stimulation, pharmacological manipulations were conducted in 7 VNLL neurons.

Fig. 35 shows the effect of bicuculline on the response to repetitive stimulation of an adaptation neuron. During bicuculline application, the firing probability increased substantially compared to the predrug level at three different repetition rates tested (Fig. 35A, 35B). With bicuculline, the firing probability was highest at 10 Hz and decreased with increasing repetition rate (Fig. 35B). In addition, for all three repetition rates, there

was a general trend of increase in spike latency from early spikes to later spikes (Fig. 35B). This trend was more apparent at the higher repetition rate (Fig. 35B, third panel). Also, there was more random variation of spike latency or jitter, with increasing repetition rate. The extent of jitter appeared to be constant throughout the stimulus train for each repetition rate.

Fig. 36 shows the effect of strychnine on the response to repetitive stimulation of a pauser neuron. The firing probability did not change significantly after strychnine application. However, it can be seen that the extent of increase in response latency from early spikes to later spikes was larger after strychnine application than before (Fig. 36B). In addition, the variation of spike latency around the trendline, i.e., jitter, was also larger after strychnine application compared to the predrug situation (Fig. 36B). There was more jitter for the later spikes than for the early spikes (Fig. 36B).

Fig. 37 is an example of the effect of CNQX on the response to repetitive stimulation of an adaptation neuron. The firing probability was greatly reduced after CNQX application compared to the predrug level. It was interesting to note that there was a loss of spikes with short latency after CNQX was applied. Thus, the trend in increase of latency from early to later spikes was smaller after CNQX application than before.

Fig. 38 illustrates the effect of combined application of strychnine and bicuculline as well as the combined effect of strychnine, bicuculline and APV on the response to repetitive stimulation of an onset neuron. This neuron showed a prominent IPSP in normal saline and thus did not show any spike to repetitive stimulation prior to drug

application. During strychnine and bicuculline application, the cell responded with a high firing probability at a low repetition rate (Fig. 38A, first panel). The firing probability decreased as the repetition rate increased (Fig. 38A). There was a trend for latency increase from early to later spikes at 10 and 20 Hz, however this trend was more apparent for 20 Hz (Fig. 38A, first and second panels). There was some jitter around the trendline at 10 Hz, however, the extent of jittering increased at 20 Hz (Fig. 38A, first and second panels). For both repetition rates, 10 and 20 Hz, in this condition, the extent of jittering was larger for the later stimulus pulses compared to the early stimulus pulses within a stimulus train. Addition of APV to the strychnine and bicuculline solution eliminated the response of the cell at repetition rates higher than 10 Hz. Also, for 10 Hz, the extent of jittering appeared to decrease after APV application than before (Fig. 38B).

4.4.5 Summary

The firing probability and response latency of VNLL neurons to repetitive stimulation were examined. Neurons usually had a high firing probability at low repetition rates, however this probability decreased as the repetition rate increased. Pauser neurons were able to follow repetitive stimulation better than the adaptation neurons. VNLL cells exhibited a general trend of increase in response latency from early to later spikes within a stimulus train. Some neurons showed this trend at low and higher repetition rates. Other neurons showed this trend only at higher repetition rates. In addition, VNLL cells displayed a random fluctuation in spike latency or jitter to repetitive

stimulation. This random variation in spike latency usually increased with increasing repetition rate.

5. DISCUSSION

5.1 Possible Conductances Involved in VNLL Discharge Patterns

In vitro studies have focused on examining the response of VNLL neurons to intracellular depolarizing current injection. Two physiological groups of neurons were identified: sustained and onset. Sustained neurons responded with continuous firing to depolarizing current injection and consisted of firing patterns such as regular, onset-pause, adaptation and burst. Onset neurons were distinct from sustained neurons, and displayed only a single action potential at the onset of depolarization (Wu, 1999, Zhao and Wu, 2001). The present study also found a variety of temporal discharge patterns in response to intracellular depolarizing current injection in VNLL neurons. There were five main firing patterns observed: Pauser, Regular, Adaptation, Onset and Burst.

The pauser discharge pattern showed sustained firing to depolarizing current injection, and as the current strength increased, the frequency of spike firing also increased. A pause was observed to depolarizing current injection after a prehyperpolarizing current pulse of 300 ms. It has been suggested that this pattern may occur as a result of an outward rapidly activating and inactivating potassium current (I_A). At normal resting potentials, this current is mostly inactivated. However, if the neuron has been hyperpolarized long enough to reprime the I_A channel, subsequent depolarization activates this channel, thus opposing spike generation, resulting in a pause. Sustained firing begins as I_A gradually inactivates (Trussell, 2002). Thus, in the present study, since the pauser pattern was identified with a depolarization following a prehyperpolarization of the cell membrane, the pause may have been due to the activation of I_A . Sustained firing resumed once I_A began to gradually inactivate.

Regular firing neurons showed a sustained firing pattern with consistent interspike intervals throughout the current injection. Previous studies have found that in the IC, this nonadapting sustained firing pattern with consistent interspike intervals is determined by a delayed rectifying 4-AP sensitive potassium current (Sivaramakrishnan and Oliver, 2001). The rate of repolarization during an action potential in these cells was sensitive to 4-AP, since the duration of the action potential was smaller in the absence of 4-AP than with 4-AP, indicating that the rate of repolarization was slower in the presence of 4-AP. Thus, it was suggested that in regular cells, it is likely that the magnitude of the 4-AP sensitive potassium current sets the firing frequency of the cell (Sivaramakrishnan and Oliver, 2001). Since the regular firing pattern observed in the VNLL is very similar to the pattern seen in the IC, it is possible that regular firing neurons in the VNLL may also reflect a delayed rectifying 4-AP sensitive potassium conductance.

Adapting neurons showed a firing pattern where action potentials were more frequent during the onset of depolarizing current injection but progressively became less frequent during the remaining current injection. Research focussing on the IC has found that adaptation cells have underlying transient potassium currents that activate and inactivate within the time period during which adaptation occurs. For example, Sivaramakrishnan and Oliver (2001), found that in the presence of apamin (which blocks the small-conductance, voltage-independent $I_{K(Ca)}$) (Hughes et al., 1982), adaptation firing was blocked. The authors maintained that the apamin-sensitive $I_{K(Ca)}$ contributed to the adaptation firing pattern. VNLL adaptation cells display the same pattern as the

adaptation neurons in the IC, thus, it is possible that the apamin-sensitive $I_{K(Ca)}$ may also be involved in the VNLL adaptation firing pattern.

Onset cells showed only a single action potential at the beginning of a depolarizing current pulse. This firing pattern has also been found in octopus cells of the PVCN (Golding et al., 1999). One mechanism which may account for this type of response involves the interaction of outwardly rectifying potassium conductances and inwardly rectifying mixed cationic conductances. The combined action of these channels results in a high resting membrane conductance, thereby requiring large inward currents to reach threshold for action potential initiation. These influences result in only an initial spike and suppress repetitive firing (Golding et al., 1999). This mechanism may also be responsible for the onset responses observed in the VNLL cells.

Bursting cells show a burst of action potentials overriding a slow depolarization. Studies of the dorsal cochlear nucleus (DCN) have also found some neurons generated a burst of action potentials on a slow depolarization (Hirsch and Oertel, 1988). The authors maintained that the action potentials are sodium-dependent and the slow depolarization is calcium-dependent. In the present study, it can also be speculated that there are at least two components mediated by different ionic channels in the burst response: fast burst spikes and a slow depolarization which may also be attributed to sodium and calcium conductances respectively.

Although neurons in the VNLL may exhibit the same type of discharge patterns found in other auditory structures such as the IC, PVCN and DCN, further investigation

of the specific types of ionic conductance present in the VNLL for each of the firing patterns would be required.

5.1.1 Functional Significance of Firing Patterns

The cells with different firing patterns may also reflect some functional significance with respect to auditory processing.

Sustained regular cells fire with consistent interspike intervals throughout the duration of a depolarizing current pulse, and thus may provide information about the duration of the input signal (Sivaramakrishnan and Oliver, 2001). Sustained regular cells also have a linear rate level function since they increase their spike frequency with increasing depolarizing current. Thus, these cells may also code for intensity.

In onset cells, since the inward and outward rectifying conductances prevent the cell from sustained firing, the onset firing pattern is not likely to change with stimulus duration and intensity. Thus, although onset cells may not be well suited to code stimulus duration and intensity, they are ideal for the precise temporal coding of sound. These cells have been found to have a low membrane input resistance in response to depolarizing current injection (Zhao and Wu, 2001), a finding which was supported by the present study. The low membrane input resistance when the cell is excited (involving outward potassium conductance) (Manis and Marx, 1991) results in a rapid repolarization of the cell membrane, which in turn curtails incoming excitatory synaptic currents and prevents summation in time. Thus, the temporal pattern of excitation in these cells can reflect precisely the temporal firing pattern of inputs from the afferents. These attributes

are necessary for these neurons to precisely encode auditory temporal information and the onset of sound (Oertel, 1991;1997).

The pauser pattern may code for sound intensity, since it has a linear rate level function, and in addition, this pattern may also register the pairing between excitatory and inhibitory inputs to the cell. IPSPs, either single or summated, can remove the inactivation of the A-current. Therefore, the EPSPs of pauser cells that are preceded by IPSPs will depend on the magnitude of the IPSP (Sivaramakrishnan and Oliver, 2001).

5.2 Synaptic Inputs to the VNLL

The primary input to the VNLL comes from the contralateral cochlear nucleus (Glendenning et al., 1981; Zook and Casseday, 1985; Covey and Casseday, 1986), where the major projection is from the contralateral PVCN and AVCN (Vater et al., 1997). Bushy, multipolar and octopus cells in the contralateral VCN provide significant sources of projection to the VNLL (Friauf and Ostwald, 1988; Schofield and Cant, 1997; Thompson, 1998; Warr, 1982). The octopus cells of the PVCN send thick axons that terminate as large Calyces of Held in the VNLL (van Noort, 1969; Warr, 1969, 1982; Adams and Warr, 1976; Adams, 1983; Glendenning et al., 1981; Huffman and Covey, 1995) where these calyceal endings are excitatory (Adams, 1997; Smith et al., 1998). Other cells in the VCN such as spherical bushy and multipolar cells send thin axons that terminate as boutons in the VNLL (Schofield and Cant, 1997; Schwartz, 1992; Covey, 1993). These cells in the VNLL may receive axon collaterals from the thinner axons of AVCN neurons, whose main axons provide excitatory input to the IC (Oliver, 1987)

suggesting that the projections from the AVCN to the VNLL may also be excitatory. Studies have found that CN efferents that project to the contralateral VNLL may use excitatory amino acids as a transmitter (Suneja et al., 1995).

The VNLL also receives a smaller projection from the ipsilateral MNTB and periolivary nuclei (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 and periolivary nuclei to the VNLL is inhibitory, where inputs from the MNTB to the VNLL are glycinergic and inputs from the VNTB and LNTB are possibly GABAergic (Moore and Moore, 1987; Helfert et al, 1989; Vater et al., 1992).

In the present study, stimulation of the lateral lemniscus elicited EPSPs and/or IPSPs in pauser, adaptation, onset, regular and burst cells. Thus, the EPSPs found in the present study may originate from the contralateral PVCN or AVCN. The IPSPs may originate from the ipsilateral MNTB and/or periolivary nuclei. Previous studies also found that electrical stimulation of the lateral lemniscus could elicit EPSPs and/or IPSPs in onset, regular, adaptation, onset-pauser and burst cell types (Wu, 1999; Zhao and Wu, 2001).

In the present study, in normal saline, regular, adaptation, pauser and burst cells showed synaptic responses of EPSPs only more than IPSPs only or both EPSPs and IPSPs. The incidence of both EPSPs and IPSPs was higher for adaptation, pauser and onset neurons compared to regular and burst neurons. Wu (1999) found that in sustained neurons, there were the same number of cells exhibiting IPSPs only and a mixture of EPSPs and IPSPs. Sustained neurons showing only EPSPs were fewer in number. Also,

the incidence of both EPSPs and IPSPs from neurons in the onset-pause group was higher than in the adaptation and regular groups.

Zhao and Wu (2001) found that across all cell types, there was a similar proportion of cells exhibiting EPSPs only and a mixture of both EPSPs and IPSPs. However, proportions of cells displaying only IPSPs were less.

In the present study, onset cells showed a higher number of IPSPs only than both EPSPs and IPSPs. There was no evidence of EPSPs only in onset cells. Wu (1999) found there were a similar number of onset cells exhibiting only EPSPs and a mixture of both EPSPs and IPSPs. Onset cells showing only IPSPs were few.

The proportions of excitatory and inhibitory responses in the present study are different from those observed in previous studies. The present results were obtained in normal saline, however following pharmacological intervention, underlying EPSPs or IPSPs were revealed in some cells in which only a single excitatory or inhibitory synaptic response was observed prior to drug application. Thus, after drug application, there was a higher proportion of onset, regular, adaptation and pauser neurons displaying both EPSPs and IPSPs as compared to what was observed in normal saline.

The results showed that all cell types, except the burst neuron, responded with both EPSPs and IPSPs in normal saline and additional neurons revealed alternate synaptic responses after pharmacological intervention, indicating that these neurons also displayed both excitatory and inhibitory responses. Thus, it can be suggested that many cells in the VNLL receive a mix of excitatory and inhibitory input from the lower auditory

brainstem. There is possibly a high convergence of excitatory and inhibitory input coming from the contralateral CN and the ipsilateral SOC onto single VNLL neurons.

5.3 Excitatory Neurotransmission in the VNLL

Most VNLL neurons showed EPSPs with two components, an early short duration component which could be blocked by the AMPA receptor antagonist, CNQX, and a late long duration component which could be blocked by the NMDA receptor antagonist, APV. Some neurons showed only a short duration EPSP which was blocked by CNQX, however IPSPs were also observed for most of these neurons. The IPSPs observed in these cells may have obscured or truncated a long duration EPSP component (Fig. 21), indicating that these cells may have also had both early and late EPSP components. It can be suggested that the EPSPs in the VNLL showing both early and late components are mediated by glutamate through both AMPA and NMDA receptors which are present and activated in the same neuron.

In two neurons showing short duration EPSPs, IPSPs were not observed. In one of the neurons, the EPSP was totally blocked by CNQX and in the other neuron, APV was applied but did not have any effect on the EPSP, thus suggesting these EPSPs were mediated by AMPA receptors only. In these neurons, it may be that NMDA receptors were not activated by stimulation, possibly because the stimulation intensity was not high enough, thus preventing the threshold for activation of NMDA from being reached. It may also be possible that NMDA receptors were not expressed in these cells. Also, in two other short duration EPSPs, application of APV blocked the EPSP. In one of these

neurons, an interaction between the EPSP and an IPSP was observed. Thus, the IPSP may have truncated the EPSP, indicating that this cell may have actually had an NMDA receptor mediated excitatory response. There was no IPSP observed in the other neuron. In these rare cells, synaptic excitation may have been mediated by only NMDA receptors.

Studies of other auditory brainstem nuclei have also found EPSPs with an AMPA and NMDA receptor mediated component. For example, Ma et al. (2002) examined the synaptic mechanisms underlying excitation in the rat's central nucleus of the inferior colliculus (ICC) by making whole-cell patch clamp recordings in brain slice preparations of the auditory midbrain. The excitatory responses in either current or voltage clamp mode consisted of two distinct components, an early fast component that could be blocked by AMPA receptor antagonists (CNQX and NBQX) and a later slow component that could be blocked by NMDA receptor antagonists (APV or CPP). The authors concluded that synaptic excitation in the rat's ICC is mediated by both NMDA and AMPA receptors with different time courses of activation, where the AMPA receptor-mediated responses had much faster rise and decay times than the NMDA receptor-mediated responses.

In their study, Ma et al. (2002) found that both AMPA and NMDA receptor-mediated responses were present at resting potential and each component could be isolated pharmacologically by application of excitatory receptor antagonists. In these cells, it was possible to evoke NMDA and AMPA receptor-mediated responses independently of one another. For example, NMDA receptor-mediated excitatory responses could be elicited after pharmacological block of AMPA receptors, indicating

that activation of NMDA receptors in the ICC could be accomplished without any preliminary depolarization by activation of AMPA receptors. Following isolation of NMDA-receptor mediated responses, voltage-clamp experiments revealed that the NMDA receptor-mediated current was voltage dependent and increased in magnitude as the cell membrane was depolarized. In the present study, however, since APV was applied before CNQX in most cases, the late NMDA component could not be isolated. Further studies are needed to attempt to isolate the NMDA receptor in the VNLL and therefore to examine properties such as voltage dependence of NMDA receptors in the VNLL.

5.3.1 Functional Significance of Excitation in the VNLL

With reference to excitatory responses in the auditory system, it has been suggested that while the AMPA receptor may be well-suited for processing auditory information when rapid responses to sensory signals are necessary, a functional significance of the NMDA receptor may be to set the gain of responses to peripheral stimuli. Since the NMDA receptors are voltage dependent, they may progressively make a larger contribution to excitatory responses as stimulus intensity is increased (Kelly and Zhang, 2002).

NMDA receptors may also be involved in the temporal integration of auditory information. ICC neurons, for example, receive inputs from a number of lower auditory brainstem structures at different times thus inevitably resulting in multiple synaptic delays. Therefore, the relatively long-lasting excitation provided by NMDA receptors

may be necessary for the temporal integration of information arriving to the IC from different sources at different times (Kelly and Zhang, 2002). The VNLL also receives input coming from various sources of the lower auditory brainstem. Thus, NMDA receptors in the VNLL may also be used to temporally integrate these inputs.

AMPA and NMDA mediated excitation in the VNLL may also have synaptic influence on their target neurons. Since most VNLL neurons are immunoreactive for either GABA or glycine or both (Riquelme et al., 2001), their excitation by neurons in the contralateral VCN, would be expected to result in the inhibition of structures to which they project, such as the IC. The early and late components of excitatory responses in the VNLL may be translated into early and late inhibitory action on target neurons in the IC. A similar situation was suggested to occur in the binaural nuclei of the DNLL (Kelly and Kidd, 2000).

The NMDA receptor may also play a prominent role in development. In Ma et al.'s (2002) study, the relative contribution of NMDA and AMPA receptor types, progressively declined between the ages of 9 and 13 days. It was suggested that NMDA receptors in the ICC may play an important role in synaptic response plasticity during development (Sanes et al., 2000) since NMDA receptor-mediated responses in the rat's ICC are more prominent during early development. Other authors have suggested that there are more binding sites for the NMDA receptor during early development (McBain and Mayer, 1994) and NMDA receptor-mediated responses in general are more prominent in developing tissue than in the mature brain (Tsumoto et al., 1987). Thus, the presence of NMDA receptors in the central auditory system is prominent during the

developmental stage where neural circuitry undergoes much change and mature synaptic responses are being established (Sanes and Walsh, 1998; Cant, 1998). In the present study, since most of the pharmacological data was obtained from rats between the ages of 14 and 16 days, it could be that in this age group, the VNLL is still undergoing developmental processes and requires activation of a sufficient amount of NMDA receptors.

5.4 Inhibitory Neurotransmission in the VNLL

With respect to inhibitory neurotransmission, some neurons in which only strychnine was applied, showed a complete block of the IPSP, thus indicating these IPSPs were mediated by glycine receptors only. Also, in some other neurons where only bicuculline was applied, there was a complete block of the IPSP, suggesting that these IPSPs were mediated by GABA receptors only. Some neurons which received strychnine only or bicuculline only showed a partial block and a combination of strychnine and bicuculline showed a complete block of their IPSP, indicating that these IPSPs were mediated by both glycine and GABA receptors.

Anatomical studies have shown that the VNLL receives both glycinergic and GABAergic inhibition from the lower auditory brainstem, most probably from MNTB neurons which are immunoreactive for glycine (Helfert et al., 1989) and the VNTB and/or LNTB which are possibly GABAergic (Moore and Moore, 1987; Helfert et al, 1989; Vater et al., 1992).

The results about the IPSPs in the present study may be due to different pathways being activated in the slice preparation. For example, due to positioning of the stimulating electrode or the angle at which the brain slice was cut, it is possible that in some instances only MNTB glycinergic neurons were activated and at other instances only LNTB or VNTB GABAergic neurons were activated.

Also, it can be speculated, that anatomically, neurons in the MNTB and periolivary nuclei may provide input to different populations of neurons in the VNLL. For example, some neurons in the VNLL may receive inhibition from only the MNTB. Other neurons may receive input from the LNTB or VNTB and still another group of neurons may receive inputs from both the MNTB and the periolivary nuclei.

In one neuron, there was a complete block of the IPSP by strychnine alone and bicuculline alone. This occurrence was also found in a previous study in the VNLL where an IPSP was totally blocked by application of either bicuculline only or strychnine only (unpublished observation). This type of neuron may possibly reflect a dual inhibitory receptor. It is possible that this type of receptor may only be activated by the presence of both glycine and GABA. Although the majority of research maintains that MNTB neurons are immunoreactive for glycine (Helfert et al., 1989), there is some possibility that MNTB neurons may also be immunoreactive for GABA. For example, Dupont et al. (1990), found immunoreactivity for GABA in a number of cell bodies of the MNTB. Helfert et al. (1989) maintained that although most MNTB neurons are GABA(-), there are a few neurons which are lightly GABA(+). This suggests the possibility of co-localization of GABA and glycine in some MNTB neurons. In addition,

the possibility of colocalization of GABA and glycine in the VNTB has also been raised (Helfert and Aschoff, 1997). Therefore, such co-localization and ultimate co-release of GABA and glycine from the lower auditory brainstem may activate a possible dual receptor in the VNLL.

5.4.1 Functional Significance of Inhibition in the VNLL

The VNLL is a prominent source of inhibitory input to the ipsilateral IC (Saint Marie and Baker, 1990). One possible function of inhibition in the VNLL may be to disinhibit responses in the IC. Thus, any incoming inhibitory input to the VNLL from either the MNTB or periolivary nuclei would disinhibit neurons in the IC, ultimately resulting in modulation and control of information in the IC. The degree of disinhibition would depend on the number and strength of inhibitory inputs. Additional functional significance of inhibition in the VNLL may be to modify excitatory input coming from the contralateral CN, which in turn can be directed to the IC.

5.5 Repetitive Stimulation

In response to repetitive stimulation of the lateral lemniscus, VNLL neurons showed a high firing probability at low repetition rates, however, they decreased their firing probability as the repetition rate increased. The pauser and regular neurons had a higher firing probability compared to the adaptation neurons. Also, VNLL neurons generally showed a trend of increase in spike latency from early spikes to later spikes throughout a stimulus pulse train. This trend was usually more apparent for higher

repetition rates. In addition, VNLL neurons displayed a random fluctuation in spike latency, i.e., jitter, which usually increased with increasing repetition rate.

5.5.1 Firing Probability During Repetitive Stimulation

Previous studies have also examined the response of auditory neurons to repetitive synaptic stimulation. Zhang and Trussell (1994) studied the responses of chick nMAG neurons to eighth nerve stimulation and found that in response to 300 stimulus pulses, the firing probability was high at repetition rates of 1 Hz to 50 Hz. At higher frequencies, such as 100 and 200 Hz, however, the firing probability decreased. Wu and Kelly (1993) studied the responses of MNTB neurons to repetitive stimulation. They found that for 10 stimulus pulses, MNTB neurons responded 90% of the time to stimulation of the contralateral trapezoid body at rates as high as 667 Hz. In the present study, it was found that for most neurons tested with repetitive stimulation at 10 – 250 Hz, the firing probability was highest at 10 Hz, and then decreased at higher repetition rates. Thus, VNLL neurons were not able to respond to rates as high as neurons in the nMAG (Zhang and Trussell, 1994) or the MNTB (Wu and Kelly, 1993). The difference between the firing probability between MNTB neurons and VNLL neurons may be due to the larger number of pulses administered in the present study, since a higher number of stimulus pulses would result in less firing. However, additional mechanisms may also be responsible for the low firing probability of VNLL neurons.

5.5.1.1 Possible Mechanisms Involved in Decreased Firing Probability:

Previous studies of the nMAG suggest that this nucleus is contacted by calyceal endings from the auditory nerve resulting in secure synaptic transmission and a high release of synaptic vesicles (Zhang and Trussell, 1994). nMAG neurons would therefore be able to follow repetitive stimulation well. Also, calyceal endings may also make synaptic contact with MNTB neurons (Wu and Kelly, 1993). The proportion of calyceal endings in the rat VNLL is not very well established and it is possible that in the present study, many neurons from which responses to repetitive stimulation were obtained did not receive such endings. In the present study, responses to repetitive stimulation were obtained from more adaptation, pauser and regular neurons compared to onset neurons. Previous research suggests that onset neurons may receive calyces from the octopus cells of the PVCN (Zhao and Wu, 2001; Schofield and Cant, 1997) and it is speculated that adaptation, pauser and regular neurons possibly receive bouton terminals from spherical bushy and multipolar cells from the VCN (Schofield and Cant, 1997). Wu and Kelly (1993) also showed that the MNTB neurons which received calyceal endings had a higher firing probability than the LSO neurons which received bouton terminals. Therefore, it is possible that the VNLL neurons tested in the present study did not receive calyceal endings from octopus cells in the PVCN, resulting in a lower firing probability.

In the present study, the results of single pulse stimulation of VNLL neurons indicated there is an interaction between excitatory and inhibitory input from the lower auditory brainstem. Therefore, such an interaction may result in an IPSP suppressing the EPSP and ultimately preventing spike initiation in response to stimulation. In the present

study, VNLL neurons showed a lower firing probability following inhibition. For example, Fig. 35 shows that the firing probability increased for all frequencies tested after the application of the inhibitory antagonist bicuculline. Thus, inhibition may play an important role in decreasing the firing probability.

An additional possible mechanism involved in the decrease of firing probability of VNLL neurons to repetitive stimulation may be synaptic depression arising from the desensitization of AMPA receptors (Otis et al., 1996) and/or presynaptic mechanisms resulting in a decrease in neurotransmitter release (Zhang and Trussell, 1994). An additional source of synaptic depression may come through the activation of GABAergic inputs. For example, Otis and Trussell (1996) found that activation of GABA_B receptors inhibited glutamate release in the nMAG. However, further study will need to examine this possibility in VNLL neurons.

Therefore, a combination of factors such as an interaction between excitatory and inhibitory inputs from the lower auditory brainstem, anatomical differences in synaptic input and synaptic depression may account for the low firing probability found in VNLL neurons.

It should be noted that in the present study, pauser and regular neurons had a higher firing probability and thus were better able to follow repetitive stimulation than the adaptation neurons. The pauser and regular neurons have more rapid firing rates in response to intracellular depolarizing current injection compared to adaptation neurons. This characteristic may account for the ability of pauser and regular neurons to follow repetitive stimulation better than adaptation neurons.

5.5.2 “Jittering” of Response Latency During Repetitive Stimulation

A variation in the spike latency was observed during the course of repetitive stimulation. Zhang and Trussell (1994) also found in their study of chick nMAG neurons, that spikes were able to follow repetitive stimulation very well at 1 Hz, however, at higher frequencies, (e.g., 10 – 50 Hz), there was an increase in jitter. At even higher frequencies, such as 100 and 200 Hz, the jitter was very pronounced. These results are comparable to the findings of the present study.

One mechanism possibly involved in jittering may be the interaction between AMPA and NMDA receptors. As shown in present study, most VNLL neurons showed an EPSP with an early AMPA component and a late NMDA component in response to single pulse stimulation. The NMDA receptor-mediated excitatory response is usually of a long duration compared to the AMPA receptor-mediated response. The peak of the EPSP and thus the timing of action potential occurrence may vary since the synaptic potentials mediated by NMDA receptors are longer and slower, as opposed to AMPA receptors which mediate rapid and brief EPSPs (Ma et al., 2002). Thus, an interaction between these two receptors may lead to high variability in the timing of spike occurrence. In the present study, it was found that application of the NMDA receptor antagonist, APV, resulted in a slight reduction in the jittering compared to before application of APV (Fig. 38A and B for 10 Hz). Jittering may increase with increasing repetition rate, possibly because of activation of more NMDA receptors and thus a higher interaction between AMPA and NMDA receptor mediated excitation (Fig. 38A, 20 Hz). Also, at high frequencies, jittering may increase due to an interaction between a

suppression of the AMPA receptor mediated excitation and a summation of NMDA receptor mediated excitation resulting in variable spike occurrence (Ma et al., 2003).

Another possible mechanism involved in jittering may be synaptic inhibition. Fig. 36, for example, illustrates that the amount of jittering increased significantly after strychnine application. It is possible that inhibition shapes excitatory responses to repetitive stimulation in the VNLL and allows them to preserve and transmit temporal information more precisely (Ma et al., 2003).

The jitter in the VNLL affects the auditory processing in the IC. Research has shown that IC neurons have a lower extent of synchronization to SAM stimulation compared to the VNLL neurons (Langner, 1992). Since the VNLL projects to the IC, the jitter in the VNLL is carried to the IC resulting in less synchrony in IC neurons.

5.5.3 Possible Mechanism Involved in Increasing Response Latency

In Zhang and Trussell's (1994) study, an increase in spike latency during a pulse train was observed at 33 Hz and at higher frequencies. This finding is also comparable to results from the present study.

A possible mechanism which may be involved in increasing the spike latency from early to later spikes during repetitive stimulation, may be the suppression of EPSPs in response to a train of pulses. Such a suppression of the EPSP may result in a smaller amplitude of the peak of the EPSP, possibly leading to a longer latency in the activation of the action potential (Ma et al., 2003).

5.5.4 Spontaneous Firing of VNLL Neurons

It has been suggested that in the absence of inhibitory input, a neuron can fire continuously at a fixed interval. However, with inhibition, some action potentials are inhibited resulting in a distinctive pattern of impulses. This effect of inhibition on the firing of a neuron is called sculpturing (Kandel and Siegelbaum, 2000). In the present study, during spontaneous firing, neurons showed firing over the course of time without stimulation. Repetitive stimulation altered this spontaneous firing in that a silent period was observed after the initial spiking before the neuron resumed continuous firing. Since the time course for this silent period is similar to that displayed by an IPSP, it is most likely that the pause in firing represented inhibition. Thus, it is likely that repetitive stimulation on spontaneous firing represented the phenomenon of sculpturing.

5.6 Summary

The present findings show that the VNLL displays different discharge patterns to depolarizing and hyperpolarizing current injection.

The VNLL receives both NMDA and AMPA receptor mediated excitation in addition to glycine and GABA receptor mediated inhibition from the lower auditory brainstem. Thus, the VNLL may be viewed as an integrative nucleus which combines and modifies excitatory and inhibitory responses for further processing of auditory information in the IC. The neuronal circuits through the VNLL may be involved in the integrative processes that underlie recognition of temporal patterns.

Results from our study of repetitive stimulation in VNLL neurons indicate that most VNLL neurons can follow repetitive stimulation at low repetition rates (10 – 20 Hz). Also, at low repetition rates, there is little jittering of spike latency observed in VNLL neurons. The firing probability decreases and the jitter increases at higher repetition rates.

The VNLL is a large source of inhibition to the IC. At low repetitive rates, since the jitter is low in the VNLL, the inhibitory input from the VNLL to the IC may provide temporally precise inputs to the IC. As a result, the inhibitory inputs can shape individual excitatory responses of IC neurons, allowing them to preserve and transmit temporal information more precisely (Ma et al., 2003). However, at higher repetitive rates, since the jitter of spike latency increases in the VNLL, inhibitory inputs from VNLL neurons arrive in an IC neuron at different times, thus spatially summated inhibitory responses in IC neurons would also summate temporally, resulting in sustained inhibition. In this way, IC neurons can effectively integrate information within a wide range of time.

Table 1. **Distribution of synaptic responses in VNLL cell types.** Table entries represent the number of cells in each category. Responses were obtained in normal saline.

Table 1: Distribution of Synaptic Responses in VNLL Cell Types

Synaptic Response	VNLL Cell Type				
	Onset	Regular	Adaptation	Pauser	Burst
EPSP only	0	8	26	15	4
IPSP only	5	1	5	2	0
EPSP and IPSP	3	1	5	4	0

Table 2. Pharmacological effects of excitatory antagonists on VNLL neurons.
Table entries represent proportions of cells in each category.

Table 2: Pharmacological Effects of Excitatory Antagonists on VNLL Neurons

Antagonist	Pharmacological Effect			
	Early EPSP		Late EPSP	
	Total Block	No Effect	Total Block	No Effect
CNQX	14/15	1/15	—	1/15
APV	2/13	10/13	9/13	1/13

Table 3. Pharmacological effects of inhibitory antagonists on VNLL neurons.
Table entries represent proportions of cells in each category.

Table 3: Pharmacological Effects of Inhibitory Antagonists on VNLL Neurons

Antagonist	Pharmacological Effect		
	Total Block	Partial Block	No Effect
Strychnine only	7/9	2/9	0/9
Bicuculline only	3/4	1/4	0/4
Strychnine + Bicuculline	3/3	0/3	0/3

Figure 1. Diagram showing input to the VNLL from the contralateral CN. The CN contains octopus, globular and stellate cells.
Adapted from Helfert, R. H. and Aschoff, A. 1997. In: *The Central Auditory System*.

Figure 1

Figure 2. Schematic diagram of the coronal section of a rat brain slice. R: recording electrode; S: stimulating electrode.

Figure 2

Figure 3. Discharge pattern of a pauser neuron. A1-A4: responses to intracellular injection of current pulses at 0.8 nA, 0.2 nA, -0.3 nA and -0.5 nA for 400 ms. B1-B3: responses to intracellular injection of depolarizing current pulses at 1.0 nA, 0.7 nA and 0.3 nA for 300 ms after a prehyperpolarizing current pulse at -0.6 nA for 300 ms.

Figure 3

PAUSER NEURON

A

B

Figure 4. Discharge pattern of an adaptation neuron. A1-A4: responses to intracellular injection of current pulses at 0.4 nA, 0.1 nA, -0.2 nA and -0.6 nA for 400 ms. B1-B3: responses to intracellular injection of depolarizing current pulses at 0.7 nA, 0.5 nA and 0.3 nA for 300 ms after a prehyperpolarizing current pulse at -0.6 nA for 300 ms.

Figure 4

ADAPTATION NEURON

Figure 5. Discharge pattern of a regular neuron. A1-A4: responses to intracellular injection of current pulses at 0.7 nA, 0.5 nA, -0.4 nA and -0.6 nA for 400 ms. B1-B3: responses to intracellular injection of depolarizing current pulses at 1.0 nA, 0.8 nA and 0.6 nA for 300 ms after a prehyperpolarizing current pulse at -0.6 nA for 300 ms.

Figure 5

REGULAR NEURON

Figure 6. Discharge pattern of a burst neuron. 1-4: responses to intracellular injection of current pulses at 0.3 nA, 0.2 nA, -0.2 nA and -0.4 nA for 400 ms. Inset 1: detailed view of action potentials on depolarization for 1; Inset 2: detailed view of action potentials on depolarization for 2.

Figure 6

BURST NEURON

Figure 7. Discharge pattern of an onset neuron. 1-4: responses to intracellular injection of current pulses at 0.6 nA, 0.4 nA, -0.4 nA and -0.6 nA for 100 ms.

Figure 7

ONSET NEURON

1

2

3

4

20 mV
50 ms

Figure 8. Synaptic responses of a pauser neuron. 8A: suprathreshold postsynaptic response at 60 v; 8B: suprathreshold postsynaptic response with longer latency at 50 v; 8C: double peaked EPSP at 50 v; 8D: EPSP at 40 v. Arrow: stimulus artifact.

Figure 8

PAUSER NEURON

A

B

C

D

Figure 9. Synaptic responses of a second pauser neuron. 9A: suprathreshold postsynaptic response at 20 v; 9B: EPSP at 10 v. Arrow: stimulus artifact.

Figure 9

PAUSER NEURON

A

B

20 mV
20 ms

Figure 10. Synaptic responses of a third pauser neuron. 10A: EPSP followed by IPSP at 90 v; 10B: EPSP followed by IPSP at 80 v; 10C: small EPSP followed by IPSP at 60 v. Arrow: stimulus artifact.

Figure 10

PAUSER NEURON

Figure 11. Synaptic responses of an adaptation neuron. 11A: suprathreshold postsynaptic response at 22 v; 11B: EPSP at 18 v; 11C: EPSP at 16 v. Arrow: stimulus artifact.

Figure 11

ADAPTATION NEURON

Figure 12. Synaptic responses of a second adaptation neuron. 12A: IPSP at 20v; 12B: IPSP at 8 v; 12C: IPSP at 5 v. Arrow: stimulus artifact.

Figure 12

ADAPTATION NEURON

Figure 13. Synaptic responses of a third adaptation neuron. 13A: small EPSP followed by IPSP at 40 v; 13B: EPSP followed by IPSP at 30 v; 13C: suprathreshold postsynaptic response at 20 v; 13D: EPSP at 9 v. Arrow: stimulus artifact.

Figure 13

ADAPTATION NEURON

A

B

C

D

Figure 14. Synaptic responses of a regular neuron. 14A: EPSP followed by a suprathreshold postsynaptic response at 60 v; 14B: double-peaked EPSP at 30 v; 14C: EPSP at 20 v; 14D: EPSP at 10 v. Arrow: stimulus artifact.

Figure 14

REGULAR NEURON

A

B

C

D

Figure 15. Synaptic responses of a second regular neuron. 15A: IPSP at 50 v; 15B: IPSP at 30 v; 15C: IPSP at 20 v. Arrow: stimulus artifact.

Figure 15

REGULAR NEURON

Figure 16. Synaptic responses of a third regular neuron. 16A: double action potential at 50 v; 16B: double-peaked EPSP at 40 v; 16C: EPSP at 15 v; 16D: EPSP at 12 v; 16E: small IPSP at 10 v. Arrow: stimulus artifact.

Figure 16

REGULAR NEURON

Figure 17. Synaptic responses of a burst neuron. 17A: two action potentials on a slow EPSP at 50 v; 17B: two action potentials on a slow EPSP at 40 v; 17C: EPSP at 20 v; 17D: EPSP at 10 v. Arrow: stimulus artifact.

Figure 17

BURST NEURON

A

B

C

D

↑

20 mV
20 ms

Figure 18. Synaptic responses of a second burst neuron. 18A: two action potentials on a slow EPSP at 40 v; 18B: two action potentials on a slow EPSP at 20 v; 18C: EPSP at 10 v. Arrow: stimulus artifact.

Figure 18

BURST NEURON

A

B

C

Figure 19. Synaptic responses of an onset neuron. 19A: IPSP at 60 v; 19B: IPSP at 30 v; 19C: IPSP at 20 v; 19D: IPSP at 10 v. Arrow: stimulus artifact.

Figure 19

ONSET NEURON

20 ms 20 mV

Figure 20. Synaptic responses of a second onset neuron. 20A: IPSP at 60 v; 20B: IPSP at 30 v; 20C: IPSP at 20 v. Arrow: stimulus artifact.

Figure 20

ONSET NEURON

A

B

C

Figure 21. Synaptic response showing initial block by CNQX followed by block by APV in an adaptation neuron. 21A: a short duration EPSP in normal saline at 9 v; 21B: elimination of EPSP after CNQX application at 9 v; 21C: large IPSP revealed after CNQX application at 30 v; 21D: elimination of IPSP and long EPSP revealed at 30 v after strychnine is added to CNQX; 21E: elimination of long EPSP at 30 v after APV is added to CNQX and strychnine; 21F: EPSP returned after postwash at 30 v. Arrow: stimulus artifact.

Figure 21

Figure 22. Synaptic response showing initial block by APV followed by block by CNQX in a pauser neuron. 22A: an EPSP showing an early short component followed by a late long component in normal saline at 20 v; 22B: elimination of the late EPSP at 20 v after APV application; 22C: elimination of the short EPSP at 20 v after CNQX is added to APV; 22D: IPSP revealed after CNQX is added to APV at 30 v to show that the cell is still stable. Arrow: stimulus artifact.

Figure 22

Figure 23. Synaptic response of a short EPSP after APV application in an adaptation neuron. 23A: early short EPSP in normal saline at 10 v; 23B: EPSP at 10 v after APV application. Arrow: stimulus artifact.

Figure 23

Figure 24. Synaptic response of late suprathreshold response after APV application in an onset neuron. 24A: a double peaked suprathreshold response showing an early component followed by a late component at 100 v in bicuculline and strychnine; 24B: elimination of the late suprathreshold response at 100 v after APV is added to bicuculline and strychnine. Arrow: stimulus artifact.

Figure 24

Figure 25. Synaptic response after CNQX application in a pauser neuron. 25A: an early suprathreshold response in normal saline at 20v; 25B: elimination of suprathreshold response at 20 v after CNQX application. Arrow: stimulus artifact. Note: cell was still stable after drug application since membrane potential was -51 mV.

Figure 25

A

B

Figure 26. Synaptic response after strychnine application in a pauser neuron. 26A: IPSP at 30 v after CNQX application; 26B: elimination of the IPSP at 30 v after strychnine is added to CNQX. Arrow: stimulus artifact. Note: cell is stable since membrane potential was approximately -52 mV.

Figure 26

Figure 27. Synaptic response after bicuculline application in a pauser neuron. 27A: IPSP in normal saline at 10 v; 27B: elimination of IPSP and EPSP revealed at 10 v after bicuculline application; 27C: IPSP returned at 30 v after postwash. Arrow: stimulus artifact.

Figure 27

Figure 28. Synaptic response showing partial block by one antagonist in an onset neuron. 28A: IPSP in normal saline at 40 v; 28B: partial block of the IPSP at 40 v after strychnine application; 28C: elimination of the IPSP and EPSP revealed at 40 v after bicuculline is added to strychnine. Arrow: stimulus artifact.

Figure 28

Figure 29. Synaptic response after bicuculline and strychnine application in an adaptation neuron. 29A: IPSP in normal saline at 90 v; 29B: elimination of the IPSP and suprathreshold response revealed at 90 v after bicuculline application; 29C: IPSP returned after postwash at 90 v; 29D: elimination of the IPSP and suprathreshold response revealed at 90 v after strychnine application. Arrow: stimulus artifact.

Figure 29

Figure 30. Response of a pauser neuron to repetitive stimulation. A: scatterplots of the spike latency as a function of the order of stimulating pulse in a pulse train for 10, 50, 100, and 200 Hz. Each circle represents a single spike. Solid line: the slope of spike latency from early to late spikes; dotted line: standard deviation of the spike latency for every 10 consecutive pulses. B: firing probability plotted as a function of the repetition rate. C: slope of spike latency plotted as a function of the repetition rate.

Figure 30

A

B

C

Figure 31. Response of an adaptation neuron to repetitive stimulation. A: scatterplots of the spike latency as a function of the order of stimulating pulse in a pulse train for 10, 33, 200, and 250 Hz. Each circle represents a single spike. Solid line: the slope of spike latency from early to late spikes; dotted line: standard deviation of the spike latency for every 10 consecutive pulses; dashed line: slope of spike latency for the first 30 pulses B: firing probability plotted as a function of the repetition rate. C: Solid line: slope of spike latency for the entire train of pulses plotted as a function of the repetition rate; dashed line: slope of spike latency for the first 30 pulses plotted as a function of the repetition rate.

Figure 31

A

B

C

Figure 32. Firing probability of adapting, regular and pauser neurons to repetitive stimulation. A: firing probability plotted as a function of repetition rate for adapting neurons. B: firing probability plotted as a function of repetition rate for regular neurons. C: firing probability plotted as a function of repetition rate for pauser neurons. Each curve was derived from the results of a single neuron.

Figure 32

Figure 33. Slope of spike latency for the first 30 pulses for adapting, regular and pauser neurons. A: slope of spike latency plotted as a function of repetition rate for adapting neurons. B: slope of spike latency plotted as a function of repetition rate for regular neurons. C: slope of spike latency plotted as a function of repetition rate for pauser neurons.

Figure 33

Figure 34. Firing distribution histogram of a regular VNLL neuron with repetitive stimulation at 10, 20, 33 and 50 Hz. This neuron showed spontaneous firing. The number of spikes are plotted as a function of time. Arrow: stimulus pulse.

Figure 34

Figure 35. Effect of bicuculline on responses to repetitive stimulation of an adaptation neuron. Spike latency was plotted as a function of order of pulse at 10, 20 and 50 Hz. A: scatterplot of spike latencies for predrug condition. B: scatterplot of spike latencies during bicuculline application.

Figure 35

Figure 36. Effect of strychnine on responses to repetitive stimulation of a pauser neuron. Spike latency was plotted as a function of order of pulse at 10 Hz. A: scatterplot of spike latencies for predrug condition. B: scatterplot of spike latencies during strychnine application.

Figure 36

Figure 37. Effect of CNQX on responses to repetitive stimulation of an adaptation neuron. Spike latency was plotted as a function of order of pulse at 20 Hz. A: scatterplot of spike latencies for predrug condition. B: scatterplot of spike latencies during CNQX application.

Figure 37

Figure 38. Effect of strychnine, bicuculline and APV on responses to repetitive stimulation of an onset neuron. Spike latency was plotted as a function of order of pulse. A: scatterplot of spike latencies during strychnine and bicuculline application for 10, 20 and 33 Hz. B: scatterplot of spike latencies during strychnine, bicuculline and APV application for 10 Hz.

Figure 38

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