**BEHAVIOURAL LIMITS OF AUDITORY TEMPORAL RESOLUTION IN THE RAT:
DURATION DISCRIMINATION AND THE ROLE OF THE VENTRAL NUCLEUS
OF THE LATERAL LEMNISCUS**

By

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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**

Master of Science

Department of Psychology

Carleton University

Ottawa, ON

July, 2005

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ISBN: 0-494-10138-5

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ISBN: 0-494-10138-5

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Abstract

Two experiments were conducted to investigate the ability of rats to detect a change in the duration of an auditory stimulus. The first experiment determined the behavioural limits of the rat for detecting incremental increases in noise duration. The data showed that discrimination thresholds for duration in the rat increase monotonically with standard duration over the range of 100 to 1000 ms, a trend consistent with other mammals. The second experiment investigated the role of the ventral nucleus of the lateral lemniscus (VNLL) in the rat's ability to perform an absolute threshold task and a duration discrimination task at standard durations of 125, 250 and 500 ms. Rats were made functionally monaural by blocking one ear with ear mold compound. Lesions were made either ipsilateral or contralateral to the healthy ear. Animals with complete lesions of the VNLL had absolute threshold scores that fell within the normal range and had no significant deficit in their ability to detect incremental increases in noise duration. Complete lesions of the VNLL and the intermediate nucleus of the lateral lemniscus (INLL) produced no deficits in absolute thresholds, but did produce a deficit in discrimination threshold at 500 ms, significant at the 0.05 level.

Acknowledgements

Firstly, to Dr. Jack Kelly, thank you for being a great supervisor and teaching me the importance of reasoning and asking questions.

To Dr. Huiming Zhang, thank you for all your time spent explaining concepts and techniques. Your help eased my transition into the field of auditory neuroscience.

To Dr. Shu Hui Wu, thank you for your assistance and input with my thesis.

To Jim Cooke, thanks for taking a kid from the Maritimes under your wing and teaching him the ropes.

To Kelly, thank you for being there for me. Successes, failures and all.

To Marv, thanks for always listening and supporting me no matter the situation.

Lastly, to my parents, A-Belle and P-Wog, thank you for all the support over the years and providing me with the opportunities needed to pursue my dreams.

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1. INTRODUCTION

Hearing allows animals to communicate with conspecifics, avoid predation and find prey. For many sounds of biological significance, such as species specific calls, information is carried in the changes of sound quality. The auditory system processes acoustic information in spectral, spatial and temporal domains. Temporal acoustic features encompass aspects of sound that relate to changes of acoustic parameters over time. Time is an important dimension in hearing since almost all sounds fluctuate over time.

Temporal resolution in the central auditory system arises from several processes that collectively analyse changes in acoustic stimuli over time. The anatomical arrangement and physiological properties of neurons dictate how incoming information is modified through intrinsic membrane properties of individual neurons and synaptic interactions that help shape neuronal responses.

The ventral nucleus of the lateral lemniscus (VNLL) is a collection of auditory neurons in the brainstem located amongst fibers in the ventral portion of the lateral lemniscus (Figure 1). Studies have suggested that the VNLL is important for analyzing and encoding information carried by temporal changes of sounds (Covey and Casseday, 1986, 1991, 1999). Electrophysiological studies in both the bat and the cat (Covey and Casseday, 1991; Guinan et al., 1972a) have revealed that VNLL neurons have little or no spontaneous activity, they are broadly tuned and have short integration times. These properties make VNLL neurons ideal candidates to encode both the onset and ongoing properties of sound. Anatomical studies have displayed the contribution of inhibitory

Figure 1: Schematic representation of the location of nuclei and connections that exist within the auditory brainstem. IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; DCN: dorsal cochlear nucleus; VCN: ventral cochlear nucleus; LSO: lateral superior olive; VNTB: ventral nucleus of the trapezoidal body; MSO: medial superior olive; SPN: superior para-olivary nucleus; MNTB: medial nucleus of the trapezoidal body. Auditory connections are shown from the left cochlea, projecting to the contralateral side only. Dashed lines indicate inhibitory connections; solid lines indicate excitatory connections (Zhang et al., 2005).

(Zhang et al., 2005)

mechanisms from the VNLL to neural processing in higher structures (Zhang et al., 1998). The VNLL provides inhibitory ascending projections to the inferior colliculus (IC), with the ipsilateral projection being major and contralateral being minor. Covey and Casseday (1999) state this inhibition plays an important role in altering temporal response patterns, as these inputs participate in tuning IC neurons to temporal features of sound.

Although physiological studies have examined the contribution of neurons in the VNLL to temporally modulated sounds (Casseday et al., 2000) there has been limited behavioural study regarding the role of the VNLL in temporal processing. The purpose of the present study was to determine the contribution of the VNLL to the detection of temporally modulated sounds using a behavioural experiment.

1.1 Duration Processing

Researchers utilize stimuli that change over time in order to test an animal's ability to resolve temporal aspects of sound. Duration is an identifying feature of many biological sounds. Changes in sound duration are an important aspect of communication sounds such as speech, frog calls and echolocation calls (Casseday et al., 2000). As a result of physiological evidence that duration-tuned neurons exist in the IC (Covey and Casseday, 1999; Faure et al., 2003; Casseday et al., 2000; Brand et al., 2000), researchers have directed interest towards detailing the mechanism of duration tuning. Duration tuning is a common phenomenon in the vertebrate auditory system. Specifically, in bats, duration tuning has been discussed as an adaptation for echolocation (Covey and

Casseday, 1999), and in frogs, it has been observed as an adaptation for mating behaviour (Gooler and Feng, 1992).

1.1.1 Duration Tuning Models

The ability of single neurons to encode temporal information within the IC has been well characterized. Different cellular response types convey different information regarding the stimulus. Duration tuned neurons generally display either sustained firing that indicates real-time information regarding the duration of the sound stimulus or transient firing which sends onset or offset information about the stimulus (Brand et al., 2000; Casseday et al., 2000; Fuzessery and Hall, 1999). If sustained responses were the only neural representation of sound duration, then all neurons with sustained responses would represent all durations, and no neuron could represent any particular duration (Casseday et al., 2000). Analysis of duration-tuning behaviour of single IC neurons in the mouse indicates that a minority of neurons are entirely duration dependent (Brand et al., 2000). Tuning characteristics are typically modified as a function of changes in stimulus parameters such as intensity and frequency and therefore indicate context dependent temporal processing in duration-tuned neurons.

Duration tuning is not found at lower levels of the central auditory system (Condon et al., 1991; Hall and Feng, 1991), suggesting that duration tuning first emerges at the level of the midbrain. Evidence suggests duration tuning is produced at neurons in the IC through the specific temporal sequence of excitatory and inhibitory inputs

(Casseday et al., 2000; Covey and Casseday 1999; Fuzessary and Hall, 1999). The IC is the target of multiple excitatory and inhibitory inputs, with the major inhibitory projections arising from neurons in the ipsilateral VNLL (Oertel and Wickesberg, 2002). Because of the anatomical connections the VNLL is believed to play a fundamental role in the processing of duration related auditory cues. Neurophysiological studies in which antagonists of γ -aminobutyric acid (GABA) or glycine were applied to duration-tuned neurons in the IC of the big brown bat, *Eptesicus fuscus*, showed that blocking this inhibitory input eliminated duration tuning (Casseday et al., 1994).

There are two different theories surrounding how the interplay of excitatory and inhibitory inputs to target cells of the IC underlie duration tuning. The model proposed by Casseday et al. (2000) (Figure 2) postulates three input components: onset inhibition, onset excitation and offset excitation. Synaptic delay lines provide a wide range in the latencies of these inputs to the duration-tuned neuron. Onset inhibition reflects the sustained inhibitory postsynaptic potential (IPSP) leading excitatory input and lasts the duration of the stimulus. The initial excitation received by the neuron is transient and correlated with stimulus onset, but rendered subthreshold by sustained inhibitory input. Stimulus offset induces a second excitatory influence termed offset excitation which is representative of a rebound from inhibition. A spike occurs when the duration of the sound allows the onset excitation to coincide with the offset depolarization. Based on the

Figure 2: Coincidence model for duration tuning. Neuron tuned to a 5 ms duration stimulus. Black bars indicate duration of acoustic stimulation. Neither onset excitation nor offset excitation is in itself sufficient to elicit spikes. A: onset inhibition is followed by an excitation at the offset of the stimulus (middle trace). A second delayed excitation occurs in response to the onset of sound (lower trace). The duration of the 5 ms sound allows the delayed onset excitation to coincide with the offset excitation and generates a response (upper trace). B: onset inhibition is sustained for the duration of the sound and subsequently the offset excitation occurs later than in A (middle trace). The latency of onset excitation is the same for both the 30 and 5 ms sounds. As a result, the onset excitation (lower trace) does not coincide with the offset excitation and no response is generated (upper trace). The best duration is determined by the latency of the delayed onset excitation. This model proposes that onset excitation stems from excitatory input, whereas offset excitation is a rebound from inhibition (Casseday et al., 2000).

(Casseday et al., 2000)

model, longer or shorter delays in onset excitatory input could permit tuning to longer or shorter stimulus durations. These delay lines are facilitated by the latencies observed in neurons of the lateral lemniscus, which are sufficient to account for alterations in duration-tuning (Covey and Casseday, 1999)

The coincidence model, which centres on synaptic delay lines to duration-tuned IC cells, is supported by two lines of evidence regarding the specific temporal sequence of excitatory and inhibitory inputs (Casseday et al., 2000; Ehrlich et al., 1997). Firstly, there is evidence that the IC is targeted by both excitatory and inhibitory inputs that respond with a broad range of latencies (Casseday and Covey, 1995). Second, direct evidence exists regarding the time course of synaptic excitation and inhibition at the cell membrane of IC neurons. Covey et al. (1996) have shown that duration-tuned cells typically respond to sound with a net outward current, followed by a net inward current. The outward current is temporally locked to the onset of sound and the inward, locked to the offset.

Casseday et al. (2000) concede that the present model cannot account for duration-tuning observed in all IC neurons. This is not surprising considering the wide variety of inputs to neurons in the IC. A minority of neurons have been observed that maintain sustained excitatory input upon blocking of inhibition with bicuculline. In order to accommodate sustained, onset-correlated excitation in such neurons the researchers suggest employing a combination of early inhibition from one source and late inhibition

from another (Figure 3). This modification would create a temporal gap during which excitation could depolarize the cell upon presentation of a short duration stimulus. If the duration of the stimulus were to increase so would the resulting duration of the early inhibition. This early inhibition would gradually overlap with the onset of the late inhibition and ultimately close the gap for suprathreshold excitation.

The second model of duration-tuned neurons in the IC is termed the anti-coincidence model (Fuzessery and Hall, 1999). This model mirrors the coincidence model in many regards but differs in that it does not require a second excitation, rebound from inhibition, in order to elicit spiking. Fuzessery and Hall (1999) recognize that the coincidence model provides an effective mechanism for bandpass duration selectivity but falls short in comprehensively accounting for short-pass duration selectivity. Similar to the coincidence model both excitatory and inhibitory inputs are triggered at stimulus onset. Inhibitory input arrives to the IC first and lasts the duration of the stimulus. The excitatory component follows and has both a fixed time course and latency. It is postulated that at short durations, the inhibitory input is complete prior to the arrival of the excitatory component and therefore spiking is elicited. Based on the model, an increase in stimulus duration would result in a reduction in spiking because of the corresponding overlap of inhibitory and excitatory components. Duration-tuned neurons of the IC therefore fire maximally when excitatory and inhibitory inputs do not coincide.

Figure 3: Modified coincidence model for duration tuning. Neuron tuned to a 5 ms duration stimulus. Black bars indicate duration of acoustic stimulation. A: for short sounds, early (1) and late (2) inhibition (middle trace) are short. Delayed onset excitation (lower trace) falls in the gap between the two inhibitory events. The excitation is suprathreshold and generates spiking (upper trace). B: for sounds of longer duration, the gap between early and late inhibition is closed (middle trace). Onset excitation is subsequently offset by inhibition and no response is generated (Casseday et al., 2000).

(Casseday et al., 2000)

1.2 Anatomy of the VNLL

The nuclei of the lateral lemniscus are composed of cells embedded in the fibers of the lateral lemniscus, which stretch from the rostral border of the superior olivary complex to the base of the IC (Malmierca et al., 1998; Aitkin et al., 1970). The nuclei of the lateral lemniscus constitute two functionally separate systems of auditory processing: the dorsal nucleus of the lateral lemniscus (DNLL) and the VNLL. The DNLL receives binaural input and projects bilaterally to the IC, while the VNLL projects ipsilaterally and receives predominantly monaural input (Covey and Casseday, 1991; Merchán and Berbel, 1996). Some researchers recognize a third division, the intermediate nucleus of the lateral lemniscus (INLL), located at the dorsal end of the VNLL. Anatomical studies indicate that this region may receive binaural input (Van Adel, 1998) and project ipsilaterally to the IC.

1.2.1 Cytoarchitecture

Studies regarding the cytoarchitecture and organization of the VNLL are controversial. Variations exist regarding the divisions of the VNLL across different species of mammals. Covey and Casseday (1991) have described three major cytoarchitectonic areas for the bat in the region ventral to the DNLL: the INLL, the multipolar and the columnar regions of the VNLL. These nuclei are bounded on the medial and lateral sides by ascending fibers of the lateral lemniscus and are morphologically distinct subdivisions of the bat VNLL. Anatomical evidence suggests

that each nucleus represents a separate parallel pathway from the ventral cochlear nucleus (VCN) to the midbrain. Studies of anterograde transport show that projections from the anteroventral cochlear nucleus (AVCN) diverge to each of the nuclei ventral to the DNLL, while retrograde transport shows that each structure subsequently sends separate projections to the IC (Covey and Casseday, 1991).

Each subdivision of the bat VNLL contains a distinctive cell population (Figure 4). Within the bat INLL, neurons are predominantly elongate with several long dendrites extending from the soma in a plane orthogonal to the ascending fibers of the lateral lemniscus and parallel to the fibers that enter the nucleus (Covey and Casseday, 1991). In the columnar region, neurons are densely packed and distributed in columns between bundles of lateral lemniscal fibers. Neurons of the columnar region are structurally similar to spherical bushy cells in the AVCN due to their round to oval shape and the presence of one large, thick dendrite that branches profusely some distance away from the cell body. The branches of neurons in the columnar region appear to extend parallel to the ascending fibers (Covey and Casseday, 1991). Lastly, the multipolar region of the bat VNLL contains neurons that are multipolar in shape. Typically, multipolar neurons of this region are larger than neurons of the columnar region and have several sparsely branching thick dendrites. Unlike the INLL and columnar region, cell bodies and dendrites of neurons in the multipolar region have no consistent orientation relative to the fibers of the lateral lemniscus (Covey and Casseday, 1991).

Figure 4: Examples of the location, size and appearance of the main cell types in the INLL, VNLLc and VNLLm of the big brown bat, *Eptesicus fuscus* (Casseday and Covey, 1991).

(Casseday and Covey, 1991)

Anatomical studies in the rat have found two morphological types of neurons in the VNLL; bushy and stellate (Zhao and Wu, 2001). Stellate cells can be further divided as stellate 1, 2 or elongate cells based on their somatic and dendritic morphologies. The distribution of these different morphological types of cells in the rat differs depending on cell type. Bushy and stellate 1 cells are typically localized to the ventral area of the VNLL whereas stellate 2 and elongate cells are located in the dorsal area.

The dendritic morphology of neurons in the rat VNLL is an important indicator of heterogeneous neuronal groups. In general, the dendritic tree of the bushy cell is smaller, more compact and highly branched compared to that of stellate cells. The morphology of the bushy cell closely resembles the bushy cell in the bat columnar region, while the dendritic features of stellate 2 cells resemble neurons in the multipolar region in the bat (Zhao and Wu, 2001).

Merchán and Berbel (1996) suggest that variations in morphology of neurons may be due to changes in the orientation of fibrodendritic coiling within a helicoid topographical arrangement of the VNLL. This suggestion is based on retrograde tracers injected into the IC and their subsequent distribution in the ipsilateral VNLL upon three-dimensional reconstruction. Based on analysis of their results, Merchán and Berbel suggest that the VNLL of the rat contains a single neuronal type consisting of flat stellate neurons. These stellate neurons and their fibers are oriented in parallel and form a series of fibrodendritic coils. The coils are topographically organized, with neurons in

peripheral laminae projecting to dorsolateral, low frequency regions of the IC and neurons in central laminae projecting to ventromedial, high frequency regions (Merchán and Berbel, 1996). Merchán and Berbel (1996) therefore suggest that cytoarchitectural differences across studies can be explained by assuming that the orientation of cells in the coiling laminae can cause cells to appear morphologically different from each other. Where it appears that there are four different cell types, there may in fact be one cell type with four different orientations.

In addition to the proposal by Zhao and Wu (2001), evidence contrary to the single neuronal type suggested by Merchán and Berbel does exist. Fusiform neurons immunoreactive to glutamic acid decarboxylase (GAD) have been shown to coexist with larger multipolar neurons not immunoreactive to GAD in the VNLL of the rat (Moore and Moore, 1987). Such findings suggest it is possible that several subtypes of flat stellate neurons and/or neurons with other morphologies may coexist.

1.2.2 Topography

When stained with cresyl violet the rat VNLL appears to have a relatively simple cytoarchitectural pattern (Merchán and Berbel, 1996) that is less differentiated than the bat. The nuclei are dispersed amongst fibers of the lateral lemniscus in a tightly woven pattern that spirals in a helical manner (Merchán and Berbel, 1996; Willard and Martin, 1983). This helicoid structure is supported by immunohistochemical findings of horizontal bands of spindle fibers interspersed between fascicles of ascending lemniscus

axons (Moore and Moore, 1987). Studies by Kelly et al. (1998) and Merchán and Berbel (1996) also suggest a spiral pattern by revealing fibers in the lateral lemniscus oriented in a ladder rung pattern congruent with a helicoid structure.

Kelly et al. (1998) revealed a complex pattern of retrograde labeling in the VNLL upon injection into the central nucleus of the inferior colliculus (ICC). In particular Kelly et al. discovered that after small injections of tracer into low frequency sites in the ICC, a horizontal ladder distribution of labeled cells appeared as multiple bands across the lemniscal pathway. According to 3-D reconstruction by Merchán and Berbel (1996), each turn of the helix appears as a mediolaterally oriented band of cells in coronal sections, with low frequencies represented peripherally and high frequencies centrally. The banding pattern is much less pronounced when retrograde tracers are placed in tonotopically high or middle regions of the ICC (Kelly et al., 1998).

1.2.3 Projections to the VNLL

Anatomical studies in various species, including the rat, have revealed that the major inputs to the VNLL are excitatory projections. These inputs primarily arise from principal cells in the contralateral ventral cochlear nucleus (VCN) (Huffman and Covey, 1995; Oertel and Wickesberg, 2002; Zook and Casseday, 1985). In the central nervous system, glutamate is a major excitatory neurotransmitter and is usually the source of postsynaptic excitation. This excitation is partly mediated in the VNLL via perisomatic calyceal synaptic endings (Wu, 1991; Vater et al., 1997). More specifically, thick fibers

arising from octopus cells of the posteroventral cochlear nucleus (PVCN) enter the VNLL and terminate with boutons similar to calyces of Held (Sunjea et al., 1995; Wu, 1999). Specialized endings of this type provide a substrate for fast robust signal transmission. Bushy and multipolar cells of the VCN also project to the VNLL in the rat (Wu, 1999). Excitatory input to the VNLL from these cells arise from medium sized boutons projecting from the AVCN (Wu, 1999).

Studies in the bat have shown that minor inputs to the VNLL also include inhibitory projections from the ipsilateral periolivary nuclei and the lateral, ventral and medial nuclei of the trapezoid body (Huffman and Covey, 1995; Vater et al., 1992). Glycine and γ -aminobutyric acid (GABA) are the two major inhibitory neurotransmitters in the central nervous system. Within the central auditory system, some uncertainty exists regarding the primary inhibitory neurotransmitter. For example, inhibition from the MNTB could be either glycinergic or GABAergic (Wu, 1999; Campos et al., 2001). The MNTB has many glycinergic cells, but the VNLL has been found to contain $\alpha_n\beta_3\gamma_2\text{L}$ (where $n = 1, 2$ or 3) GABA_A receptor types, indicating a GABAergic input (Campos et al., 2001).

In the big brown bat, subnuclei of the VNLL vary in the proportion of input they receive from various groups of cells in the VCN (Huffman and Covey, 1995). A systematic ventral to dorsal change in relative proportions exists, whereby the multipolar region receives mostly direct projections from the AVCN and the INLL receives

primarily indirect projections from the cochlear nucleus via the medial nucleus of the trapezoidal body (MNTB). The columnar region of the VNLL, which lies between the multipolar region and INLL, receives a balance of indirect and direct input from the VCN (Huffman and Covey, 1995). These differing proportions of input to each subnuclei of the lateral lemniscus suggest that signal processing is different in each nucleus.

1.2.4 Projections from the VNLL

The major output of neurons in the VNLL are inhibitory ascending projections to the IC (Kelly et al., 1998; Oertel and Wickesberg, 2002; Whitley and Henkel, 1984; Zook and Casseday, 1987). Inhibition plays an important role in altering temporal response patterns. The main inhibitory neurotransmitters in the auditory brainstem are glycine and GABA. In the rat, cell bodies and fibers have been labeled by antibodies against GAD, the enzyme that catalyzes the synthesis of GABA (Moore and Moore, 1987). 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. Similar projections have been observed in the big brown bat (Vater et al., 1997) and the mustached bat, *Pteronotus parnellii* (Winer et al., 1995). Across these species, the VNLL contains glycine positive neurons that receive excitatory input from bouton terminals of the VCN. The VNLL thus transforms the rapid excitatory input derived from the contralateral VCN into inhibitory output that shapes duration-tuning in the auditory midbrain.

Connections from many brainstem auditory nuclei to the IC follow a tonotopic organization, but projections from the VNLL are diffuse and non-specific, lacking any obvious topography related to sound frequency. This is consistent across all species as restricted injections of retrograde label into the IC or anterograde label into the VNLL result in distributed labeling (Covey and Casseday, 1986; Malmierca et al., 1998; Merchán and Berbel, 1996; Whitley and Henkel 1984). The tonotopic organization of the VNLL has been difficult to resolve as electrophysiological recordings have yielded complex results that are not easily generalized across species. A tonotopic organization in the VNLL of the rat should not however be excluded. Friauf (1992) observed c-fos immunoreactive horizontal bands, after pure tone stimulation, which resemble banding encountered by Merchán and Berbel (1996) after iontophoretic injection. Merchán and Berbel suggest that these bands could be representative of isofrequency planes and therefore show that the tonotopicity of the rat VNLL may be helicoidal in structure. Such a system of fibrodendritic laminae would be compatible with the organization of isofrequency planes observed in other auditory nuclei.

In addition to the major inhibitory projections to the IC, the VNLL has minor projections to the thalamus, the DNLL, the dorsomedial periolivary nucleus (DMPO) and the ventral nucleus of the trapezoid body (VNTB) (Oertel and Wickesberg, 2002).

1.3 Physiological Responses of Cells of the VNLL

An animal's ability to analyze temporal properties of sound depends on the anatomical arrangement of neurons within the nucleus and on the physiological properties of those neurons. Electrophysiological studies have been conducted in several species including bats (Covey and Casseday, 1991; Huffman et al., 1998), cats (Aitkin et al., 1970; Guinan et al., 1972a,b), and rabbits (Batra and Fitzpatrick, 1997, 1999, 2002). These studies have revealed that VNLL neurons respond to acoustic stimulation with various temporal firing patterns. It is the combination of synaptic input and the intrinsic membrane properties of a cell that ultimately dictate the cell's response and subsequent output.

1.3.1 Discharge Patterns of Neurons in the VNLL

Two patterns of responses to tones have been observed in recordings from the VNLL. Firstly, in both bats and cats, multipolar neurons have been shown to be sharply tuned and respond to tones with regular, sustained firing throughout the stimulus duration. The second pattern consists of neurons that are broadly tuned with sharply timed action potentials locked to tone onset (Aitkin et al., 1970; Covey and Casseday, 1991; Oertel and Wickesberg, 2002). In the columnar region of the big brown bat VNLL, neurons respond to tone bursts with spikes that are locked to stimulus onset with exceptionally precise timing (Covey and Casseday, 1991).

Studies across species have displayed that the majority of neurons in the VNLL maintain either a sustained or transient response to contralateral stimuli. Although many researchers consider the VNLL to be monaural, physiological studies have shown that responses of VNLL neurons to contralateral stimuli can be modified by ipsilateral stimulation (Aitkin et al., 1970; Guinan et al. 1972a; Batra and Fitzpatrick, 1997, 1999; Zhang, personal communication January 19th 2005). The sensitivities of neural responses to interaural level and time disparities have been studied (Batra and Fitzpatrick, 1997, 1999, 2002; Covey and Casseday, 1991). Using dichotic tone bursts with interaural level disparity, Covey and Casseday found that virtually all neurons in the bat's VNLL are monaural. In contrast, using tone bursts with interaural temporal disparity, Batra and Fitzpatrick found that many neurons in the rabbit's VNLL are binaural. Research in the rat using binaural level stimuli has found that many neurons in the VNLL display EI and/or EE binaural interactions (Zhang, personal communication January 19th 2005). These findings suggest that differences may exist across species regarding the binaural interaction of VNLL neurons.

The bat is considered to have a specialized VNLL due to its echolocating abilities (Covey and Casseday, 1986). Neurons in the columnar region of the big brown bat VNLL fire a single action potential with constant latency relative to pure tone onset across a wide range of frequencies and intensities (Covey and Casseday, 1991; Huffman et al., 1998; Rosenberger et al., 2003). This type of discharge pattern provides a precise marker

for sound onset (Huffman et al., 1998). Frequency tuning curves of columnar region neurons rapidly become much broader as stimulus intensity is increased above threshold (Covey and Casseday, 1991; Vater et al., 1997). The onset type response and broad tuning curves of columnar region neurons could result from the pattern of synaptic arrangements in the subdivision. Dendrites of many columnar region neurons cross isofrequency planes and are more susceptible to inhibitory input (Vater et al., 1997). The resulting lack of frequency tuning in the columnar region suggests that this region could provide accurate representation of temporal changes in sounds (Covey and Casseday, 1991).

Characterization of neurons in the multipolar region of the VNLL is not as easy as those in the columnar region. Some multipolar region neurons respond similarly to the columnar region, while others respond with a sustained discharge pattern (Batra and Fitzpatrick, 1997; Huffman et al., 1998; Covey and Casseday, 1991). The sustained responses elicited in the multipolar region vary widely. Four response patterns that have been observed are chopper, tonic, primary-like and pauser (Covey and Casseday, 1991). Chopper neurons are characterized by spiking at regular intervals throughout the duration of the stimulus. The responses of tonic cells are poorly locked to stimulus onset and display continuous spiking throughout the stimulus. Primary-like cells respond with a decline in spiking as the duration of the stimulus elongates. The response of pauser neurons is represented by spiking to the onset of sound, followed by a momentary stop and then a resumption in firing.

Multipolar region neurons are believed to be able to convey accurate information regarding stimulus duration and intensity because they respond in a graded manner over a broad, dynamic range (Huffman et al., 1998). Unlike the frequency tuning curves of columnar region neurons, the tuning curves of multipolar region neurons become gradually broader as intensity is increased and are often symmetrical (Covey and Casseday, 1991).

Response properties of VNLL neurons in the rat are similar to those in the VNLL of the bat and the rabbit (Zhang, personal communication January 19th 2005). Zhang has demonstrated that the inherited diversity in the temporal firing patterns of cells is consistent with anatomical evidence that VNLL neurons receive excitatory inputs from various major types of neurons in the VCN (Huffman and Covey, 1995; Oertel and Wickesberg, 2002; Vater et al., 1997; Zook and Casseday, 1985). Among the VCN neurons providing inputs to the VNLL, spherical-bushy cells and globular-bushy cells exhibit primary-like responses patterns, while octopus cells display onset response patterns and stellate cells show chopping firing patterns (Rhode and Greenberg, 1992). All of these firing patterns were observed in the VNLL, suggesting that the temporal characteristics of inputs from the VCN are inherited by some VNLL neurons. The response characteristics inherent in VNLL neurons are not a simple reflection of those represented in the VCN. VNLL responses may be the result of converging excitatory

inputs from different neurons in the VCN, coupled with inhibitory inputs from the subnuclei of the trapezoidal body (Zhang, personal communication January 19th 2005).

In vitro intracellular recording methods have been utilized to study the physiological response properties of neurons in the VNLL of the rat. At the cellular level, neurons of the VNLL display two sets of intrinsic electrical characteristics. Wu (1999) studied the current-voltage relationships, firing patterns and synaptic responses of the VNLL and was able to differentiate two types of neurons; Type 1 and Type 2. Type 1 neurons responded in a sustained manner to positive current throughout the duration of the stimulus and possessed a linear current-voltage relationship. The Type 1 neurons typically display three different types of firing patterns; regular, onset-pause and adaptation. The regular firing pattern is represented by spiking throughout the duration of injected current and accounted for approximately 40 percent of all Type 1 cells. The onset-pause firing pattern is characteristic of approximately 25 percent of neurons and represented by an initial onset burst of action potentials followed by a pause and then subsequent firing throughout the stimulus. Lastly, during adaptation the number of action potentials subsides throughout the duration of the sound. The adaptation firing pattern accounted for approximately 35 percent of all Type 1 neurons. Type 2 neurons respond to positive current with limited depolarization and limited action potentials. This pattern of spiking suggests that Type 2 neurons may possess membrane properties that permit processing of information regarding the onset of a sound. The regular, onset-pause and

adaptation patterns in Type 1 neurons and the onset pattern in Type 2 neurons resemble chopper, pauser, primary-like and on responses, respectively, as defined in *in vivo* VNLL studies (Wu, 1999).

The differing linear and non-linear current-voltage relationships found between Type 1 and 2 cells are similar to the type of cells that provide input to the VNLL. The non-linear current-voltage relationship of Type 2 cells is similar to bushy cells in the VCN and principal cells in the MNTB. The linear current-voltage relationship of Type 1 cells is similar to stellate cells in the VCN and principal cells in the lateral superior olive (LSO). These findings suggest that the VNLL plays multiple roles in auditory processing and is an important integrative nucleus rather than a simple relay station connecting the lower brain stem with higher centres. While the responses of the neurons of the VNLL are similar to those of the neurons that provide input, they are not a simple reflection of these cells. Intrinsic membrane properties and a convergence of excitatory input dictate the final response of a VNLL neuron.

1.3.2 Intrinsic Membrane Properties of VNLL Neurons

In addition to the organization of synaptic inputs, the intrinsic membrane properties of a neuron play a large role in shaping the firing pattern of auditory neurons. The ion channels of a nerve cell are ultimately responsible for the cell's intrinsic properties. Specifically, voltage sensitive potassium conductances are particularly important in determining discharge patterns due to their ability to affect both action

potential duration and frequency (Li et al., 2001). Potassium conductances can therefore shape auditory responses designed to preserve the timing features of acoustic signals (Li et al., 2001).

Li et al. (2001) found a high expression of Kv3.3 potassium channel subunits in the rat VNLL. These types of channels are high voltage-activating and therefore are likely to open only in response to strong depolarizations, such as the peak of an action potential or the very large excitatory synaptic potential at calycal synapses. The rapid activation kinetics and steep voltage sensitivity of Kv3.3 subunits ensure quick opening once threshold is reached (Li et al., 2001). Kv3.3 subunits also display rapid channel inactivation via an amino terminal mechanism that essentially blocks the channel pore. The rapid inactivation minimizes the refractory period and thus, facilitates initiation of another spike. Kv3 channels are also found in many other nuclei with cells known for their narrow action potentials and high frequency firing such as those in the MNTB (Wu and Kelly, 1993).

Low threshold potassium currents such as those created by Kv1.1 subunits have been shown to contribute to precise phaselocking and transient onset responses that provide information regarding temporal features of sound in the columnar region of the big brown bat (Rosenberger et al., 2003). Prominent expression of Kv1.1 subunits have also been found in cells of other species known to respond to auditory signals with high temporal precision. The subunit inactivates slowly and acts to shorten the excitatory

postsynaptic potential duration (Li et al., 2001). Kv1.1 is capable of maintaining the membrane potential of the neuron at a subthreshold level during sustained depolarization (Li et al., 2001). As a result, the high concentration of Kv1.1 subunits coincides with the columnar region's onset-type response pattern.

The summation of excitatory and inhibitory influences and the contribution of intrinsic membrane properties result in VNLL neurons eliciting output that is uniquely different from its input. Researchers have suggested several roles for the VNLL. Firstly, it has been theorized that the VNLL creates a delay line that contributes to the circuitry that analyzes the temporal features of sound. In this case, inhibition from the VNLL provides a brief (4-5 ms) increase in latency postsynaptically (Huffman et al., 1998; Vater et al., 1997). A second suggestion is that the VNLL suppresses ongoing ICC activity in order to allow sound onset to be more prominent. In this regard, the VNLL could suppress spontaneous activity postsynaptically so that incoming auditory information becomes more conspicuous (Vater et al., 1997).

Minimal research has been conducted to evaluate the contribution of the VNLL to the detection of temporally modulated sounds at the behavioural level. Using a conditioned avoidance procedure, the goal of this thesis was to evaluate the laboratory rat's (*Rattus norvegicus*) ability to discriminate changes in duration of white noise after destruction of its VNLL. The laboratory rat served as a good candidate for this present

study: its auditory pathway is well characterized and readily accessible and it is easily trained for behavioural tests.

Based on physiological and anatomical studies, one would expect lesions of the VNLL to result in an impairment in temporal processing and a disruption in the ability of animals to discriminate between sounds that differ in duration. Determination of the contribution of the VNLL to the animal's ability to resolve temporally modulated sounds was conducted in two phases. During the first phase normative data was collected which outlined the behavioural limits of temporal resolution in the rat using varying durations of noise bursts. The second phase of the experiment involved making lesions of the VNLL and determining the effects of these lesions on the animal's ability to detect changes in stimulus duration.

2. METHODS

2.1 Subjects

Subjects were male Wistar rats obtained from Charles River Company (St. Constant, Quebec, Canada). They were approximately 70 days old, 275 grams at the beginning of the experiment and had not been involved in previous research. Rats were individually housed in the Life Sciences Building at Carleton University. The animal holding room was maintained on a 12/12-hour light/dark cycle with lights on at 8:00 a.m. and off at 8:00 p.m. Animals were water deprived, by regulated access to water during each experimental session, and had continuous access to Purina Rat Chow in their home

cages. Rats were examined and weighed daily to ensure that they were in good health. The animal's outer and middle ears were checked regularly for obstructions and/or irregularities that might have affected their ability to hear.

The experimental group, consisting of 26 animals, was tested for their ability to discriminate incremental increases in noise duration across several different standard durations. Sixteen of these animals were tested before and after lesions of the VNLL. The animals' ears were blocked using an earmold compound (see section 2.5). Eleven animals received the VNLL lesion contralateral to their unblocked ear while the other 5 had the VNLL lesion ipsilateral to their unblocked ear. As a control for the efficacy of the ear blocking technique, three additional animals received bilateral ear blocks and were tested on an absolute threshold task to determine the threshold for detection of white noise.

2.2 Apparatus

The experimental setup is illustrated in Figure 5. A computer program designed by Avalon DSP (Ottawa, ON) controlled the sound stimulus and recorded the response of subjects. A Tucker-Davis Technologies (TDT) System 3 Real Time Processor was linked to the computer to produce the sound stimulus consisting of broad band noise bursts.

The TDT unit was subsequently connected to a Lafayette constant current shocker (Model A-615AR) that delivered a pulse of current sufficient to cause the subject to withdraw from the spout upon termination of the warning trial. Current levels varied across animals, depending on a rat's size and sensitivity, ranging from 0.8 mA to 1.0 mA.

Figure 5: Schematic of the experimental apparatus. The test cage (E) and speaker (C) were located in a sound-attenuated chamber. A computer (B) was hooked up to a processor (A) that elicited the sound stimulus from the speaker. The rat stood on the metal plate (G) in the centre of the test cage and drank from the water spout (F). This completed a circuit that feed back to the computer to facilitate behavioural scoring. The circuit also initiated the water pump (D) leading to the water spout (Adapted from Heffner and Heffner, 1995).

(Adapted from Heffner and Heffner, 1995)

Psychoacoustical testing was conducted in a sound attenuated chamber (Eckel Industries, Belleville, Ontario). A Kef speaker (Model T27) was suspended within the chamber, horizontal to the cage, at a distance of approximately one meter from the front of the cage and connected to the TDT processor. The TDT processor was calibrated via a Breull & Kjaer Sound Level Meter, with a half-inch condenser microphone, to elicit 50dB SPL white noise at the spout. The dimensions of the experimental cage measured 30 x 20 x 20 cm, a spout was located 3 cm medial from the front of the cage extending 2.5 cm from the cage floor and a 13 x 8 cm metal plate was located centrally on the floor (Figure 5). By standing on the metal plate and drinking from the spout, the rat completed an electrical circuit that allowed water to be pumped via a Yale Apparatus syringe pump (Model YA-12) to the spout at a rate of 50 ml/hour. The electrical circuit allowed for both the shock to be administered and the rat's responses to be recorded. Completion of the electrical circuit allowed contact with the spout to be registered by the computer and indicated whether the rat had recognized the change in noise duration and withdrawn from the spout to avoid a shock.

The number of hits was defined as the number of times the animal accurately identified the change in noise duration and broke contact with the spout. The number of false positives was defined as the number of times the animal broke contact with the spout when there was no change in noise duration. The responses of the rat to the alterations in noise duration were stored in the computer for later analysis.

2.3 Stimuli

2.3.1 Duration Discrimination

The experiment analyzed a rat's ability to process sound in the temporal domain by manipulating the durations of noise bursts. Broad band noise served as the carrier rather than pure tones because it contains energy at all frequencies and is minimally affected by onset and offset transients.

A standard duration (D), representing a safe stimulus, served as the control to which a warning stimulus sound was compared. The duration of safe stimuli remained constant throughout the course of each experimental session. Warning stimuli consisted of D and some additional predetermined stimulus time (ΔD). As a result, warning stimuli ($D + \Delta D$) varied in duration and were longer than safe stimuli. The inter-stimulus interval (ISI), defined as the interval of silence between each stimulus administration, remained constant across all trials.

The method for measurement of hits remained consistent across all warning trials. By varying the delta durations using the method of descending limits, from longest to shortest delta duration, a threshold was obtained for each standard duration. The threshold was taken to be the delta duration at which the rat obtained a proportion correct of 0.5.

The methodology for trial administration is illustrated in Figure 6. Five hundred ms prior to trial administration, the computer verified the rat was on the spout before

Figure 6: Schematic representation of trial administration. A: computer monitoring technique used for both safe and warning trials (safe trials do not have a shock); warning trial is illustrated. Five hundred ms prior to trial administration the computer initiated a spout check (Spout Precheck) to ensure the rat was drinking from the spout. The trial consisted of two stimuli, each having a duration of $D+\Delta D$, with an interstimulus interval of 500ms (Trial), the final 200 ms of which was monitored to determine whether the animal had stopped licking from the spout (Spout Check). Upon completion of the trial, a 300 ms shock was administered to the water spout (Shock). B: representation of a trial sequence with the warning trial signal occurring on trial 3. Trial presentation was paused following a warning trial until the animal returned to the water spout.
(Heffner and Heffner, 1995)

(Heffner and Heffner, 1995)

initiating the trial sequence (Spout Precheck Figure 6). The computer program automatically paused if the rat broke contact with the spout and resumed the ensuing trial once the connection was reestablished. The warning trial lasted 2000 ms, the final 200 ms of which were monitored by the computer to determine whether the animal had detected the stimulus (Spout Check Figure 6). At the end of the warning trial, a shock was administered to the spout for 200 ms. Safe trials were administered in a similar fashion but there was no change in stimulus duration and no accompanying shock.

Warning trials consisted of two administrations of the corresponding $D+\Delta D$ stimulus. These warning trials were interspersed with differing numbers of safe trials (Figure 6). The number of safe trials administered between each warning trial was randomly chosen by the computer and ranged in value from zero to eight.

Three standard durations of 125, 250, and 500 ms served as representatives for short, middle and long durations, respectively. Psychophysical curves were then constructed for each animal before and after lesions of the VNLL, using these three values. Any deficits in threshold observed on the psychophysical curve of an animal were therefore attributed to the damage of neurons within the VNLL.

2.3.2 Absolute Intensity

An absolute threshold task was administered to each animal in order to evaluate its ability to hear the noise stimulus. The task consisted of a silent period with periodic white noise bursts as warning stimuli. The intensity of the noise stimulus varied using

the method of descending limits from high intensity to low intensity noise bursts. Psychophysical curves were constructed showing performance by intensity for each animal. The absolute threshold for each rat was defined as the intensity for which the animal received a proportion correct of 0.5. This task was utilized for two reasons: to measure absolute threshold in animals with lesions and in animals with bilateral ear blocks.

Three animals with bilateral ear blocks were tested for absolute threshold using white noise bursts against a silent background. The task was performed before and after double ear block surgery to ensure the ear block technique created a level of responding in warning trials equal to that of safe trials at 50 dB SPL. Figure 7 depicts the average shift in absolute intensity curves for these 3 animals after the double ear block surgery. The average detection threshold prior to surgery was +15 dB SPL, whereas the average threshold after the double ear block surgery shifted to +68 dB SPL. Figure 7 displays that at 50 dB SPL post surgery the animals possess a proportion correct level of 0.26. This level is equivalent to statistical chance ($p > 0.01$) (Heffner and Heffner, 1995), as defined by statistical comparison with performance expected when no noise was present (ie. safe trials). As a result, the ear block was effective in preventing the animals from detecting a sound through the occluded ear.

Figure 7: Absolute threshold shift as a result of double ear block procedure. The threshold of detection for the presence of a white noise burst against a silent background was 15 dB SPL pre-blockage and 68 dB SPL post ear block. Error bars are representative of the standard error.

2.4 Procedure

The animals in these experiments were conditioned via an avoidance procedure previously described by Heffner and Heffner (1995). They were trained to associate an increase in noise duration (duration discrimination task), and/or the presence of a noise burst (absolute intensity task), with a mild shock.

When the animal stood on the metal plate and drank from the water spout, an electrical circuit was completed. As previously mentioned the circuit both activated the water pump and provided feedback to the computer as to whether the rat had removed itself from the spout, upon detection of a duration change, to avoid shock. This feedback was then integrated by the computer into a formula: $\text{performance} = \text{hit rate} - (\text{hit rate} \times \text{false positive rate})$, where $\text{hit rate} = \text{number of hits} / \text{number of warning trials}$ and $\text{false positive} = \text{number of false positives} / \text{number of safe trials}$.

Animals were water deprived for 24 hours prior to testing and water was delivered in small amounts through a spout in the test apparatus as a reward to maintain steady contact with the spout during a daily test session. Training began by familiarizing the rat with the experimental cage. The first two days of training thus consisted of the rats being placed in the cage with a standard duration background noise (125 ms bursts). They were given free access to water from the spout with a pump rate set at 80 ml/hour to develop a strong association between the spout and water delivery. As training proceeded, the pump rate was reduced to the experimental rate of 50ml/hour to support

longer periods of time in contact with the spout. This rate was sufficient to maintain the animal at the spout for thirty minutes per session. A duration of thirty minutes per experimental session had been chosen because it allowed an adequate amount of time to obtain data while promoting steady drinking behaviour throughout the test session. The animals were then trained to develop an association between the sound stimulus and the shock. This was accomplished by presenting large stimulus changes initially (large Δ Ds in the duration discrimination task and high intensity noise bursts in the absolute intensity task), gradually making the detectable stimulus smaller and smaller (progressively smaller Δ Ds and noise intensity) until the threshold values were approached.

The following example illustrates these steps with the duration discrimination task at a standard duration of 125 ms. For the first two days of training the animals were placed in the test cage and exposed to a background stimulus at a standard duration of 125 ms. This served to develop the association that water was readily accessible from the spout in the cage. The pump rate was maintained at a high flow rate during the initial phase of the experiment. On the third day of training the animals were exposed to two obvious warning trials, administered manually by the experimenter, with a Δ D of 1000 ms. The shocker was not turned on during these warning trials. On day four, animals were exposed to five additional trials with an easily detectable warning stimulus. The shocker was then turned on and water was pumped at a rate sufficient to maintain the animal at the spout for 30 minutes. This served to develop the association between a

change in noise duration and administration of a shock. On day five, animals were exposed to ten manually administered trials with the Δ Ds becoming progressively smaller, from 1000 to 100 ms in decrements of 100 ms. Day six, the animals were tested at smaller Δ Ds, from 500 to 50 ms, and the trials were administered by the computer rather than by the experimenter. On day seven, animals were tested at Δ Ds from 125 to 25 ms in decrements of 25 ms. These values were then used throughout the remainder of the experiment with the standard duration of 125 ms until performance reached optimal level (standard error of data points < 0.1). The computer facilitated the administration of trials to the animals at random time intervals.

2.5 Surgical Procedure

Animals were anaesthetized using a cocktail of xylazine (0.5 ml/kg) and ketamine (0.1 ml/kg) injected intramuscular. A midline incision was made in the scalp, a small craniotomy performed, and the dura mater retracted. The VNLL was located using a stereotaxic instrument, and a microelectrode (15 – 20 μ m tip) filled with kainic acid (1.0 mg/ml in phosphate-buffered saline) was lowered into the middle of the VNLL (from lambda: 0.68 mm laterally, 0.08 mm rostrally, 0.75 mm depth). Two microlitres of kainic acid were released into the VNLL by pressure injection. The electrode was retracted and the craniotomy filled using Gelfoam (Upjohn Canada). Kainic acid is an excitatory neurotoxin that kills cells through over stimulation and periodically caused seizure

activity in our animals. In the event of seizure activity, diazepam (0.5 ml/kg), a tranquilizer, was injected intraperitoneally.

After the VNLL was lesioned, one of the animal's ears were blocked to create a profound hearing loss in that ear. Five animals had the ear ipsilateral to the lesion blocked, and 11 animals had the ear contralateral to the lesion blocked. This facilitated a test of whether functional deficits were related to ipsilateral or contralateral central auditory projections.

The ear block consisted of rupturing the tympanic membrane and removing the middle ear ossicles with a pair of thin forceps (Fine Science Tools, Jacksonville). The middle ear was subsequently filled with 0.7 ml of Dreve brand Otoform-A/X compound (Widex Canada), and a suture was made under the skin, around the ear canal holding the solid Otoform in place using non-absorbable sutures (Johnson and Johnson). The incision in the scalp was sutured using non-absorbable sutures (Johnson and Johnson). The animals were given 0.5 ml of acetaminophen rectally and 0.5 ml of bupivacaine topically for their analgesic properties. All animals were given two weeks to recover after surgery before being re-tested.

After completion of the experiment, animals were perfused by injection of 4% paraformaldehyde transcardially. The brains were removed and sectioned in the frontal plane at a thickness of 40 microns using a microtome, stained with cresyl violet and cover

slipped. The brains were then examined using a light microscope (Zeiss, Germany) and a qualitative analysis was conducted for neuronal loss within the VNLL.

3. RESULTS

3.1 Normal Thresholds for Duration Detection

The purpose of the experiment was to characterize the normal behavioural limits of auditory temporal resolution in the rat, by determining the thresholds for detecting an increase in the duration of a sound. Twenty-six animals were tested for their ability to discriminate between noise bursts of different durations. Figure 8 shows the mean psychophysical curve for performance at a standard duration of 250 ms and a sound pressure level of 50 dB SPL. Performance was high for large values, decreased gradually at lower values and reached a lower limit of ≈ 0.2 . The threshold as defined by the 0.5 performance level for detecting an increase in noise duration (ΔD) was 163.65 ms. The results were highly reliable as indicated by the small standard error values in Figure 8.

Figure 9 shows the mean psychophysical curves for each of the standard durations (D) studied. In every case performance levels were low (≈ 0.2) with small ΔD values and high (≈ 0.8) for large ΔD values. For all standard durations, the general level of performance fell to chance as defined by statistical comparison with performance expected when no increase was present (ie. safe trials) (Heffner and Heffner, 1995). At long ΔD s the animals maintained a high level of responding in warning trials and low

Figure 8: Psychophysical curve of all 26 animals tested at a standard duration, D , of 250 ms. The animals were tested with delta durations, ΔD of 25, 125, 225, 325 and 425 ms. The mean score at each ΔD is plotted and the discrimination threshold is taken as the point at which an animal obtains a proportion correct of 0.5. Error bars are representative of the standard error.

Figure 9: Mean psychophysical curves for all 26 animals relating performance and noise burst discrimination for standard durations between 100 and 1000 ms. Duration discrimination sensitivity decreased as the standard duration increased.

level of responding in safe trials. This is reflected in the consistently high performance of animals at long ΔD s for all standard durations.

The psychometric functions created for each standard duration had performance levels that increased from statistical chance to an asymptotic maximum. The steepest area of the curve was typically around the 0.5 performance level, which was utilized for threshold estimation. This minimized variance of the estimates of ΔD around threshold and increased the accuracy of threshold estimation. The ranges of ΔD selected were sufficient to maintain the animals under stimulus control throughout the experiment and therefore provide a valid representation of the animals' behavioural limits for duration discrimination.

The mean difference thresholds for detecting an increase in noise duration (ΔD) with standard duration values ranging from 100 to 1000 ms are plotted in Figure 10. This psychophysical curve plots the thresholds of all animals tested at each standard duration and represents the behavioural limits of the rat for detecting an increase in noise duration. The ΔD thresholds increased monotonically over the range of standard durations. The data from the rat are plotted together with those of other mammals in Figure 10. Across all species a general trend exists whereby the size of the difference threshold (ΔD) increases with standard duration over the range of D values from 100 to 1000 ms. In absolute terms, the difference thresholds obtained for the rat are similar to those reported

Figure 10: Summary psychophysical curves of thresholds for detecting an increase in the duration of a sound for the rat (current experiment), the mouse (Klink and Klump, 2004), the rhesus monkey (Sinnott et al., 1987) and humans (Abel, 1972). The increasing trend of the psychophysical curves as standard duration increases is consistent across all species. The three arrows on the figure indicate standard durations of 125, 250 and 500 ms. These standard durations were selected to represent short, middle and long durations for subsequent use in the lesion portion of the experiment.

for the mouse (Klink and Klump, 2004) but are substantially greater than those for primates (Abel, 1972; Sinnott et al., 1987). The corresponding Weber fractions, $\Delta D/D$, (Table 1) reflect this finding. Weber fractions for rats are similar to those of mice, but larger than those for humans and rhesus monkeys. All species, however, appear to have very little variation in their respective Weber fractions at different standard durations. This indicates that the difference threshold is proportional to the magnitude of D at these standard durations. In general, the increment in duration required to produce any particular level of discriminability is a constant proportion of standard duration (Church et al. 1976).

3.2 VNLL Lesions: Anatomical Results

Sixteen rats were re-tested on the duration discrimination task after kainic acid lesions of the VNLL. Once behavioural testing was completed, the brains of all animals were sectioned, stained with cresyl violet and examined to determine the extent and severity of the lesion. An example of the effects of kainic acid injection into the VNLL is shown in Figure 11. Cresyl violet stain of frontal sections taken through the VNLL on the side of the kainic acid injection shows a complete absence of nerve cells (Figure 11A). The pattern of neuronal loss within the VNLL is typical of all complete lesion cases. The majority of animals with lesions also sustained some minimal damage to the reticular formation adjacent the VNLL. This was a result of the spherical diffusion of kainic acid

Table 1: The Weber fractions ($\Delta D/D$) of various durations for the rat (current experiment), the mouse (Klink and Klump, 2004), the rhesus monkey (Sinnott et al., 1987) and humans (Abel, 1972). The Weber fractions for rats are similar to those of mice but larger than those of primates.

Table 1 - $\Delta D/D$

D	Rat	Mouse	Rhesus Monkey	Human
100	0.47	0.62	0.31	
125	0.74			
160				0.13
200		0.55	0.27	
250	0.65			
300				
320				0.11
400			0.25	
500	0.80			
600				
640				0.09
1000	0.72			0.06
1200				
Mean =	0.68	0.59	0.28	0.10

Figure 11: Sample VNLL lesion with cresyl violet staining. Boxed regions on frontal section correspond to adjacent photomicrographs. VNLL located within outlined regions of photomicrographs. SC: superior colliculus; IC: inferior colliculus; CG: central grey; VNLL: ventral nucleus of the lateral lemniscus; RF: reticular formation; PN: pontine nucleus (A) Photomicrograph of a 40 micron section through the VNLL lesion at 10 X magnification. (B) Photomicrograph of a 40 micron section through the healthy VNLL at 10 X magnification.

(A) Lesion

(B) No Lesion

upon pressure injection. The corresponding sections through the brainstem on the opposite side show a normal complement of neurons in the VNLL (Figure 11B).

3.2.1 Contralateral Lesions

Two rats (R1, R7) had no damage or cell loss within the VNLL or surrounding brainstem structures after placement of the pipette in the VNLL. The lack of any damage to the VNLL is likely due to a blockage of the glass pipette tip that prevented the delivery of any kainic acid. These cases therefore provide a control for the effects of the surgical procedure on duration discrimination.

The extent of the kainic acid lesions delivered contralateral to the healthy ear varied across the 9 animals that did have damage. As a result, animals were grouped based on the extent and placement of lesions into 2 groups; lesions that included INLL and VNLL and those that were restricted to VNLL. A reconstruction of the extent of damage of the kainic acid lesions in the 4 rats with unilateral damage of the VNLL and INLL contralateral to the healthy ear are shown in Figure 12. Areas of degeneration are superimposed on standard frontal sections through the rat's brainstem. Shaded areas represent a total absence of nerve cells and checkered areas represent regions of partial cell loss. Two of 4 rats (R4, R5) sustained complete loss of the VNLL and INLL (Figure 12), while an additional 2 rats (R12, R15) had complete lesions of the VNLL and partial cell loss in the INLL (Figure 12).

Figure 12: Representative frontal sections illustrating the extent of damage in rats with kainic acid lesions of the VNLL, INLL contralateral to the healthy ear. Shaded areas indicate complete loss of nerve cells and stippled areas indicate partial cell loss. The small numbers below each section indicate the caudal to rostral position through the VNLL in millimeters.

Rat #4

Rat #5

Rat #12

Rat #15

Five rats sustained damage restricted to the VNLL. A schematic representation of the extent and severity of the lesions for 4 rats (R8, R9, R13, R23) that had complete neuronal loss in the VNLL are shown in Figure 13. Histological analysis showed that the VNLL of these rats was completely destroyed on the side of the brain contralateral to the healthy ear. The remaining rat (R19) in this group had a partial VNLL lesion with complete cell loss in the caudal region of the VNLL but cells present in more rostral sections as indicated by arrows in Figure 13.

3.2.2 Ipsilateral Lesions

One rat (R24) had no damage or cell loss within the ipsilateral VNLL or surrounding brainstem structures after surgical intervention, likely due to blockage of the pipette tip. As a result, this case serves as a control for surgical intervention ipsilateral to the healthy ear. The location of the ipsilateral kainic acid lesions varied across the 4 animals. Three of the four animals were grouped into a VNLL, INLL lesion group. Within this group, 2 animals (R17, R26) received complete lesions of the VNLL and INLL on one side of the brain. The third rat (R10) received a complete lesion of the VNLL and partial lesion of the INLL (Figure 14A). The remaining rat (R11), the only one with a lesion restricted to the VNLL, received a partial lesion of the VNLL in the rostral region of the brainstem but no damage in areas more caudal as indicated by arrows in Figure 14B.

Figure 13: Representative frontal sections illustrating the extent of damage in rats with kainic acid lesions of the VNLL contralateral to the healthy ear. Shaded areas indicate complete loss of nerve cells. Arrows indicate areas with partial lesion. The small numbers below each section indicate the caudal to rostral position through the VNLL in millimeters.

Rat #8

Rat #9

Rat #13

Rat #23

Rat #19

Figure 14: Representative frontal sections illustrating the extent of damage in rats with kainic acid lesions of the VNLL ipsilateral to the healthy ear. (A) Kainic acid lesion localized within the VNLL and INLL. (B) Partial kainic acid lesions localized within the VNLL. Shaded areas indicate complete loss of nerve cells and stippled areas indicate partial cell loss. The small numbers below each section indicate the caudal to rostral position through the VNLL in millimeters.

(A)

Rat #10

Rat #17

Rat #26

(B)

Rat #11

3.3 Absolute Threshold Task Post VNLL Lesions

An absolute threshold task was conducted to determine the effects of a VNLL lesion on auditory sensitivity to a noise stimulus. Thirteen animals with kainic acid lesions contralateral or ipsilateral to a healthy ear were tested to determine their respective absolute thresholds for detecting a 3 s noise burst. The absolute intensity curve of R7 is displayed in Figure 15. This curve is within the range of absolute threshold for normal rats and designates a threshold, as defined by the 0.5 performance level, of 14 dB SPL. The thresholds for the remaining 12 animals are consistent with that for R7 regardless of the extent and placement of the unilateral lesion (Figure 16). The animals with lemniscal lesions displayed no deficit in their abilities to detect noise bursts within the normal range. As a result, the task served to eliminate the possibility that any potential deficits observed on the duration discrimination task could be the result of the animal's inability to detect sound.

3.4 Duration Discrimination Post VNLL Lesions

For the purpose of testing the effects of lesions on duration discrimination, the standard durations 125, 250 and 500 ms were selected as representative short, middle and long durations respectively. Figure 17 shows a sample of the mean psychophysical curves for performance at standard durations of 125, 250 and 500 ms before and after VNLL lesions combined with a unilateral ear block. The shifts in threshold were expressed as

Figure 15: Absolute threshold curves for Rat 7 after the VNLL lesion and unilateral ear block and a normal sample of animals (n=10). All experimental animals were tested to determine whether or not kainic acid lesions of the VNLL produced shifts in absolute threshold. Rat 7 was able to reliably detect an experimental white noise burst at 50 dB SPL. Error bars are representative of the standard error.

Figure 16: Mean absolute thresholds grouped based on extent and location of kainic acid lesion. Dotted line represents the mean absolute threshold of a normal sample of rats (n=10), 17 dB SPL. The shaded regions above and below the dotted line encompass a standard deviation about the mean. Error bars located on the bar graph are also representative of standard deviation.

Figure 17: Sample psychophysical curves, pre and post complete kainic acid lesion of the VNLL and ear block (R8), at standard durations of 125, 250 and 500 ms.

Standard Duration - 125 ms

Standard Duration - 250 ms

Standard Duration - 500 ms

the increase in ΔD as a proportion of the standard duration, D . Shifts shown in Figure 17 are 0.02 at 125 ms, 0.01 at 250 ms and 0.06 ms at 500 ms. A chart summarizing the difference in threshold for every animal at each standard duration can be found in Table 2.

3.4.1 Statistical Results

The effects of lemniscal lesions were analyzed by comparing the performance curves before and after surgery. A difference score was obtained for each rat by subtracting the preoperative threshold from the post-operative threshold at each of the standard durations and dividing by the respective standard duration. The magnitudes of the difference scores for rats with kainic acid lesions ipsilateral to the healthy ear did not vary greatly. Comparison between rats with no lesion (R1, R7, R24), VNLL lesion (R11) and INLL/VNLL lesion (R10, R17, R26) clearly show that animals are able to perform after surgery at a level equivalent to pre-operative levels. This would be expected because the primary projections of the cochlear nucleus are contralateral. Therefore, destruction of the VNLL ipsilateral to the healthy ear would have no effect on acoustic processing because information is only processed along the normal pathway. Any improvements observed in performance levels can be attributed to practice effects over the course of the experiment.

Table 2: Discrimination threshold shifts after VNLL lesions and ear blocks. All numbers are threshold differences as a proportion of the standard duration. Positive numbers indicate that the threshold of the animal at that standard duration decreased after the lesion and ear block. Negative numbers indicate that the threshold of the animal increased after the lesion and ear block. (A) Threshold shifts for animals with VNLL lesions made contralateral to the healthy ear. (B) Threshold shifts for animals with VNLL lesions made ipsilateral to the healthy ear.

Table 2

(A)

Discrimination Threshold Shifts Contralateral Injection

Lesion	Rat #	125 ms	250 ms	500 ms
VNLL, INLL	4		0.09	0.49
	5		0.41	0.70
VNLL, Partial INLL	12	0.14	0.11	0.23
	15	0.03	0.30	0.22
VNLL	8	0.02	0.01	0.06
	9	0.13	-0.07	0.09
	13	-0.63	0.03	-0.15
	23	-0.27	0.03	
Partial VNLL	19	-0.02	0.33	0.17
No Lesion	1		0.23	0.17
	7	0.23	0.18	-0.58

(B)

Discrimination Threshold Shifts Ipsilateral Injection

Lesion	Rat #	125 ms	250 ms	500 ms
VNLL, INLL	17	-0.60	-0.04	
	26	-0.20	0.20	
VNLL, Partial INLL	10	0.05	0.42	0.11
Partial VNLL	11	0.28	-0.16	-0.37
No Lesion	24	0.27	-0.29	0.16

Comparisons were also made within the contralateral group for animals with no lesion (R1, R7, R24), VNLL lesion (R8, R9, R13, R19, R23) and INLL/VNLL lesion (R4, R5, R12, R15) respectively. A Kruskal-Wallis nonparametric ANOVA showed no differences between groups at 250 ms ($H=1.47$, $p>0.05$). At 500 ms there was a significant difference between groups ($H=7.05$, $p=0.03$). Thus, at 500 ms there was an effect of the lesion, which was limited to the long duration noise bursts. Lesions restricted to the VNLL appear to have little effect on the duration discrimination abilities of the animals, whereas lesions that included both the VNLL and cells of the INLL resulted in deficits at longer durations.

4. DISCUSSION

The behavioural limits of the rat's ability to discriminate an increase in the duration of a noise burst provides basic information regarding temporal processing in this species. The results obtained indicate that discrimination thresholds for duration in the rat increase monotonically with standard duration, a trend consistent with other mammals (Klink and Klump, 2004; Sinnot et al., 1987; Abel, 1972). A valid and reliable description of the behavioural limits of duration discrimination is necessary to develop a comprehensive understanding of temporal processing in the rat. These results will therefore complement existing anatomical, behavioural and physiological knowledge to help further clarify the current understanding of temporal processing in the auditory system (Casseday et al., 2000; Malmierca, 2003; Kelly et al., submitted).

Electrophysiological data from the bat have suggested that the VNLL plays a role in temporal processing by providing a major source of inhibitory input to the IC that shapes neuronal responses to temporal auditory stimulation. Specifically, the duration tuning model proposed by Casseday et al. (2000) states that the VNLL plays a significant role in shaping temporal response patterns in the IC by creating delay lines that facilitate the measurement of sound duration. The current study explored the contribution of the VNLL to the rat's ability to complete a duration discrimination task. In contrast to propositions made for the bat (Casseday et al., 2000), the present results suggest that inhibitory projections to the IC from the VNLL only moderately influence duration processing in the rat.

4.1 Behavioural Limits of Normal Rats

The psychophysical curve obtained for standard durations ranging from 100 to 1000 ms revealed a monotonic relation, with difference thresholds that increase as a function of the standard duration. Similar to other mammals (Fay, 1988), the increases in difference thresholds are proportional to the standard duration for durations >100 ms.

It has been observed in humans (Buus, 1997) and mice (Ehret, 1976) that at durations ≤ 100 ms, the duration discrimination task can become a loudness task if the sound pressure level of the stimulus is held constant. This phenomenon occurs as a result of temporal summation and allows animals to discriminate stimuli based on the perceived sound pressure level. Essentially, the animal's sensitivity to sound increases with sound

duration and results in a reduction of the animal's absolute threshold for hearing (Moore, 2003). The data obtained in the current experiment at a standard duration of 100 ms (threshold = 48 ms) may therefore reflect the animals' perception of an increasing increment in loudness due to temporal summation.

The thresholds for detecting an increment in noise duration in the rat are similar to the results reported in the mouse (Klink and Klump, 2004). The threshold for duration discrimination in the mouse at a standard duration of 250 ms was approximately 148 ms, whereas the rat had a discrimination threshold of 163 ms. The Weber fractions for these animals remain relatively constant across the examined range of standard durations (>100 ms). The consistency of the Weber fractions indicates that the thresholds of discrimination obtained for these values are a reflection of the rat's ability to discriminate the duration of the stimulus, independent of temporal summation. Church et al., (1976) reported considerably larger discrimination thresholds for duration in the rat. Specifically, the Weber fraction at 480 ms was 1.55. This inflated Weber fraction could be the result of sampling error considering the small number of animals tested in their study (n=3). The results also suggest that the animals were not under adequate stimulus control during the experiment.

Although the discrimination threshold curve of the rat displays a trend consistent with other mammals, the absolute values for threshold are high compared to those for primates (Sinnott et al., 1987; Abel, 1972). One possible explanation is that differences

exist among species in their ability to detect an increase in duration, as a result of differing selective pressures. Another possible explanation is that the reported species differences might be complicated by procedural and methodological factors (ie. stimulus frequency and intensity levels). Kelly et al. (submitted) and Syka et al. (2002) have investigated the effects of stimulus parameters on detection thresholds for temporal processing tasks. Kelly et al. reported that the magnitude of difference thresholds in a duration discrimination task was dependent on the sound pressure level of the stimulus. Specifically, as the intensity of the stimulus increased, the corresponding detection thresholds decreased proportionally. Syka et al. showed that the threshold of detection for a silent gap in sound varied depending on the intensity and frequency of the stimulus. Therefore, it is possible that small differences in stimulus parameters across studies might make some contribution to the species differences seen in psychophysical curves. Further studies are needed to determine whether cross-species comparisons are affected by differences in the quality of experimental stimuli.

4.2 VNLL Lesions: Absolute Threshold

Audiograms for the albino rat have indicated that the absolute thresholds for tones range from 0 to 70 dB SPL across the rat's normal frequency hearing range (0.5 to 50 kHz) (Kelly and Masterton, 1977). The present study showed the threshold of detection for a white noise sound burst in a silent background to be 17 dB SPL in normal animals (n=10). This threshold fits within the intensity range described by the audiogram and

therefore appears to be a reasonable representation of the rat's ability to perform the absolute threshold task.

Kainic acid lesions of the VNLL did not produce a shift in absolute threshold for detection of a white noise sound burst. Absolute threshold curves for animals with kainic acid lesions fell well within the normal range of audibility for the rat. It can be concluded that unilateral lesions of the VNLL do not produce general auditory deficits in the rat. This result is not unexpected because the kainic acid lesions destroy cells while preserving the fibers of passage in the auditory pathway (Rooney et al., 1991; Kelly et al., 1996). When the VNLL is lesioned, the primary projection paths of lower structures such as the cochlear nucleus and superior olive complex are still preserved. Therefore processing of auditory signals, such as intensity, are still carried out by the central auditory system.

4.3 Emotional and Motor Responses After Lesions of the VNLL

It is possible that deficits in performance, following brainstem lesions, may be due to emotion or motor dysfunctions. However, the results of our study show this is not the case. The fact that all animals in the absolute threshold task performed at a level similar to the normal group after they had received lesions of the VNLL shows that they were capable of high levels of performance without showing any impairment in motor or emotional response. As a result, it is reasonable to assume that any deficits that would be observed on subsequent tasks involving kainic acid lesions of the VNLL would reflect an

animal's inability to perform a specific sensory task (ie. duration discrimination) and would not be due to the animal's inability to make a general emotional or motor response.

4.4 VNLL Lesions: Duration Discrimination

Animals were tested further in an experiment to evaluate duration processing abilities at short, middle and long standard durations before and after lesions of the VNLL. VNLL lesions were made either contralateral or ipsilateral to the healthy ear. The results indicate that although the VNLL is not vital in itself for duration discrimination, lesions of the VNLL and INLL do produce marginal deficits at longer durations (500 ms).

4.4.1 VNLL Lesions Do Not Produce Threshold Shifts

Electrophysiological studies in the frog (Gooler and Feng, 1992), the bat (Casseday et al., 1994; Ehrlich et al., 1997), the mouse (Brand et al., 2000) and the chinchilla (Chen 1998) have shown that the IC is the lowest auditory structure that has neurons tuned to specific durations. Studies in the big brown bat have suggested that the VNLL is an important source of inhibition for shaping the responses of duration tuned IC neurons (Casseday et al., 2000). It is believed that the various first-spike latencies of neurons in the VNLL provide neurons in the IC with inhibitory signals at different times. Casseday et al. believe that this innervation facilitates encoding of specific time delays regarding the duration of sound by target neurons in the IC. The importance of VNLL input for duration tuned IC neurons in the big brown bat is supported by pharmacological

studies in which bicuculline and strychnine eliminated or drastically altered duration tuning (Casseday et al., 2000). As a result, the VNLL is thought to shape the firing patterns of IC neurons in response to stimuli of varying durations.

Based on this logic, one would expect to observe severe deficits in an animal's ability to perform a duration discrimination task after destruction of the VNLL. This was not observed in the current study, as the ability of the rat to perform the duration discrimination task did not change after kainic acid lesion of the VNLL. This was consistent across all complete VNLL lesions contralateral or ipsilateral to the healthy ear.

The discrepancy between the current result and the proposition made for duration-tuning by Casseday et al. (2000) does not rule out the possibility that the VNLL may play some role in the processing of durations not tested in the present study. The VNLL may in fact only be utilized in the tuning of IC neurons to very short sound durations.

Casseday et al. (1994) have shown that neurons in the IC of the big brown bat are mainly tuned to very short stimulus durations. These short durations, which range from 2 to 10 ms, are representative of the durations utilized in echolocation calls. The IC neurons of the mouse (Brand et al., 2000) and the corresponding area in the frog (Gooler and Feng, 1992) however are tuned to longer durations that reflect each animals' respective vocalization repertoire. These differences indicate that species specificity exists in the duration tuning of IC neurons. Long sound durations extend beyond the scope of delay

lines facilitated by first-spike latencies in the VNLL and therefore require a different neural mechanism for duration processing.

Similar to the mouse and the frog, the rat has a range of biologically relevant vocalizations that are significantly longer than that of the bat. Vocalizations in adult laboratory rats have been shown to range from 20 to 600 ms, with a mean duration of approximately 300 ms (Brudzynski et al., 1993). It would be expected that if duration tuning occurs in the IC of the rat, then many IC neurons would be tuned to durations longer than those observed in the bat. Therefore the hypothesis proposed by Casseday et al. (2000) explaining tuning for short durations (2 to 10 ms) in the bat is likely unsuitable to account for duration tuning within the rat's normal vocalization range.

The durations of white noise bursts utilized in the present study (125, 250 and 500 ms) encompassed the duration range of rat vocalizations and did not account for the rat's resolution abilities at very short durations. Durations shorter than 125 ms were not examined and therefore it cannot be determined whether or not deficits might occur at these shorter durations. Further studies are needed to determine whether the VNLL has a significant role in duration processing in the rat at durations <125 ms.

4.4.2 INLL/VNLL Lesions Produce Marginal Duration Threshold Shifts

Kainic acid lesions of the VNLL and INLL produced marginal deficits in the ability of the rat to perform a duration discrimination task at a standard duration of 500 ms. This result suggests that cell bodies within the INLL could be of importance to

duration discrimination at longer durations. Data however were not obtained at a standard duration of 500 ms for animals with complete INLL and VNLL lesions ipsilateral to the healthy ear. Therefore, a comparison of the effect of lesions ipsilateral and contralateral was not possible for this duration. It was observed at all other standard durations (125, 250 ms) that ipsilateral and contralateral lesion cases did not differ greatly. Future research will need to be conducted in order to determine whether the deficit at longer durations (ie. 500 ms) is specific to the contralateral projection pathway.

Brand et al. (2000) have suggested the feasibility of binaural structures, such as the superior olivary complex, in facilitating duration processing. It is therefore possible that the INLL or other binaural structures such as the dorsal nucleus of the lateral lemniscus (DNLL) may play some role in duration processing. Currently no behavioural or electrophysiological studies have been conducted investigating the role of the INLL or DNLL in an animal's ability to perform a temporal processing task. Further studies are needed to determine whether these binaural lemniscal structures are involved in the temporal encoding of sound duration.

4.5 Future Directions

Future research on the VNLL should consider its possible role in discrimination of shorter durations. In order to conduct experiments with short duration sounds, it is necessary to study the effects of temporal summation on difference thresholds in the rat. The construction of a temporal summation curve would allow one to balance the effects

of temporal summation by making the necessary adjustments to the intensity of shorter sounds. As a result one could correct for the changes in loudness and resolve the behavioural limits of duration discrimination in the rat at very short durations. By doing this one could further investigate the role of the VNLL in the rat for duration processing as it relates to the theory proposed by Casseday et al. (2000).

A modification of the present study to be used in future VNLL lesion experiments would involve adjustment of the lesion technique. The current methodology involves surgical intervention with a glass pipette along the dorsal-ventral axis of the VNLL followed by a single pressure injection of kainic acid. The kainic acid is discharged at the mid-point of the VNLL and allowed to diffuse outward in a spherical fashion. This pattern of release results in a spherical region of neuronal loss within the brainstem. The VNLL however is not a spherical structure but rather a long curved cylinder. Therefore in order to ensure sufficient cell loss through the extent of the VNLL, a large amount of kainic acid must be injected into each animal. To allow for more precise damage, an injection technique utilizing a series of two or three injections along the dorsal-ventral axis is proposed. This would allow the experimenter to minimize the amount of kainic acid administered to the animal and improve the reliability of lesions.

Another area of interest regarding duration-tuning would be to conduct both behavioural lesion and electrophysiological studies investigating the roles of other structures such as the INLL, DNLL and the dorsal cortex of the IC (ICD). The ICD is of

particular interest because electrophysiological studies in the ICD have displayed offset and onset-offset firing patterns in response to noise bursts 100 ms in duration (Zhang et al., 2005). These patterns of firing are good indicators of sound duration because they provide an accurate representation of the beginning and end of the sound envelope.

4.6 Conclusions

In conclusion, it was found that the rat's behavioural thresholds for discriminating incremental increases in the duration of white noise bursts were similar to those found in other mammals; they increased monotonically with standard duration. The absolute values for the rat were similar to those of the mouse, but were larger than those for primates. Lesions ipsilateral to a healthy ear did not produce severe impairments in absolute threshold or duration discrimination. Kainic acid lesions of the VNLL contralateral to a healthy ear also produced no deficits in the rat's ability to perform an absolute threshold task or duration discriminations at standard durations of 125, 250 and 500 ms. A small deficit in duration discrimination was observed however, at 500 ms, but only when VNLL lesions were extended to include the INLL. In general, the effects of VNLL and INLL/VNLL lesions were less severe than anticipated.

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