BEHAVIOURAL LIMITS OF AUDITORY TEMPORAL RESOLUTION IN THE RAT: SINUSOIDAL AMPLITUDE MODULATION DETECTION AND THE ROLE OF THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS

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James E. Cooke

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Abstract

Temporal features of sound are important for relaying information regarding sound sources. Sinusoidal amplitude modulated sounds are an example of temporally modulated stimuli. The current thesis included two studies: one to outline the ability of the normal albino rat, *Rattus norvegicus*, to detect the presence of sinusoidal amplitude modulated noise across different modulation frequencies. The second study examined the contribution of the ventral nucleus of the lateral lemniscus (VNLL) to the rat's ability to process sinusoidal amplitude modulated noise. After outlining the behavioural limits of the rat to detect the presence of sinusoidal amplitude modulation, it was revealed that the VNLL contributes to the rat's ability to detect sinusoidal amplitude modulation. It was found that complete lesions of the VNLL resulted in large deficits in the rat's ability to detect amplitude modulation at high modulation frequencies, and that the VNLL is monaural in nature, driven from the contralateral side.

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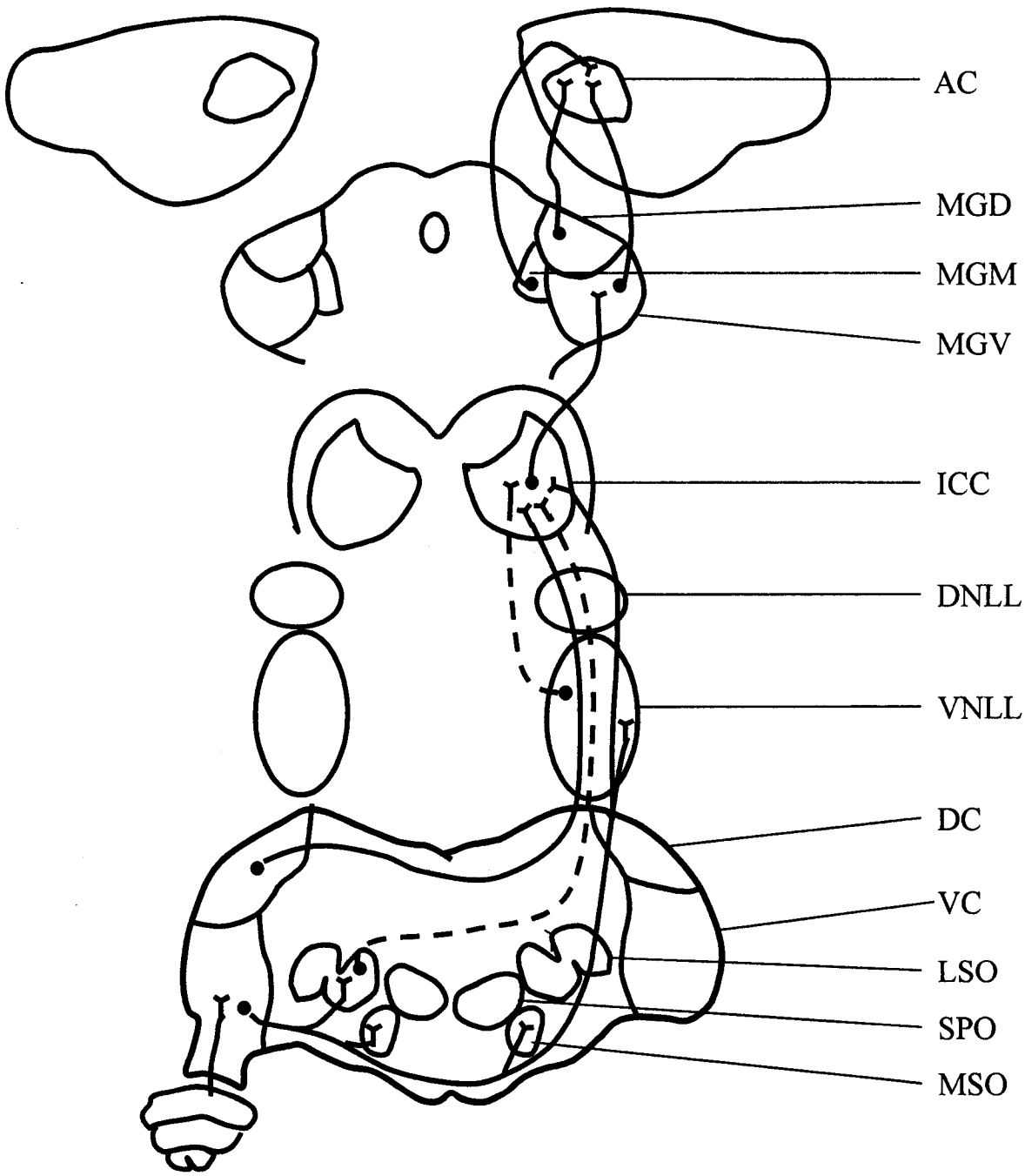
1. Introduction

An animal's ability to process sounds helps it to make sense of its surroundings, detect predators or prey, and interact with other members of its species. Sounds are analyzed by the auditory system in spectral, spatial or temporal domains. Temporal features of sound are changes of acoustic parameters over time. The central auditory system has several means by which it analyzes temporal changes in sound stimuli. The anatomical arrangement and physiological properties of neurons, including the intrinsic membrane properties of individual neurons and synaptic interactions that help shape neuronal responses, dictate how the incoming information will be modified.

The ventral nucleus of the lateral lemniscus (VNLL) is a collection of cells in the auditory brainstem (Figure 1). Recent findings have implicated the VNLL in temporal processing. Covey and Casseday (1986; 1991) found that neurons of the VNLL of the bat all have characteristics that make them well suited to encode temporal information: little spontaneous activity, broad tuning curves and short integration times. Additionally, these researchers have shown that neurons within the VNLL with different response types (onset, chopper and pauser) all converge to one specific area within the central nucleus of the inferior colliculus. Covey and Casseday believe that the convergence of different response types onto a specific area or neuron could provide the target cell with specific time delays or temporal patterns. These findings are supported by the discovery of neurons in the central nucleus of the inferior colliculus of the cat that are selective for specific rates of amplitude modulation (Langner and Schreiner, 1988).

Figure 1: The central auditory system of the rat. This figure is meant to reveal the location of nuclei within the central auditory system and to illustrate the connections that exist within the central auditory system. AC: auditory cortex; MGD: dorsal division of the medial geniculate nucleus of the thalamus; MGV: ventral division of the medial geniculate nucleus of the thalamus; MGM: medial division of the medial geniculate nucleus of the thalamus; ICC: central nucleus of the inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; LSO: lateral superior olive; MSO: medial superior olive; SPO: superior paraolivary complex; DC: dorsal cochlear nucleus; VC: ventral cochlear nucleus. Auditory connections are shown from the left cochlea, projecting to the contralateral side only. Dashed lines indicate inhibitory connections, solid lines indicate excitatory connections.


AC
MGD
MGM
MGV
ICC
DNLL
VNLL
DC
VC
LSO
SPO
MSO

While several studies have examined the physiological responses of neurons of the VNLL to temporally modulated sounds, no behavioural studies have shown a contribution of the VNLL to temporal processing. The purpose of the following study is to determine the contribution of the VNLL to the detection of temporally modulated sounds using a behavioural design. The experiment will be conducted on the laboratory rat, *Rattus norvegicus*, which is an ideal subject for several reasons: it is easily trained for behavioural tests and its auditory pathway nuclei are easily accessible.

In order to establish the contribution of the VNLL to the animal's ability to perform a task, normative data must first be obtained. Therefore, the first part of this experiment will be to define the behavioural limits of temporal resolution by the rat using sinusoidal amplitude modulation of a white noise carrier as a stimulus. Sinusoidal amplitude modulation involves changing the intensity of a sound over time, and as such is an adequate stimulus for investigating temporal processing abilities.

The second part of the experiment will involve making lesions of the VNLL and determining how these lesions affect the animal's ability to detect the presence of the sinusoidal amplitude modulation. Deficits in threshold after lesions are made can be attributed to the absence of the VNLL.

In order to illustrate why the VNLL is such an ideal candidate for temporal processing, it is necessary to review how animals process temporal information, and how the VNLL is anatomically and physiologically arranged.

1.1 Temporal Processing

In order to test an animal's ability to resolve temporal aspects of sound, researchers must use a stimulus that changes over time. Periodic changes in sound intensity, such as those produced by sinusoidal amplitude modulation (SAM) of a sound, are widely used to test an animal's ability to process temporal acoustic information (Frisina, 2001; Viemeister, 1979). In previous behavioural studies researchers presented SAM stimuli to an individual and measured their modulation threshold: the depth of modulation necessary to just allow discrimination of modulation (Viemeister, 1979). This modulation threshold provides an objective measure of temporal processing ability (Viemeister, 1979). In physiological studies researchers presented SAM tones to an animal while recording from neurons of its central auditory system. This allows researchers to study how individual neurons encode and process information about temporally modulated sounds.

The ability of single neurons to encode temporal information has been studied by examining the synchronization of firing to the envelope of a stimulus, as well as the strength of the response (Frisina, 2001). Cells that relay the temporal features of a sound with higher synchrony are well suited to encode information regarding the temporal structure of sound (Frisina, 2001; Rosenberger *et al.*, 2003). Different cellular response types relay different information about the stimulus: sustained firing indicates real-time information regarding the duration of a sound stimulus; transient firing sends onset or offset information about the stimulus.

Before making assumptions about the contribution of the VNLL to temporal processing, it is important to review the anatomical and electrophysiological information regarding the possibility of the VNLL being a temporal processing nucleus.

1.2 Anatomy of the VNLL

The nuclei of the lateral lemniscus are composed of cells that lie along the course of, and intermingle with, fibers of the lateral lemniscus as they follow a ventral to dorsal path from the superior olivary nuclei to the inferior colliculus (Malmierca *et al.*, 1998; Aitkin *et al.*, 1970). In the rat there are two areas of the lateral lemniscus, the dorsal nucleus of the lateral lemniscus (DNLL) which is binaural, and the ventral nucleus of the lateral lemniscus (VNLL) which is mainly monaural (Aitkin *et al.*, 1970). Some researchers believe that the dorsal end of the VNLL is a distinct nucleus called the intermediate nucleus of the lateral lemniscus (INLL) (Covey and Casseday, 1991; Vater *et al.*, 1997). However, there is no obvious distinction between INLL and VNLL in the rat on the basis of cytoarchitecture (Kelly *et al.*, 1998; Merchan and Berbel, 1996), therefore this paper shall henceforth refer to the entire lateral lemniscus ventral to the DNLL as the VNLL.

1.2.1 Cytoarchitecture

Merchan and Berbel (1996) suggest that the topographical arrangement of the VNLL, a helicoid pattern, may result in different orientations of neurons and give the appearance of varying cytoarchitectural shapes. The researchers also suggest that there may be several subtypes of stellate cells located in the VNLL. These different subtypes might have variations in their cellular membranes giving rise to differences in their physiological response properties and immunoreactivity to glutamic acid decarboxylase (Moore and Moore, 1987). The results of the Merchan and Berbel experiment are compelling, as they were able to indicate a single morphological cell type based on 3-D reconstructions. The 3-D reconstructions not only show a single morphological cell type

in the VNLL, but are able to explain results from previous research which seemed, at times, contradictory: the group were able to explain the discovery of several different neuronal subtypes in other animals as being due to the helicoid configuration of the VNLL, and the orientation of cells within this helix can cause the cells to appear different from each other.

The VNLL of the insectivorous bat is separated into two regions, one called the 'columnar region' and the other called the 'multipolar region' (Covey and Casseday, 1986). Each region of the bat VNLL has its own unique cell morphology. The columnar region, or VNLLc, is comprised of neurons that are very similar to spherical bushy cells of the anteroventral cochlear nucleus (AVCN), round to ovoid in shape. The multipolar area, or VNLLm, contains cells that are multipolar in shape. These findings are different from those of the rat, in which only one morphological cell type, flat stellate cells, is found (Merchan and Berbel, 1996). However, the results found in the bat may not be incompatible with those in the rat. The helical arrangement of cells within the VNLL may explain the apparent differences of cellular morphology of the bat. Where it appears that there are two different cell types, there may in fact be one cell type with two different orientations.

1.2.2 Topography

The topography of the VNLL differs substantially between species. For example, the VNLL of the rat is less sophisticated than that of the bat. When stained with cresyl violet, the rat VNLL appears to contain cells that are scattered without any specific orientation. Retrograde labelling has shown that the cells of the VNLL are arranged in a helicoid, or staircase distribution consisting of fibrodendritic laminae (Merchan and

Berbel, 1996; Kelly *et al.*, 1998). The cell bodies of VNLL neurons form a peripheral tube through which the axons of other structures, such as the cochlear nucleus or the superior olivary complexes, pass (Merchan and Berbel, 1996). These ascending axons have been shown to send collaterals into the VNLL, thereby allowing them to interact with the fibrodendritic laminae (Merchan and Berbel, 1996).

The rat VNLL receives input mainly from the contralateral cochlear nucleus (Koch and Grothe, 2000; Covey and Casseday, 1986; Covey and Casseday, 1991; Wu, 1999), although some smaller projections from the ipsilateral ventral cochlear nucleus and superior olivary complex have been seen (Wu, 1999). Specifically, thick fibers from octopus cells of the posteroventral cochlear nucleus enter the VNLL and terminate with boutons similar to calyces of Held (Wu, 1999). Bushy and multipolar cells of the ventral cochlear nucleus also project to the VNLL in the rat (Wu, 1999).

The VNLL of the rat projects to the entire central nucleus of the inferior colliculus (ICC) (Zhang *et al.*, 1998; Koch and Grothe, 2000; Kelly *et al.*, 1998). Retrograde tracing studies have shown that the 'staircased' appearance of the fibrodendritic laminae of the VNLL is only apparent after injection of the tracer into the low frequency areas of the ICC (Merchan and Berbel, 1996; Kelly *et al.*, 1998). This banding pattern is much less pronounced when retrograde tracers are placed in tonotopically high or middle regions of the ICC (over 20 KHz) (Kelly *et al.*, 1998). The fact that the banding pattern is seen in the VNLL only after injections of a retrograde tracer in the low frequency areas of the ICC raises the question of whether or not the VNLL is tonotopically organized.

The VNLL of the bat has a complexity of organization unmatched in other mammals (Covey and Casseday, 1986). In fact, the VNLL of insectivorous bats is much

more developed than that of frugivorous bats (Metzner and Radtke-Schuller, 1987). As in most mammalian species, the contralateral cochlear nucleus (CN) is the main source of projections to the VNLL (Covey and Casseday, 1986; Covey and Casseday, 1991; Zook and Casseday, 1987; Batra and Fitzpatrick, 1997; Cant and Benson, 2003; Metzner and Radtke-Schuller, 1987). In the bat, it is the ventral cochlear nucleus (VCN) specifically that provides these contralateral projections (Zook and Casseday, 1987). Retrograde tracing studies reveal that the CN also provides the medial nucleus of the trapezoid body (MNTB) with input, which in turn projects to the VNLL (Zook and Casseday, 1987; Vater *et al.*, 1997).

The VNLL in the bat is segregated into two distinct subdivisions based on topography, cytoarchitecture and physiology: a columnar division (VNLLc) and a multipolar division (VNLLm) (Covey and Casseday, 1986; Covey and Casseday, 1991; Huffman *et al.*, 1998). The VNLLc and VNLLm both receive input from the contralateral anteroventral cochlear nucleus (AVCN) as well as posteroventral cochlear nucleus (PVCN), but the projection to the VNLLc seems slightly more complicated (Huffman and Covey, 1995; Covey and Casseday, 1991). In the VNLLc, there exists a three-dimensional matrix of cells in which projections from the AVCN converge onto sheets of cells, each sheet being one cell thick (Covey and Casseday, 1991). These cells are innervated by excitatory calyceal endings resembling the end bulbs of Held in the AVCN or the calyces of Held in the MNTB (Vater *et al.*, 1997; Covey and Casseday, 1991). There is likely considerable convergence from AVCN to sheets of cells in the VNLLc. There are roughly 20 stacks of cells one cell thick, suggesting that each sheet

represents 4 KHz (Covey and Casseday, 1991). The VNLLm is less defined than the VNLLc, as the cells are not organized into sheets in a tight coil.

Both the VNLLc and the VNLLm are bounded on the medial and lateral sides by the ascending fibers of the lateral lemniscus (LL). Some of these fibers send collaterals to the VNLL, entering at right angles to the ascending fibers of the LL (Covey and Casseday, 1991). The rest of the fibers bypass the VNLL and enter the inferior colliculus. In this way parallel processing channels are established where certain features of acoustic stimuli are abstracted and preferentially coded at the expense of other features (Frisina, 2001). An example of this is the monaural pathway of the lateral lemniscus versus the binaural pathway of the superior olivary complex (SOC), where the SOC pathway preferentially codes for spatial information and the LL pathway preferentially codes for temporal information (Covey and Casseday, 1991). The auditory information is sent to higher structures in the auditory system via two pathways that each code preferentially for one type of stimulus over another.

The VNLL of the bat, as with most mammals, projects to the central nucleus of the inferior colliculus (ICC) on the ipsilateral side (Malmierca *et al.*, 1998; Covey and Casseday, 1986; Zook and Casseday, 1987; Zhang *et al.*, 1998; Koch and Grothe, 2000; Kelly *et al.*, 1998; Batra and Fitzpatrick, 1997; Ross and Pollack, 1989). Specifically, the VNLL of the moustache bat projects to the dorsoposterior division of the ICC, in which 60 KHz sounds are tonotopically represented (Ross and Pollack, 1989). Frequencies around 60 KHz are of particular importance for the moustache bat, as it uses these frequencies for echolocation.

The cat is a mammal whose VNLL has been divided into 3 subdivisions based on cell orientation (Malmierca *et al.*, 1998) which, according to Merchan and Berbel (1996), can be attributed to the helicoid pattern of arrangement. Due to the helix formation of the VNLL, the cells in the ventral and middle portion are vertically oriented, while those in the dorsal third are rostrocaudally oriented (Malmierca *et al.*, 1998). The helix of the VNLL is formed by external and internal fibers: the external fibers form a peripheral tube of cells, while the internal fibers are ascending axons of the peripheral cells (Malmierca *et al.*, 1998).

Like the bat, the VNLL of the cat receives input from the cochlear nucleus of the contralateral side (Smith *et al.*, 1991; Smith *et al.*, 1993; Whitley and Henkel, 1984). Specifically, the anteroventral and posteroventral cochlear nuclei (AVCN and PVCN, respectively) have been shown to project to the contralateral VNLL in the cat (Smith *et al.*, 1993; Whitley and Henkel, 1984). Retrograde tracing experiments have revealed that globular bushy cells, multipolar cells, and octopus cells from the ventral cochlear nucleus, as well as spherical bushy cells of cat AVCN, sent projections to the VNLL on the contralateral side (Smith *et al.*, 1993). The globular bushy cells send collateral axons to the ventral nucleus of the trapezoid body (VNTB) and lateral nucleus of the trapezoid body (LNTB) on the contralateral side, which in turn send feedback to the cochlear nucleus (Smith *et al.*, 1991).

After receiving projections from the contralateral cochlear nucleus, the cat VNLL sends projections throughout the central nucleus of the inferior colliculus (ICC) (Whitley and Henkel, 1984). However, it also sends projections to dorsal nucleus of the inferior colliculus (ICD) (Whitley and Henkel, 1984). In fact, the projections of the VNLL of the

cat imply that there may be different subdivisions within the VNLL. The dorsal area of the VNLL projects to medial and central areas of ICC, and deep layers of ICD (Whitley and Henkel, 1984). While dorsal and ventral VNLL both send some projections to the medial geniculate nucleus of the thalamus (MGN), descending projections to periolivary regions and ipsilateral projections to the DNLL (Whitley and Henkel, 1984). Whitley and Henkel (1984) believed that the tracts running through the VNLL were as follows: one track from the superior olivary complex (SOC) to the dorsal VNLL, and to deep dorsal areas of ICD; another track from contralateral AVCN and PVCN to the middle area of VNLL to the ICC. The first, binaural track involving the dorsal VNLL may in fact have been the intermediate nucleus of the lateral lemniscus (Merchan and Berbel, 1996).

1.2.3 Tonotopic Organization of the VNLL

The VNLL has never clearly demonstrated a laminar, tonotopic organization (Malmierca *et al.*, 1998). Studies have suggested that there may be some isofrequency planes within the rat VNLL corresponding to pure tone frequencies (Kelly *et al.*, 1998; Friauf, 1992), but these have not been conclusive.

The columnar area of the bat VNLL (VNLLc), unlike the rat VNLL, has shown a tonotopic organization (Covey and Casseday, 1986; Covey and Casseday, 1991). The VNLLc of the insectivorous, echolocating bat has a tonotopic map with low frequencies represented dorsally and high frequencies represented ventrally (Covey and Casseday, 1991; Covey and Casseday, 1986). The multipolar area of the bat VNLL (VNLLm) has a trend of low frequencies represented medially and high frequencies represented laterally, but this has never been clearly shown (Covey and Casseday, 1991). Interestingly, the

VNLL of the cat has shown similar trends to that of the VNLLm of the bat. The cells of the cat VNLL are arranged in clusters with similar frequencies, such that low frequencies are represented medially and high frequencies are represented laterally (Malmierca *et al.*, 1998).

Having reviewed the anatomical arrangement of neurons within the VNLL, it is now prudent to review the physiological responses of cells of the VNLL to help define the role of the VNLL further.

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 a nucleus, and on the physiological properties of those neurons. The response patterns of a cell, and subsequent transfer of information to other cells, depend on intrinsic membrane properties of a cell as well as the synaptic input to the cell.

1.3.1 Discharge Patterns of Neurons in the VNLL

The physiological response properties of neurons within the VNLL of the rat have been studied using *in vitro* intracellular recording methods. Specifically, the lab of Dr. Wu (1999) has been able to identify two types of cell in the rat VNLL based on physiological response properties, called 'type 1' and 'type 2'. Type 1 cells respond in a sustained manner to injected current, while type 2 cells respond with an onset response to injected current. Of the type 1 cells, there existed several different firing patterns. Forty two percent of type 1 cells had a 'regular' firing pattern, in which action potentials are present throughout the duration of the injected stimulus. Thirty seven percent of type 1 cells had 'adaptation' responses, in which the number of action potentials diminishes

during the current injection. Twenty five percent of type 1 cells had 'onset-pause firing' patterns, in which a pause occurs after the initial onset response (consisting of one or a few spikes), followed by continued firing after the pause. This pause in the firing of the neuron was seen at lower current values, but the pause disappears at current values greater than 1 nA. All type 1 cells discharged continuously for the entire duration of the current stimulus and have a linear current-voltage relationship.

Type 2 cells respond to positive current with a single action potential and have a non-linear current-voltage relationship. As the current injected to the cell increase, the result in cell membrane potential was that it remained constant. The cell quickly returned to its resting potential after firing an action potential. Type 2 cells made up 23.2% of all cells in the VNLL.

Both type 1 and 2 cells were found throughout the VNLL. The distinction between type 1 and 2 cells, specifically in their linear and non-linear current-voltage relationships, is 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 ventral cochlear nucleus and principle cells of the medial nucleus of the trapezoid body. The linear current-voltage relationship of type 1 cells is similar to stellate cells in the ventral cochlear nucleus and principal cells of the lateral superior olive. While the responses of the neurons of the VNLL are similar to those of the neurons that provide them with input, the responses of the VNLL neurons are not a mere reflection of the cells that provide them with input. Intrinsic membrane properties and a convergence of excitatory and inhibitory input dictate the final response of a VNLL neuron.

The bat has the most specialized VNLL among mammals due to its echolocating needs (Covey and Casseday, 1986). It is segregated into columnar and multipolar areas, each with their own distinct cell type (Covey and Casseday, 1986). Neurons in the VNLLc of the insectivorous bat respond with an 'onset' response to unmodulated pure tones (Rosenberger *et al.*, 2003; Huffman *et al.*, 1998; Covey and Casseday, 1991). These cells fire a single action potential with constant latency relative to pure tone onset across a wide range of frequencies and intensities (Rosenberger *et al.*, 2003; Huffman *et al.*, 1998; Covey and Casseday, 1991). This type of discharge pattern provides a precise marker for sound onset (Huffman *et al.*, 1998).

The frequency tuning curves of VNLLc neurons broaden rapidly above threshold for the characteristic frequency (Covey and Casseday, 1991; Vater *et al.*, 1997). It is believed that the broadening of the frequency tuning curve and the onset response of VNLLc neurons is due to the pattern of synaptic arrangements in the VNLLc, as the dendrites of many VNLLc neurons cross isofrequency planes and are more susceptible to inhibitory input (Vater *et al.*, 1997). The lack of frequency acuity in the VNLLc allows for this area to have additional accuracy in temporal processing (Covey and Casseday, 1991). However, neurons of the VNLLc do not synchronize well to the sinusoidal amplitude modulated pure tones (Huffman *et al.*, 1998). They often do not respond to the sinusoidal amplitude modulated pure tones at all (Huffman *et al.*, 1998). It is possible that the inhibitory input to the VNLLc is responsible for limiting its ability to resolve periodic amplitude modulation (Vater *et al.*, 1997).

There is much more variation in response characteristics among cells in the VNLLm. Some neurons in the VNLLm respond like those of the VNLLc, with an onset

pattern, while others respond with sustained discharge patterns (Batra and Fitzpatrick, 1997; Huffman *et al.*, 1998; Covey and Casseday, 1991). These sustained discharge patterns include 'chopper' responses, 'tonic' responses, 'primary-like' responses and 'pauser' responses (Covey and Casseday, 1991). Chopper neurons respond throughout the stimulus with spikes at regular intervals. Tonic cells fire constantly throughout the stimulus, but are poorly locked to stimulus onset. Primary-like cells respond throughout the stimulus, but the response decreases with time. Pauser cells respond to the onset of the sound, and then stop firing momentarily before they resume firing.

The neurons in the VNLLm that have sustained response characteristics tend to respond in a graded fashion over a broad dynamic range, and are therefore believed to be able to convey accurate information regarding stimulus duration and intensity (Huffman *et al.*, 1998). The tuning curves of VNLLm neurons broaden gradually as intensity is increased and they are often symmetrical (Covey and Casseday, 1991). These characteristics are unlike the rapidly broadening asymmetrical frequency tuning curves of VNLLc neurons (Covey and Casseday, 1991). When presented with sinusoidal amplitude modulated pure tones, neurons of the VNLLm respond with synchronized spike activity (Huffman *et al.*, 1998). The spike times remain synchronized to the SAM pure tones from 100 to 1000 Hz, all modulation frequencies tested (Huffman *et al.*, 1998).

Studies using unanaesthetized rabbits have suggested that the VNLL is segregated into medial and lateral parts (mVNLL and lVNLL respectively) based on cytoarchitecture and physiological response differences (Batra and Fitzpatrick, 1997; Batra and Fitzpatrick, 1999; Batra and Fitzpatrick, 2002). The neurons of the lVNLL are tightly

packed and they generally have sustained, short-latency discharge patterns in response to pure tone stimuli (Batra and Fitzpatrick, 2002; Batra and Fitzpatrick, 1999). The sustained discharge patterns include transient, inhibited, long-latency and strongly adapting types (Batra and Fitzpatrick, 2002; Batra and Fitzpatrick, 1999). These responses are similar to those seen in the VNLLm of the echolocating bat. Very few neurons of the lVNLL showed onset responses.

The neurons of the mVNLL are scattered and responded primarily with onset responses (Batra and Fitzpatrick, 1997; Batra and Fitzpatrick, 2002; Batra and Fitzpatrick, 1999). The authors argue that due to the disparity between cytoarchitecture and physiological responses to external stimuli, the lVNLL and the mVNLL should be segregated into two distinct areas, one that is monaurally driven, and one that is binaurally driven (Batra and Fitzpatrick, 1999). However, the responses obtained from the lVNLL and mVNLL of the rabbit are similar to those in the VNLLm and VNLLc regions of the bat, respectively.

A study on the VNLL of the cat (Aitkin *et al.*, 1970) revealed physiological responses to pure tone stimuli similar to the bat and the rabbit. This group of researchers found that responses of neurons of the VNLL ranged from onset responses to sustained discharge patterns. Phase-locking was seen in these cells when the modulation frequency was less than 1000 Hz, and these cells only responded to stimuli from the contralateral ear, indicating monaural, contralateral input. There was no evidence of tonotopicity in the VNLL. So the physiological responses of cells within the VNLL of the rat, bat, rabbit and cat are very similar, although the rat and cat do not seem to have any segregation of their cells into distinct regions.

1.3.2 Intrinsic Membrane Properties of VNLL Neurons

The intrinsic properties of neurons help to modify information in multiple ways. It is not only the interaction of excitatory and inhibitory signals on a cell that determine its response. Rather the membrane of the neuron may have its own capacity to modify the incoming information. It is the ion channels of a nerve cell that give it its intrinsic membrane properties. For example, voltage-sensitive potassium-conductances have been found to be important in determining discharge patterns of neurons (Li *et al.*, 2001). Specifically, potassium-conductances shape auditory responses for neurons specialized in preserving the timing features of acoustic signals (Li *et al.*, 2001).

In the rat, Li *et al.* (2001) found a pronounced expression of Kv3.3 potassium channel subunits in the rat VNLL. This type of subunit is high-threshold, requiring a large potential for activation. The Kv3.3 subunit has an amino terminal ball structure responsible for rapid inactivation. Any neuron containing Kv3.3 subunits would have its action potential duration limited by the rapid kinetics of its potassium channels, but would repolarize quickly and be able to fire another action potential shortly after the first. The expression of this type of subunit suggests that the VNLL is equipped for high-frequency firing (Li *et al.*, 2001).

A similar result has been shown in the bat. In the echolocating Big Brown Bat, Rosenberger *et al.* (2003) have identified high levels of Kv1.1 potassium channel subunit in the VNLLc. This subunit inactivates slowly and acts to shorten the excitatory post-synaptic potential duration (Li *et al.*, 2001). The Kv1.1 subunit is capable of keeping the membrane potential of a cell subthreshold during sustained depolarization (Li *et al.*, 2001). Due to its ability to keep a cell subthreshold shortly after depolarization begins, it

is not surprising that there was a high concentration of this type of receptor subunit in an area of the VNLL that shows onset-type responses. This evidence strongly suggests that the intrinsic properties of the cells themselves can modify the information without relying on synaptic input.

Clearly the intrinsic membrane properties of cells within a nucleus contribute, to some degree, to their information processing abilities. There is also the possibility that convergence of multiple synapses on a cell dictate its response.

1.3.3 Synaptic Input to the VNLL

In the central nervous system glutamate, a major excitatory neurotransmitter, is usually the source of postsynaptic excitation. Inhibition is brought about by either glycine or γ-aminobutyric acid (GABA). Perisomatic calyceal synaptic endings have been found in the VNLL of many species, including the rat and echolocating bat (Wu, 1999; Vater *et al.*, 1997). These often contain the excitatory neurotransmitter glutamate (Wu, 1999).

The VNLL of the rat receives both excitatory and inhibitory influences as well. The calyceal endings that innervate the VNLL provide excitatory glutamatergic input, possibly from octopus cells of the posteroventral cochlear nucleus (Suneja *et al.*, 1995b; Wu, 1999). Medium sized boutons in the VNLL may provide excitatory input from stellate or bushy cells of the anteroventral cochlear nucleus (Wu, 1999). The VNLL receives inhibition from the MNTB, which is either glycinergic or GABAergic (Wu, 1999; Campos *et al.*, 2001). The MNTB has many glycinergic cells, but the VNLL has been found to contain $\alpha_n B_3 \gamma 2L$ (where n = 1, 2, or 3) $GABA_A$ receptor types, indicating a GABAergic input (Campos *et al.*, 2001).

In the bat, the medial nucleus of the trapezoid body (MNTB) provides inhibitory glycinergic input to the VNLLc and VNLLm (Vater *et al.*, 1997). The MNTB also provides the rabbit VNLL with inhibition, although it may also receive inhibitory influences from the superior olivary complex (Batra and Fitzpatrick, 1999). The rabbit VNLL receives excitatory glutamatergic input from the contralateral ventral cochlear nucleus, as does the VNLL of the guinea pig (Batra and Fitzpatrick, 1999; Suneja *et al.*, 1995a).

Due to this interplay of excitatory and inhibitory influences and the contribution of intrinsic membrane properties, the response of neurons of the VNLL is not merely a reflection of its input. Incoming information is modified in the VNLL, and the resulting output is much different than the input.

The projection from the VNLL to the central nucleus of the inferior colliculus (ICC) is inhibitory in nature. Some evidence suggests that this input is GABAergic (Zhang *et al.*, 1998; Suneja *et al.*, 1995a) while others suggest it is glycinergic (Vater *et al.*, 1997). In so doing, the VNLL provides the ICC with inhibition.

The role of the VNLL could be several things: creating a delay line contributing to the circuitry that analyzes temporal features of sound. The idea behind the delay line theory is that the inhibition from VNLL neurons would provide a brief (4-5 ms) increase in latency postsynaptically or (Huffman *et al.*, 1998; Vater *et al.*, 1997). Another possible role of the VNLL is that it could suppress ongoing ICC activity so that sound onset stands out. The inhibition from the VNLL could suppress spontaneous activity postsynaptically so that incoming auditory information is more conspicuous (Vater *et al.*, 1997).

Two types of cell dominate the VNLL of every animal covered here, one type of cell encodes stimulus onset and the other sustained type conveys information regarding stimulus duration. Both of these cell types are critical for conveying temporal information of sound stimuli. This temporal information is in the form of postsynaptic inhibition at stimulus onset or throughout the duration of the stimulus. The one commonality in the responses of VNLL neurons is their involvement in temporal processing. The VNLL is clearly involved in temporal processing by modifying incoming information to an inhibitory signal of varying duration postsynaptically.

At this point no research has evaluated 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 is to evaluate an animal's ability to discriminate sinusoidally amplitude modulated white noise after destruction of its VNLL. If an animal has a significant shift in its threshold to perform the task after VNLL lesions, it will be inferred that the VNLL contributes to the animal's ability to detect sinusoidal amplitude modulated sound. In order to accomplish this, the first part of the experiment will be to define the behavioural auditory limits of the animals using sinusoidal amplitude modulation across various frequencies. Lesions will then be made in the VNLL and any deficits in threshold post lesion will be attributed to a lack of VNLL contribution.

2. METHODS

2.1 Subjects

Male Wistar rats were used in these experiments. The animals were housed in the Life Sciences Building at Carleton University. Animals 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 or irregularities that might affect their ability to hear.

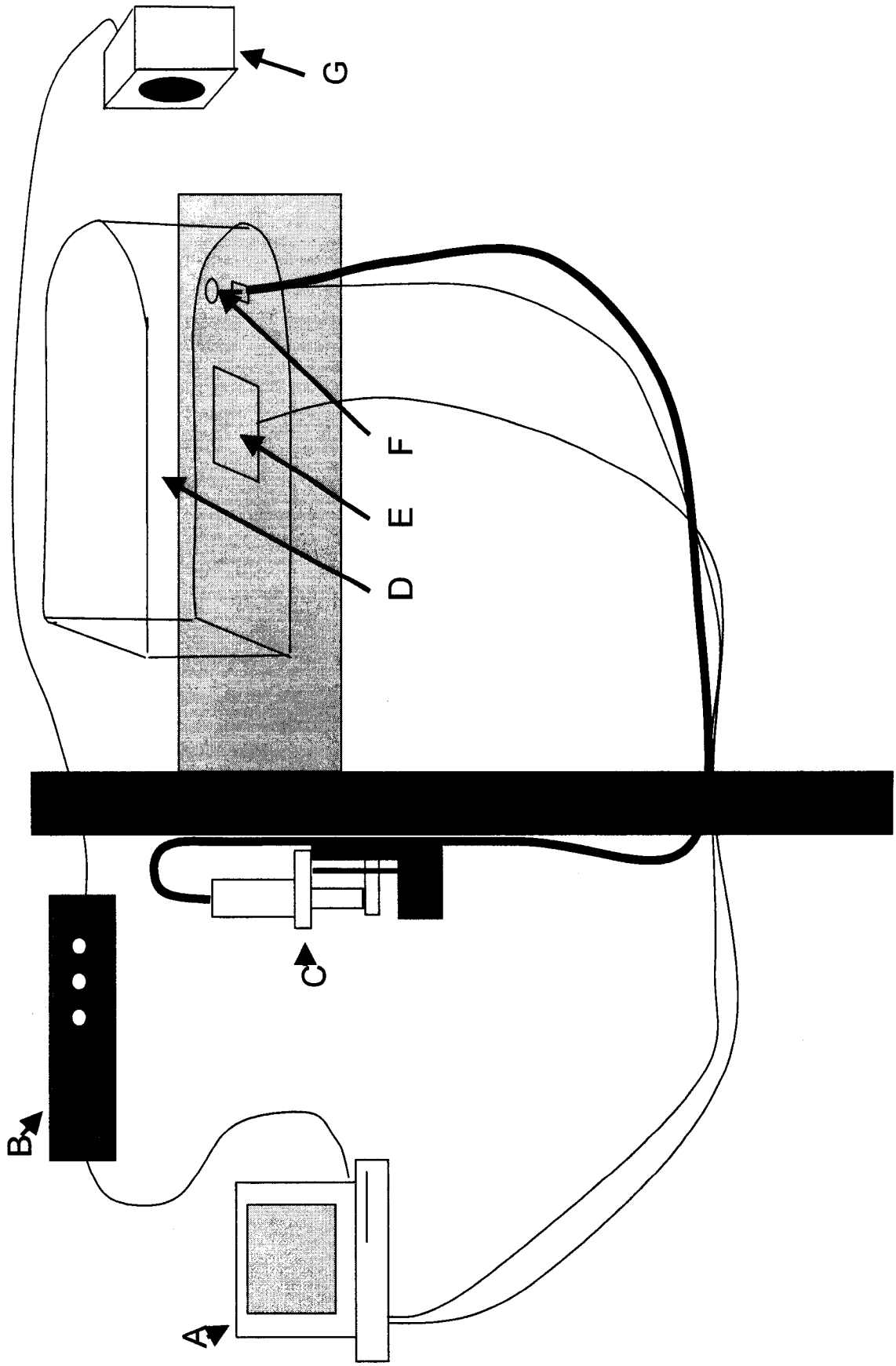
A group of twelve animals was tested for their ability to detect the presence of sinusoidal amplitude modulation across several different modulation frequencies. These animals were considered the 'normative' group. The second group of ten animals was tested for their ability to detect sinusoidal amplitude modulation before and after lesions of the VNLLs and they were considered the 'VNLL lesion' group.

In the VNLL lesion group, the animal's ears were blocked using an ear block technique (see section 2.5). Five of the animals in the 'VNLL lesion' group had the VNLL lesion contralateral to their unblocked ear while the other five had the VNLL lesion ipsilateral to their unblocked ear. As a control for the efficacy of the ear blocking technique, three animals were tested on an absolute intensity task before and after bilateral ear blocks.

2.2 Apparatus

The apparatus used is illustrated in Figure 2. The computer program, designed by Avalon DST (Ottawa, ON), mediated the sound stimulus and recorded the response of the rat. The computer was linked to a Tucker-Davis Technologies (TDT) System 3 Real Time Processor which produced the broad band noise.

Figure 2: Overview of the apparatus involved in the both experiments. The test cage (D) and speaker (G) were contained within a sound-attenuating room. The computer (A) was hooked up to a processor (B) that produced the noise that came out of the speaker. When the rat was standing on the metal plate (E) in the middle of the test cage and drinking from the water spout (F), it completed a circuit that fed back to the computer. It also started the water pump (C) which pumped water to the water spout.


G
D
E
F
C
B
A

The TDT unit was hooked up to a Lafeyette brand shocker that delivered just enough current to cause the subject to withdraw from the spout after termination of the amplitude modulated sound. Shock levels were approximately 0.8 mA, but varied from 0.7 mA to 1.0 mA depending on rat size and sensitivity.

Testing was conducted in a soundproof chamber (Eckel Industries, Belleville, Ontario). A Kef brand speaker in the soundproof chamber was connected to the TDT machine and produced the white noise at 50 dB. The sound loudness was calibrated using a Breull & Kjaer Sound Level Meter, with a half-inch condenser microphone. The speaker was placed directly in front of the test cage. The test cage measured 30 x 20 x 20 cm with a spout 3 cm from the front of the cage which extended 2.5 cm from the cage floor, and a 13 x 8 cm metal stand on the floor (Figure 2). When on the stand and drinking from the spout, the rat completed a circuit that pumped water out at a rate of 40 ml/hour from a Yale Apparatus water pump. Feedback from the spout read back to the computer which detected whether the rat recognized the amplitude modulation, stopped licking, and avoided the shock. The response was then registered in the computer.

2.3 Stimuli

2.3.1 Sinusoidal Amplitude Modulation

Experiments were conducted using a broad band noise carrier. The goal of these experiments was to analyze an animal's ability to process sounds in the temporal domain across all frequencies. For this reason, broad band noise, rather than a pure tone, was selected as the sound carrier.

The lines of minimum and maximum deflection of the broad band noise stimulus form an envelope. The size of the envelope of the broad band noise is the amplitude of

the broad band noise. The amplitude determines the intensity of the noise. If the amplitude of the noise were changed over time, it is considered modulated. If modulation of the amplitude of noise occurs in a sinusoidal fashion, such that the amplitude increases, peaks, then decreases to a minimum value, and finally increases to a peak again, this is considered sinusoidal amplitude modulation of a noise carrier (Figure 3).

When the top of the envelope meets the bottom of the envelope while being sinusoidally amplitude modulated, this is a modulation depth of 100 % (Figure 3). At this modulation depth, the intensity of the noise is 0 dB (SPL). By varying the modulation depth using the method of descending limits, from high modulation depth to low modulation depth, a threshold was obtained for each modulation frequency. The threshold was extrapolated as the modulation depth at which the animal obtains a proportion correct of 0.5.

The method that the computer used to administer trials is illustrated in Figure 4. Five hundred ms prior to administering a trial, the computer monitored the drinking spout to ensure that the animal was on the spout before initiating a trial ('Spout Precheck in Figure 4). A ramp time of 20 ms introduced the sinusoidal amplitude modulation of the white noise carrier. The warning trial lasted 3000 ms, the final 200 ms of which were monitored by the computer to determine whether the animal had detected the stimulus ('Spout Check' in Figure 4). At the end of the warning stimulus, a shock was administered to the spout for 300 ms.

Figure 3: Sinusoidal amplitude modulated noise. In this example, the amplitude of the noise was modulated at a frequency of 10 Hz. The modulation depth was 100 %, as the top of the envelope meets the bottom of the envelope. The intensity of the noise being amplitude modulated in this example was 25 dB. In the experiment, the noise had an intensity of 50 dB.

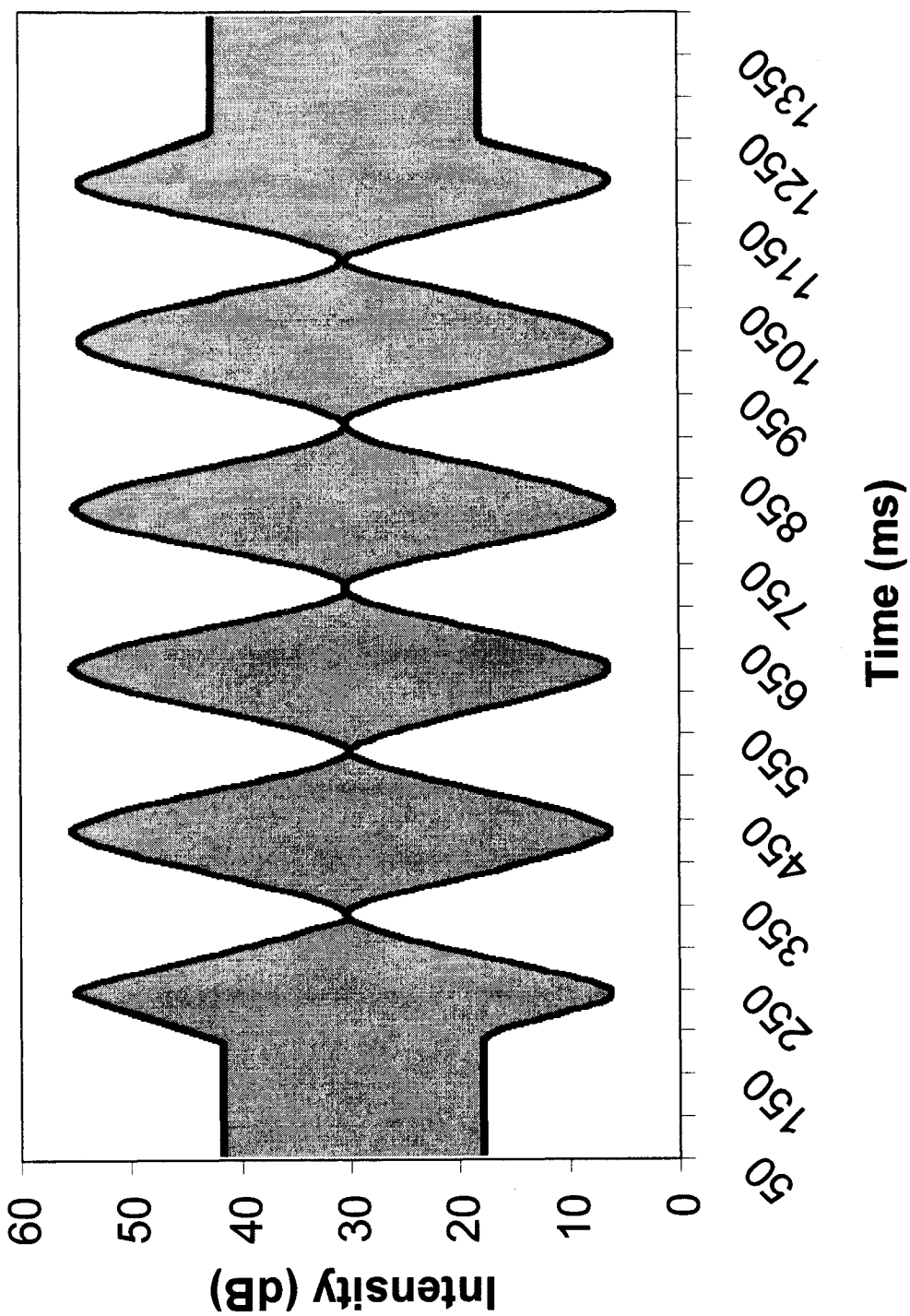
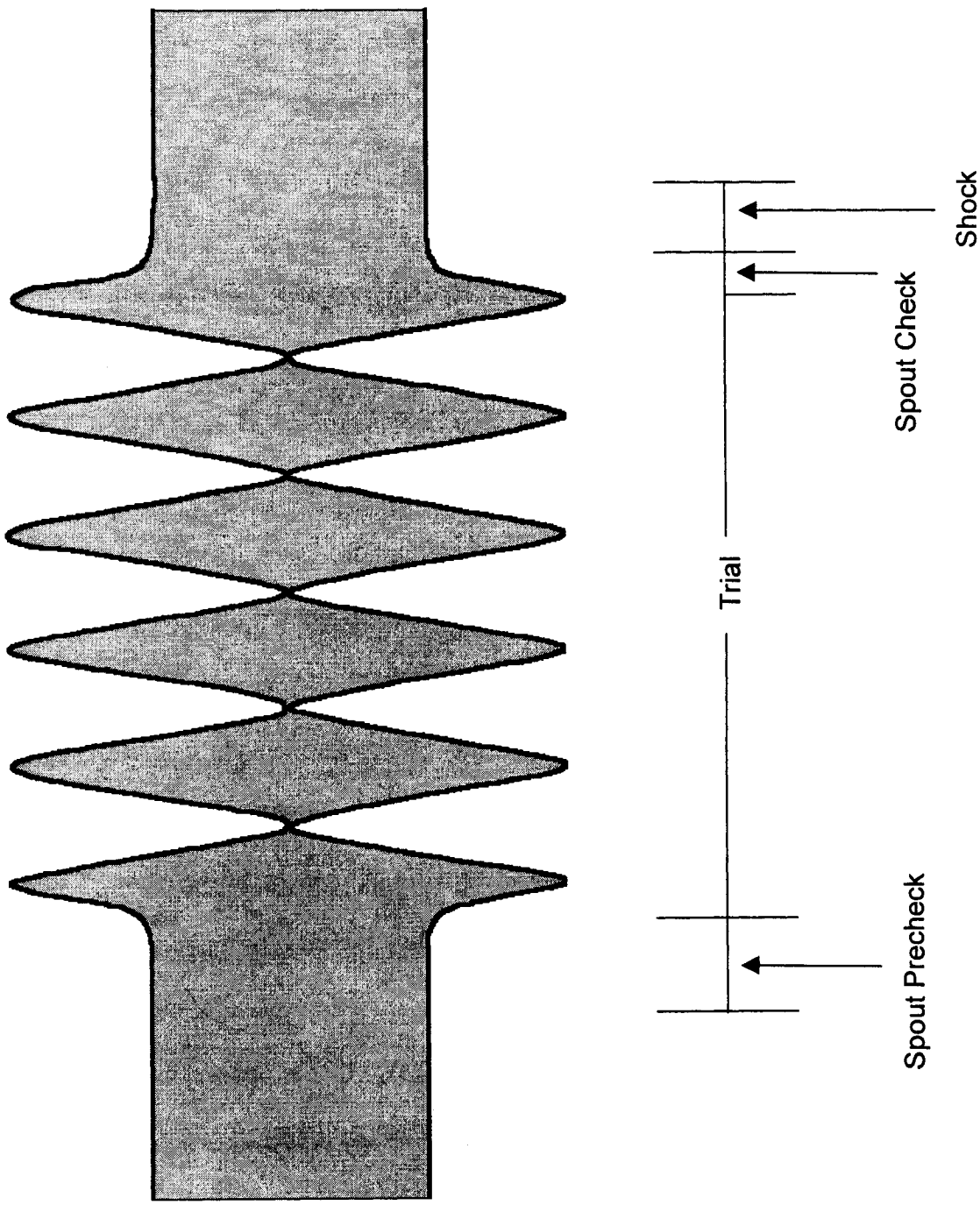

Intensity (dB)
0
10
20
30
40
50
60
Time (ms)
50
150
250
350
450
550
650
750
850
950
1050
1150
1250
1350

Figure 4: Method followed by the computer to administer trials and record responses. The depiction of the computer monitoring method lies below a sinusoidal amplitude modulated stimulus. This same monitoring technique was used for both safe and warning trials (safe trials do not have a shock); although a warning trial is illustrated here. Five hundred ms prior to the trial the computer did a spout check to ensure that the rat was drinking from the spout before initiating a trial (Precheck). The sinusoidal amplitude modulation had a duration of 3000 ms (Trial), the final 200 ms of which were monitored by the computer as a spout check (Spout Check) to determine whether the animal had stopped licking from the spout. At the end of the amplitude modulation a shock of 300 ms duration was administered to the water spout (Shock).


Shock
Spout Check
Trial
Spout Precheck

When the sinusoidal amplitude modulation was introduced to the noise carrier, the modulation began with the phase at 0 degrees and a cosine ramp set to 20 ms.

The amplitude of the noise carrier was modulated in the sinusoidal fashion for 3000 ms, at which point the modulation finished. There is no guarantee that the phase ended at 0 degrees since the time of the trials (3000 ms) plus the ramps (20 ms at onset and offset) was not necessarily equal to an integer number of cycles. The end of the phase varied with different modulation frequencies.

Modulation frequency, or rate, is the number of sinusoids completed in one second. For a modulation frequency of 10 Hz, the sine wave completes 10 cycles in one second. In the first experiment, the collection of normative data, the animals were tested on their ability to detect the presence of the sinusoidal amplitude modulation of the noise carrier at the following modulation frequencies: 5, 10, 25, 50, 100, 500, 1000, and 2000 Hz. The second group of animals were tested on their ability to detect the presence of the sinusoidal amplitude modulation at three frequencies representing low, middle and high frequency based on the data obtained with the normative group. The thresholds of the second group were obtained before and after lesions of the VNLL.

Plotting the thresholds, in modulation depth, of an animal across all modulation frequencies produces a modulation transfer function (MTF). This MTF has been shown to be a quantitative way of illustrating the temporal auditory experience (Viemeister, 1979). An MTF was constructed using the data from the 'normative' group of animals. Afterwards, three modulation frequencies of 10, 100, and 1000 Hz were selected as representatives for low, middle and high frequencies, respectively, at which the 'VNLL group' was tested. The VNLL group were then tested to create MTFs before and after

lesions of the VNLL, using these three values. Any deficits in threshold observed on the MTF of an animal were attributed to the lack of VNLL contribution due to the lesion.

2.3.2 Absolute Intensity

As a control, animals were tested to obtain absolute thresholds using an absolute intensity task. The absolute intensity task consisted of a silent background with white noise burst stimuli. The intensity of the noise stimulus was varied using the method of descending limits; from high intensity noise bursts to low intensity noise bursts. The absolute threshold of an animal was determined as the intensity at which the animal receives a proportion correct of 0.5. This procedure was used for two reasons: to ensure that the ear block technique produced a threshold shift over 50 dB to three animals with double ear blocks, effectively deafening the affected ear, and to ensure that the lesions of the VNLL did not produce shifts in absolute threshold for the VNLL lesion animals.

2.4 Procedure

These experiments used a conditioned avoidance procedure (Heffner and Heffner, 1995). The animals in these experiments were conditioned to associate the onset of sinusoidal amplitude modulation (in the sinusoidal amplitude modulation task), and the presence of a noise burst (in the absolute threshold task), with a mild shock.

When the animal stood on the metal plate and drank from the water spout, he completed an electrical circuit. This circuit was involved in testing the animal in two ways: when complete, it activated the water pump to begin pumping water. It also fed back to the computer which monitored whether the animal removed itself from the water spout in order to avoid the shock. In calculating a data point, a simple formula was used by the computer: data point = hit rate – (hit rate x false positive rate), where hit rate =

number of hits / number of warning trials and false positive = number of false positives / number of safe trials.

Number of hits was the number of times the animals accurately identified the amplitude modulation and removed themselves from the spout. Number of false positives was the number of times the animals removed themselves from the spout without the presence of the amplitude modulation.

The procedure for training the animals took six days. The animals were water deprived for 24 hours prior to testing. One of the first steps in animal training was to allow the animals to learn that they can obtain water from the water spout in the cage. At that point it was important to have the water pump set to a high rate, 70 ml/hour, so that the animals got as much water as they needed. As training proceeded, it was important to drop the pump rate to a point that had the animals licking from the water spout for thirty minutes, 40 ml/hour. Thirty minutes was an adequate amount of time to obtain data but not so long that it frustrated the animal. At the same time as the pump rate was dropped, it was important to condition the animals to associate the sound stimulus to the shock. This was done by presenting very obvious trials (large modulation depths in the sinusoidal amplitude modulation task and high intensity noise bursts in the absolute threshold task) early on, gradually making the trials more subtle with time (progressively smaller modulation depths and noise intensity). The following example illustrates these steps with the sinusoidal amplitude modulation task at a modulation frequency of 5 Hz:

On day one, the animals were placed in the test cage and were exposed to the background stimulus of broad band noise. They received no 'warning' trials on day one. At that point they learned that water was accessible from the water spout in the cage.

Water was pumped out at a rate of 70 ml/hour. On the second day, the animals were exposed to one very obvious 'warning' trial of a modulation depth of 100 %. The water was pumped out at a rate of 60 ml/hour on day two. On day three the animals were exposed to five trials of the warning stimulus at a modulation depth of 100 %. The water was pumped out at a rate of 50 ml/hour on day three. By the end of day three, the animals had associated the amplitude modulation with the shock. On day four the animals were exposed to ten trials with modulation depths becoming progressively smaller, from 100 % in decrements of 10 % to 10 %. The water was pumped out at a rate of 40 ml/hour on day four, and the pump rate remained at 40 ml/hour for the duration of the experiment. This allowed the animal to get 20 ml during the half hour testing each day. On day five the animals were tested at smaller modulation depths, from 42 % to 6 %, and the trials were administered by the computer rather than by the experimenter. On day six, the animal was tested at modulation depths from 30 % to 6 % in decrements of 6 %. These values were used throughout the remainder of the experiment at the modulation frequency of 5 Hz. The computer presented the animal with trials at random time intervals.

2.5 Surgical Procedure

The animals in the VNLL group were anaesthetized using a cocktail of xylazine (0.5 ml/kg) and ketamine (0.1 ml/kg). A midline incision was made in the scalp, a craniotomy performed, and the dura mater retracted. The VNLL was located using a stereotaxic instrument, and a microelectrode (15 – 20 μm tip) filled with kainic acid (1.0 mg/ml in phosphate-buffered saline) was lowered into the middle of the VNLL (from lambda: 0.67 mm laterally, 0.08 mm rostrally, 0.75 mm depth). One microlitre of kainic

acid was released into the VNLL by pressure. The electrode was retracted and the hole in the skull was filled using Gelfoam (Upjohn Canada). Since kainic acid is an excitatory neurotoxin that kills cells by overstimulating them, there was the possibility of kainic acid inducing seizures. In the event of seizure activity, diazepam (0.5 ml/kg), a barbiturate, was injected intraperitoneally.

After the VNLL was lesioned, one of the animal's ears was blocked to create a profound hearing loss in this ear. Five animals had the ear ipsilateral to the lesion blocked, and five animals had the ear contralateral to the lesion blocked. Having half the animals with blocks contralateral to the lesion and half with blocks ipsilateral to the lesion tested whether the VNLL is primarily driven contralaterally or ipsilaterally.

The ear block consisted of breaking the tympanic membrane and removing the middle ear ossicles with a pair of thin forceps (Fine Science Tools, Jacksonville). The middle ear was then filled with 0.5 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 bupivicaine topically for their analgesic properties. All animals were given two weeks to recover after surgery before being re-tested.

After completion of the experiment, the animals were perfused by injection of 4% paraformaldehyde transcardially. The brains were removed and sliced using a microtome, stained with cresyl violet and cover slipped. The brains were then examined using a light microscope (Zeiss, Germany) for loss of cell bodies in the VNLL.

3. RESULTS

3.1 Normative Experiment

The goal of this experiment was to establish normal behavioural auditory temporal resolution abilities in the rat, using sinusoidal amplitude modulation as a stimulus. Twelve animals were tested in the normative group across eight modulation frequencies. The frequencies tested were 5, 10, 25, 50, 100, 500, 1000, and 2000 Hz. Threshold for each modulation frequency was the modulation depth at which the animal obtains a proportion correct of 0.5.

An example of how threshold was obtained is shown in Figure 5. This is a modulation sensitivity curve for all the animals sampled at a modulation frequency of 10 Hz. From Figure 5, the threshold of all animals sampled at a modulation frequency of 10 Hz was 12.5 %. This value is extrapolated from the sensitivity curve as the modulation depth at which the animals obtain a proportion correct of 0.5.

This process was repeated for every animal at all modulation frequencies tested (Figure 6). The threshold of the animals at a modulation frequency of 5 Hz was 12.5 %. At a modulation frequency of 25 Hz, threshold was 15 %. The threshold of the animals at a modulation frequency of 50 Hz was 17.5 %. At a modulation frequency of 100 Hz, threshold was 17 %. Threshold at a modulation frequency of 500 Hz was 23 %. At a modulation frequency of 1000 Hz, threshold was 29.5 %. At the final modulation frequency tested, 2000 Hz, threshold was 45.5 %.

The thresholds for each modulation frequency were then plotted with modulation frequency on the x-axis and the threshold of the animals (in modulation depth) on the y-axis. This is called a modulation transfer function (MTF). An MTF plots the thresholds

Figure 5. Modulation sensitivity curve of all twelve animals combined at a modulation frequency of 10 Hz. The animals were tested at modulation depths of 30, 24, 18, 12 and 6 %, and the mean score at each depth is plotted here. The purpose of this figure is to illustrate how threshold is obtained for each modulation frequency. The modulation depth at which the animal obtains a proportion of 0.5 is the animal's threshold. In this figure, the horizontal and vertical lines indicate how threshold is extrapolated: the modulation depth at which the animal obtains a proportion correct of 0.5.

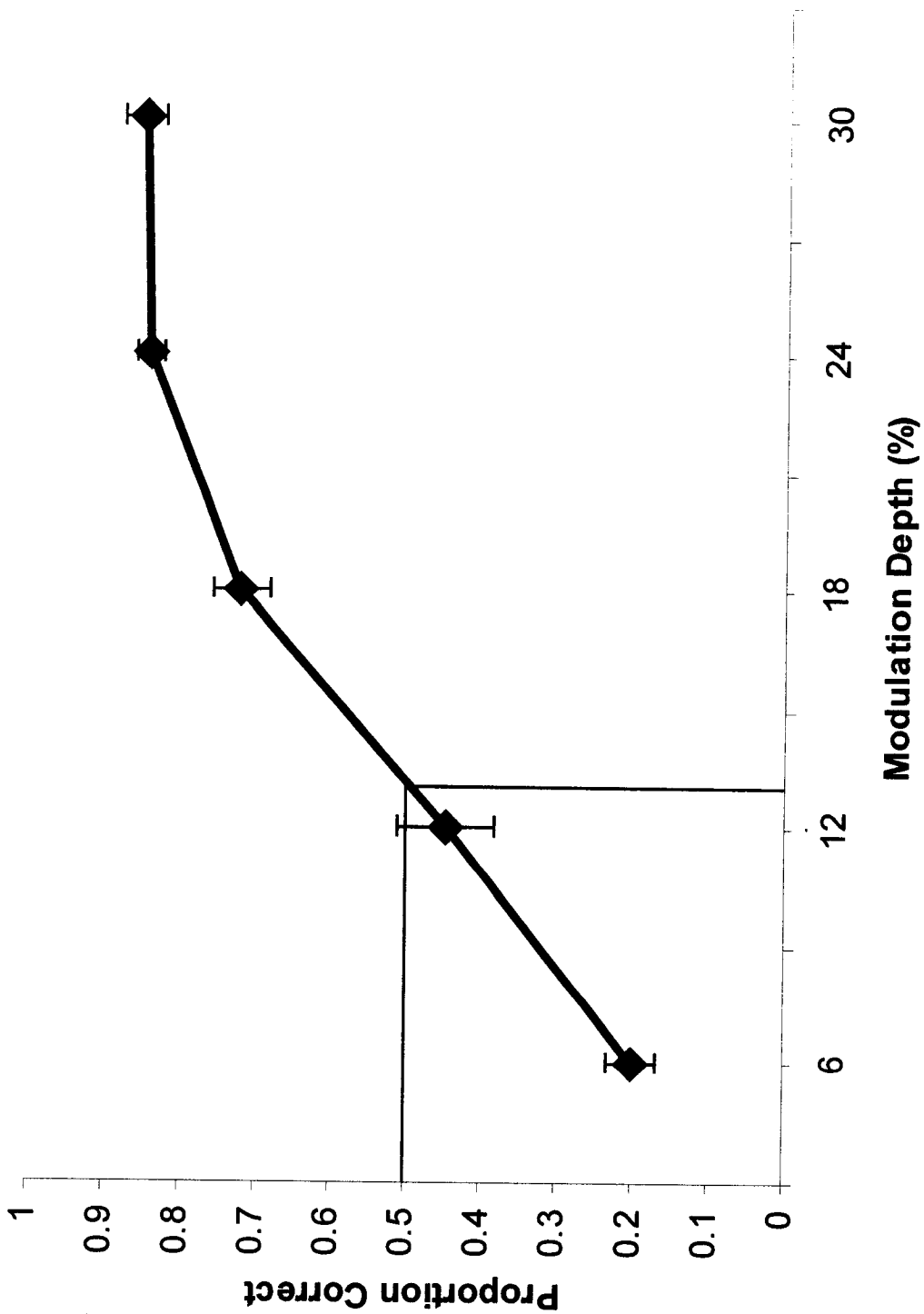
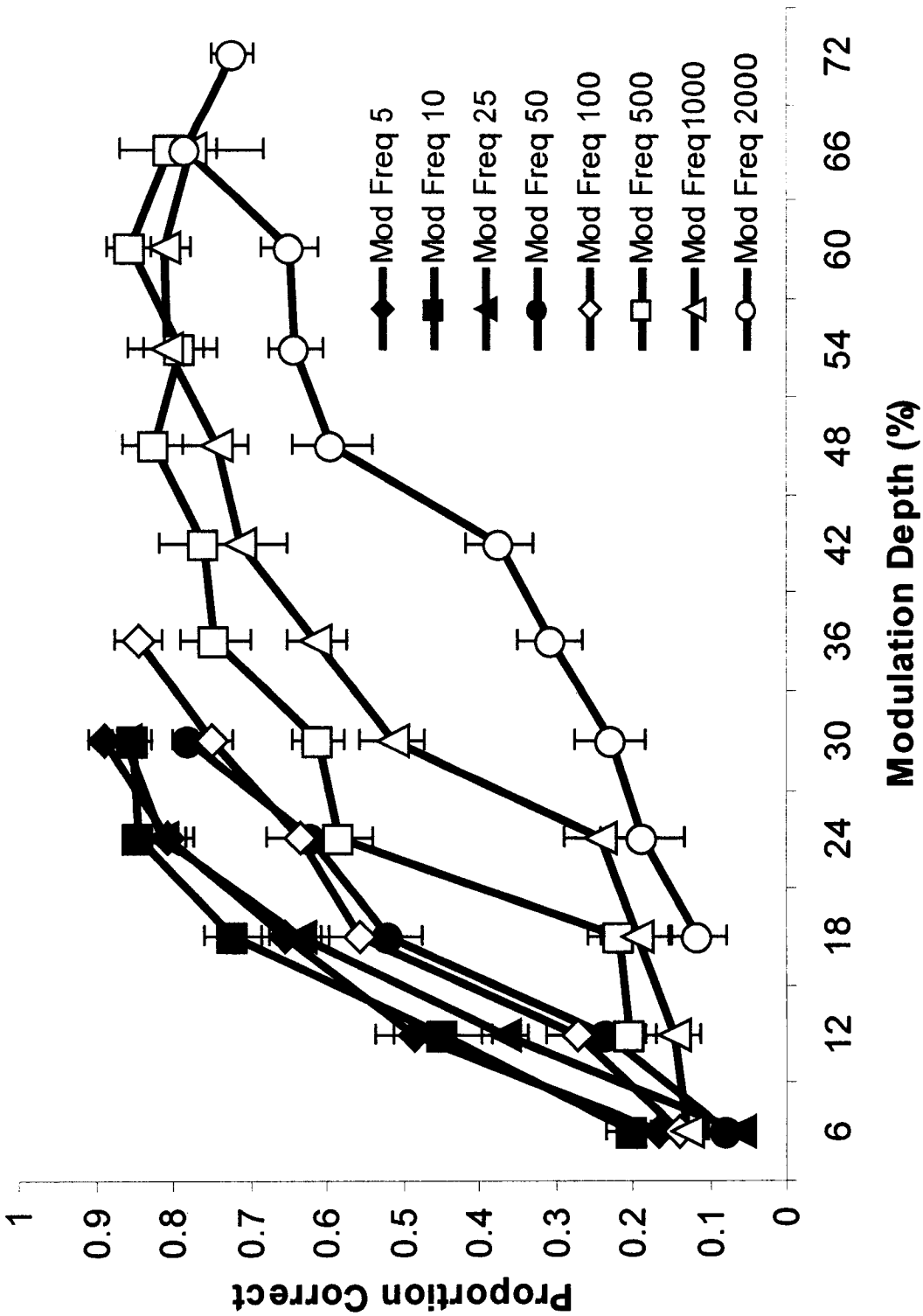

Proportion Correct
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
12
18
24
30
Modulation Depth (%)

Figure 6. Modulation sensitivity curves for all twelve animals at all eight modulation frequencies. The threshold of the animals, in modulation depth, increases as the modulation frequency increases. In the legend, Mod Freq is an abbreviation for 'modulation frequency', for example Mod Freq 5 represents 'modulation frequency of 5 Hz'.


Proportion Correct
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
6
12
18
24
30
36
42
48
54
60
66
72
Modulation Depth (%)
Mod Freq 5
Mod Freq 10
Mod Freq 25
Mod Freq 50
Mod Freq 100
Mod Freq 500
Mod Freq1000
Mod Freq 2000

of all animals tested across each modulation frequency tested, and represents the behavioural limits of the rat to sinusoidal amplitude modulation detection. An MTF was constructed from the thresholds of every animal in this group across all modulation frequencies tested (Figure 7).

The MTF obtained for the rat in the current experiment was compared to those of the chinchilla (Henderson et al., 1984) and human (Viemeister, 1979) (Figure 7). The MTFs of all these mammals are similar: as modulation frequency increases, a greater modulation depth is required for the animals to detect the presence of the amplitude modulation.

Having obtained the normal behavioural limits of the rats ability to perform the sinusoidal amplitude modulation task, three points on the MTF of the rat were selected to represent low, middle and high modulation frequencies for the VNLL experiment (Figure 7). The arrows on the MTF of the rat in Figure 7 indicate the points that were chosen: 10, 100 and 1000 Hz. The second experiment evaluated the contribution of the VNLL to the rat's ability to perform the sinusoidal amplitude modulation task.

3.2 VNLL Lesions Contralateral to the Unblocked Ear

In this experiment, the modulation frequencies 10, 100 and 1000 Hz were used as representations of low, middle and high frequency ranges respectively. Each animal was tested at all three modulation frequencies before and after an ear block and lesion of the VNLL. Five animals received a VNLL lesion on the side of the brain contralateral to the unblocked ear and five received a lesion ipsilateral to the unblocked ear.

All animals had their brains sliced, dyed with cresyl violet and examined. Figure 8 illustrates the extent of the lesion in Rat 12 with cresyl violet staining. Figure 8 shows

Figure 7. Modulation transfer functions (MTFs) for the rat (obtained during the current experiment) the chinchilla (Henderson et al., 1984), and humans (Viemeister, 1979). The shape of the modulation transfer functions for all three mammalian species is similar: as modulation frequency increases, the modulation depth necessary for the animal to detect the amplitude modulation increases. The three arrows on the figure indicate modulation frequencies of 10, 100 and 1000 Hz. These three modulation frequencies were selected to represent low, middle and high frequencies (respectively) to be used in the second experiment, the contribution of the VNLL.

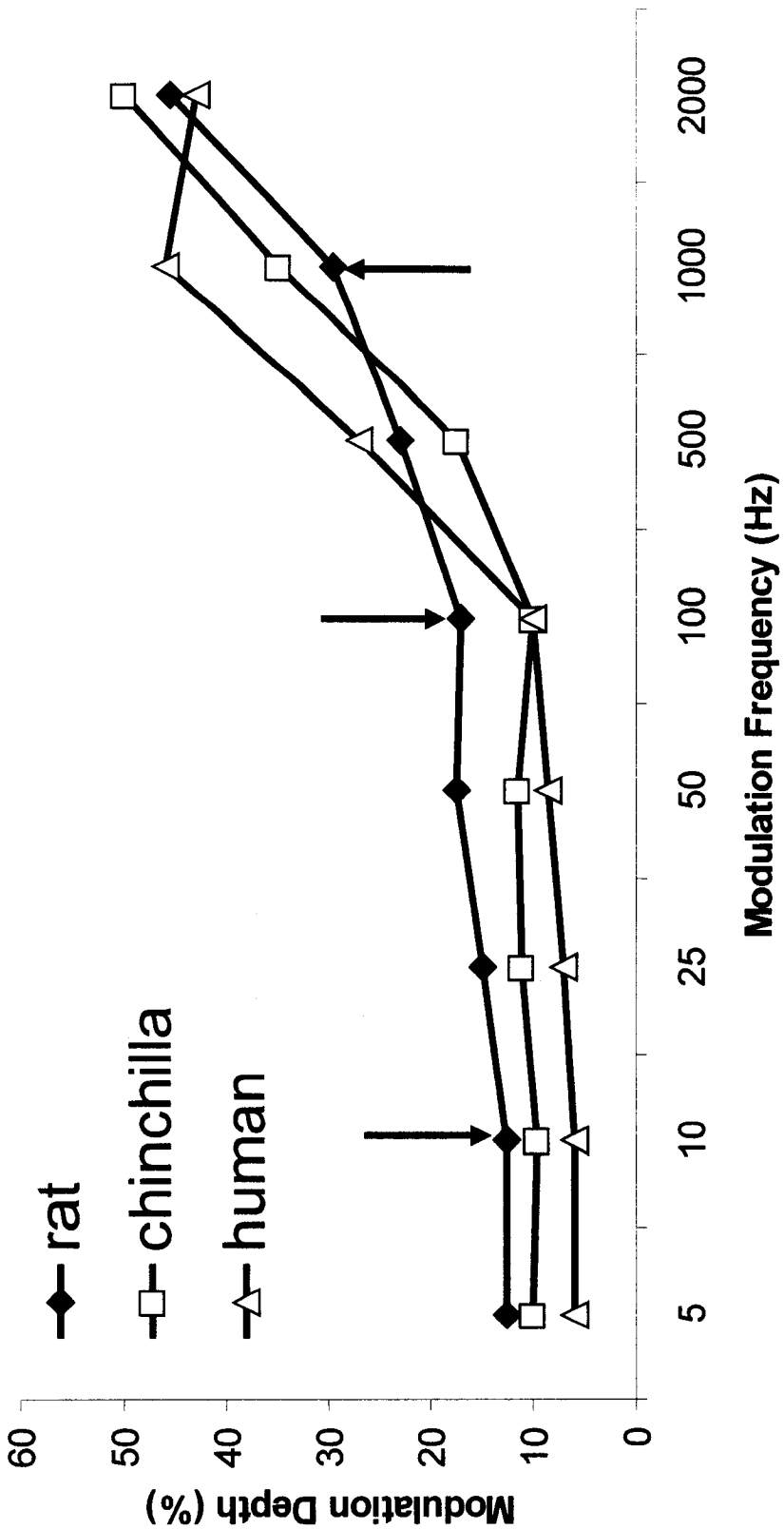
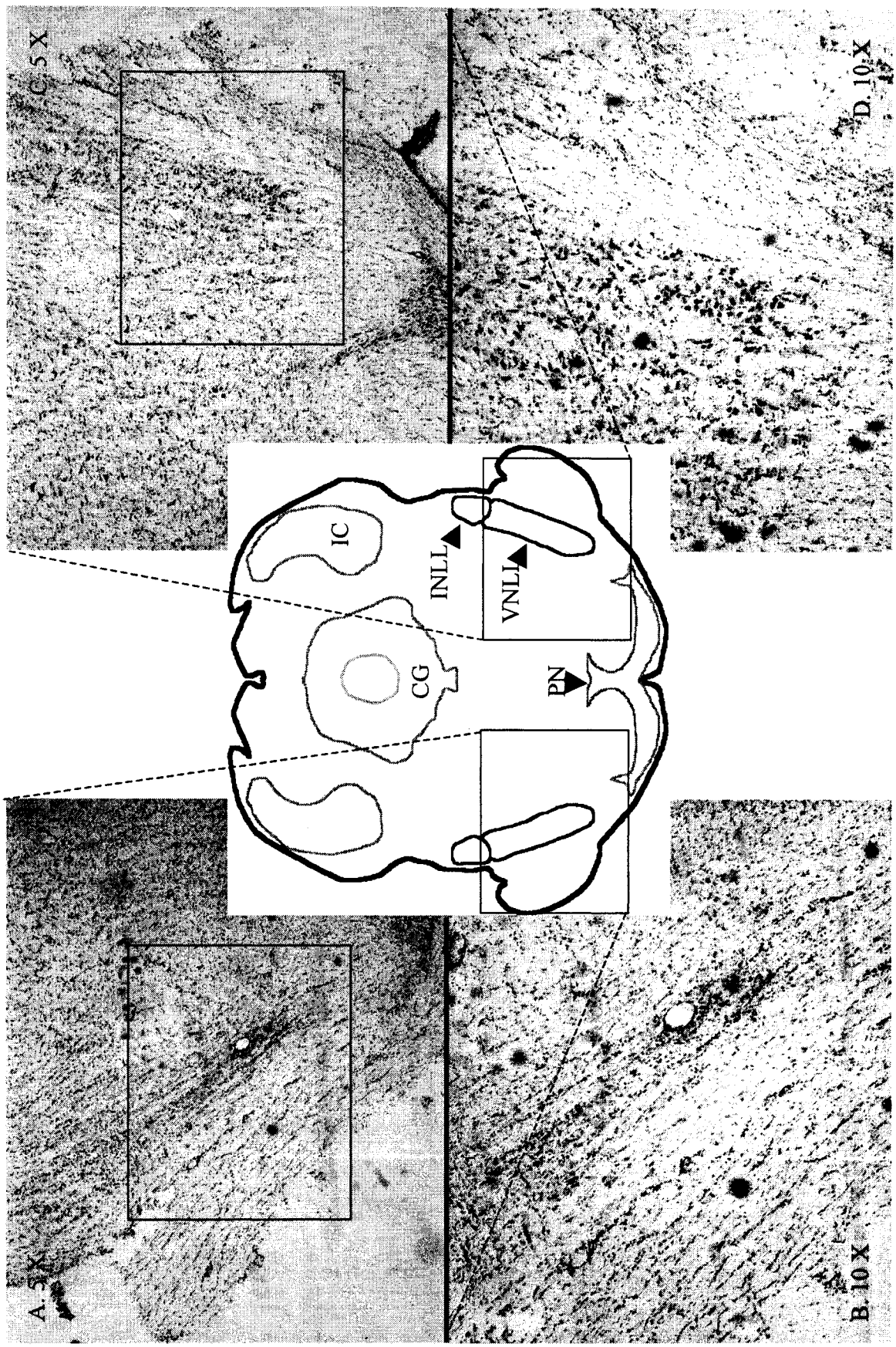

rat
chinchilla
human
Modulation Depth (%)
0
10
20
30
40
50
60
5
10
25
50
100
500
1000
2000
Modulation Frequency (Hz)

Figure 8. Example of a VNLL lesion with cresyl violet staining. The central figure is a schematic image used to represent a brain slice, where IC represents the inferior colliculus, CG represents the central grey, PN represents the pontine nucleus, INLL represents the intermediate nucleus of the lateral lemniscus, and VNLL represents the ventral nucleus of the lateral lemniscus. The two figures at the top of the page, A and C, are cresyl violet images of the VNLL at 5 X magnification. The bottom two images, B and D, represent the VNLL slices in A and C, respectively, at 10 X magnification. The boxes in A and C represent the area included in B and D. The lesioned images are on the left, A and B (note a lack of cell bodies in the VNLL), and the images on the right, C and D, are of the intact VNLL.


A. 5X
B. 10 X
C. 5 X
D. 10 X
IC
INLL
VNLL
CG
PN

what a complete lesion of the VNLL looks like, alongside the intact VNLL of the same animal.

The threshold of each animal was obtained at each modulation frequency before and after the ear block and VNLL lesion. A chart summarizing the difference in threshold for every animal at each modulation frequency can be found in Table 1.

Due to the small sample size of the study, it would be inappropriate to analyze the results of this experiment in groups. For this reason, the results obtained will be examined as individual cases.

3.2.1 Rat 01

The lesion of the VNLL of Rat 01 was large, destroying all neurons within the VNLL and some cells of the surrounding reticular area (Figure 9).

The threshold of Rat 01 at a modulation frequency of 10 Hz was 25.5 % before the VNLL lesion and ear block. After the lesion and ear block, the threshold of this animal decreased to 19.0 % modulation depth (see Figure 10). This difference of -6.5 % in threshold can be seen in Table 1 and in the modulation transfer function (MTF) for this animal (Figure 10).

The threshold of Rat 01 at a modulation frequency of 100 Hz was 24.0 % before the VNLL lesion and ear block. The threshold of this animal increased to 25.0 % after the lesion (see Figure 10). The difference of 1 % can be seen in Table 1 and in the MTF for this animal.

The threshold of Rat 01 at a modulation frequency of 1000 Hz was 28.5 % prior to the VNLL lesion and ear block. The threshold of Rat 01 increased substantially after

Figure 9. Schematic image of the VNLL lesion of Rat 01. The lesion included all of the VNLL, as well as some of the reticular area medial to the VNLL. The lesion is shaded in gray. The ventral portion of the VNLL in the caudal-most image is not included in the lesion as some cells were evident in this area. However, these cells may have been the rostral-most neurons of the lateral superior olive. For this reason, the lesion is considered complete. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

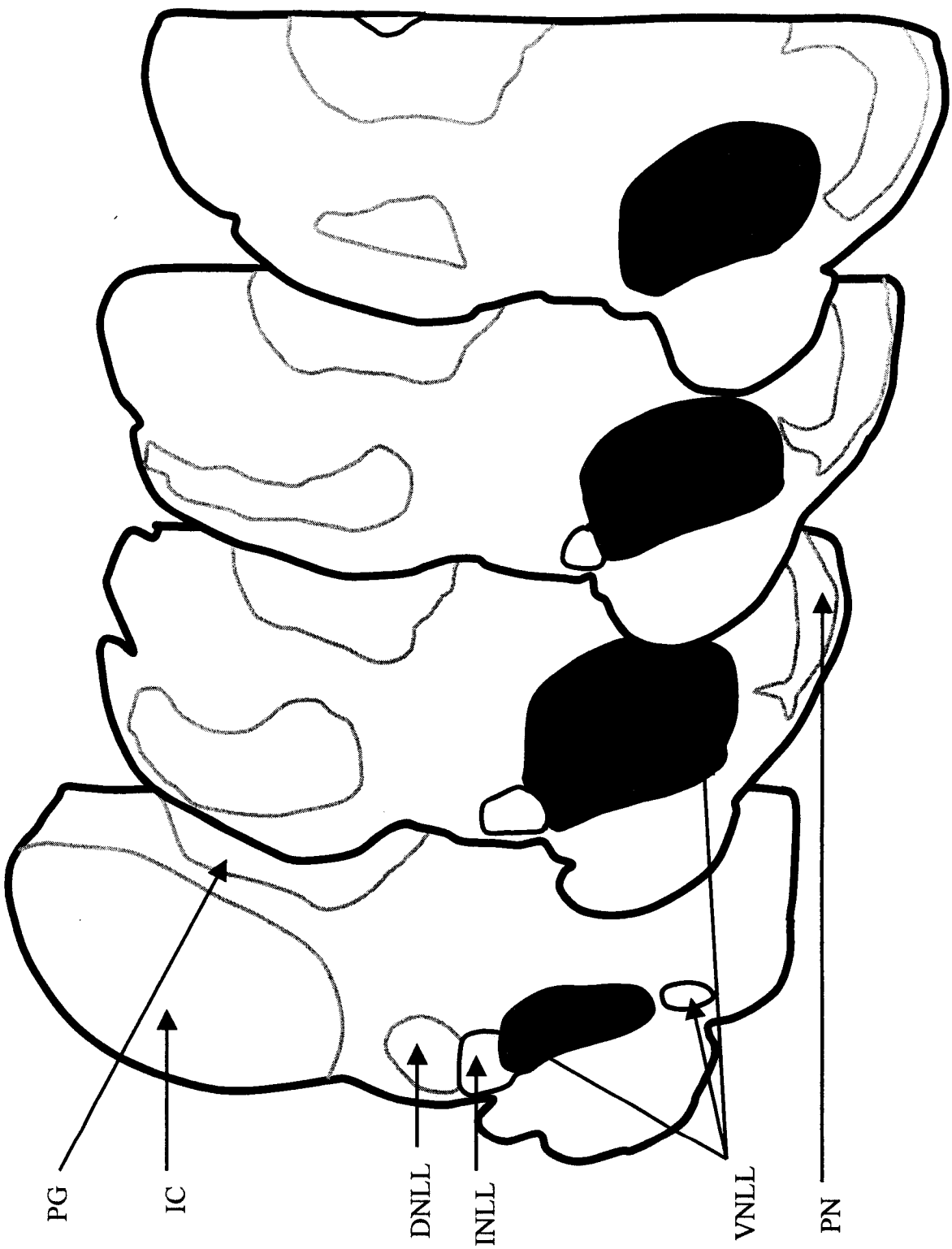
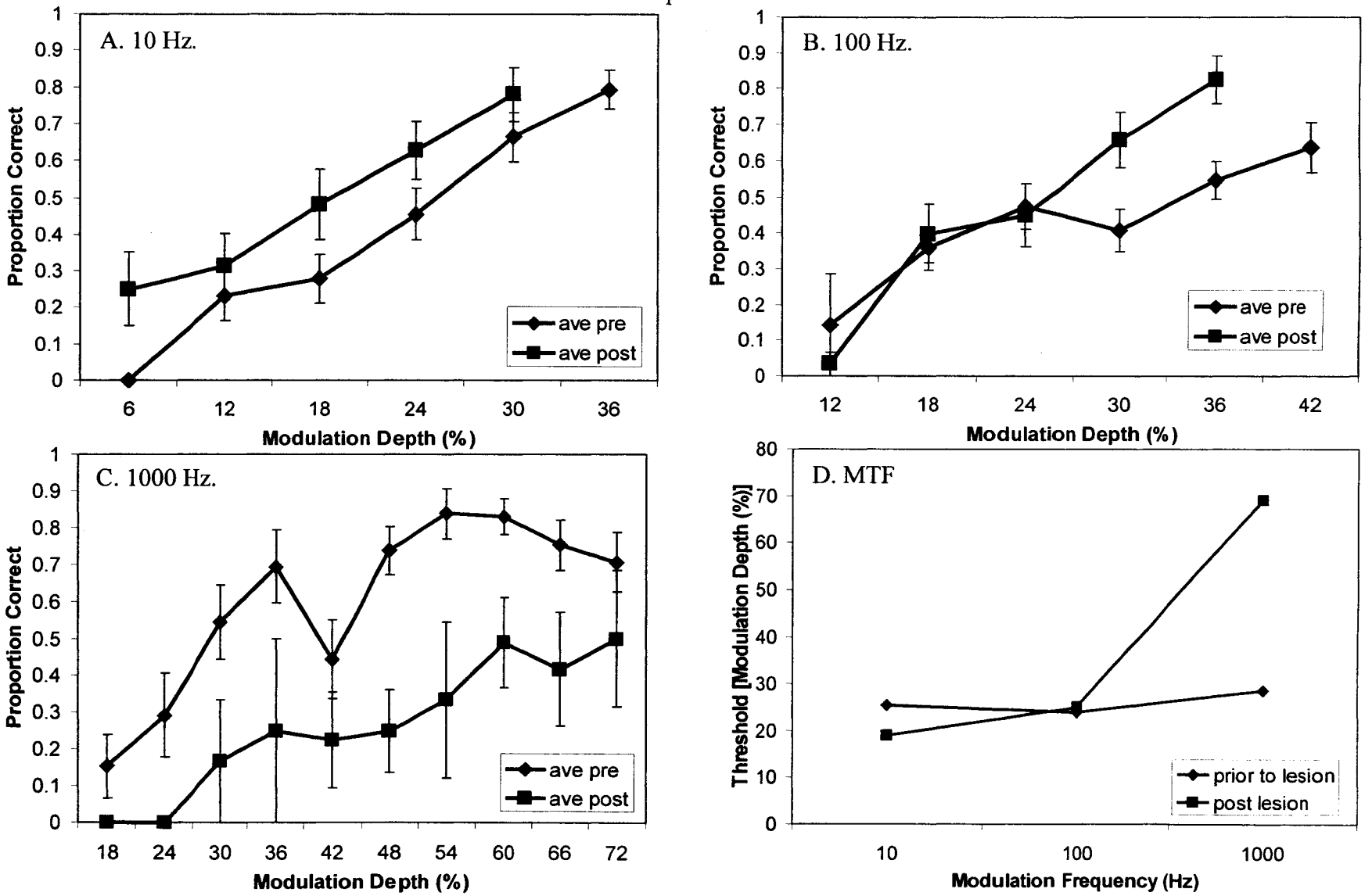

PG
IC
DNLL
INLL
VNLL
PN

Figure 10. Behavioural results of Rat 01. A: modulation sensitivity curve for Rat 01 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 01 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 01 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 01 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.

Complete Lesion



the lesion and ear block, to 69.0 % (see Figure 10). The threshold for this animal was extrapolated using a linear trendline from the data points. While the animal had difficulty detecting the presence of the amplitude modulation at all modulation depths, the animal's scores improved as the modulation depth increased from lower to higher values. The difference of 40.5 % can be seen in Table 1 and in the MTF of this animal.

3.2.2 Rat 08

There was effectively no lesion of the VNLL in Rat 08. There appeared to be some slight cell loss on the medial edge of the VNLL, but cell loss was minimal. Many healthy cells remained throughout the VNLL and the reticular area (Figure 11).

The threshold of Rat 08 before the ear block and VNLL lesion was 20.5 %. Threshold for this animal remained identical after the ear block and VNLL lesion at a modulation depth of 20.5 % (see Figure 12). The difference in threshold of 0 % can be seen in Table 1 and in the modulation transfer function (MTF) for this animal (Figure 12).

The threshold of Rat 08 before the ear block and VNLL lesion at a modulation frequency of 100 Hz was 23.0 %. The threshold of Rat 08 decreased after the VNLL lesion and ear block to 16.5 % after the lesion (see Figure 12). The difference in threshold of -6.5 % for this animal can be seen in Table 1 and in the MTF of this animal.

The threshold of Rat 08 at a modulation frequency of 1000 Hz before the VNLL lesion and ear block was 39.0 %. After the VNLL lesion and ear block, the threshold of Rat 08 was 33.0 % (see Figure 12). The difference in threshold can be seen in Table 1 and in the MTF for this animal.

3.2.3 Rat 12

Figure 11. Schematic image of the VNLL lesion of Rat 08. Cell loss was minimal and restricted to the medial edge of the VNLL, as well as some of the reticular area. The lesion is shaded in gray. A substantial amount of healthy neurons were visible in the area not included in the lesion. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

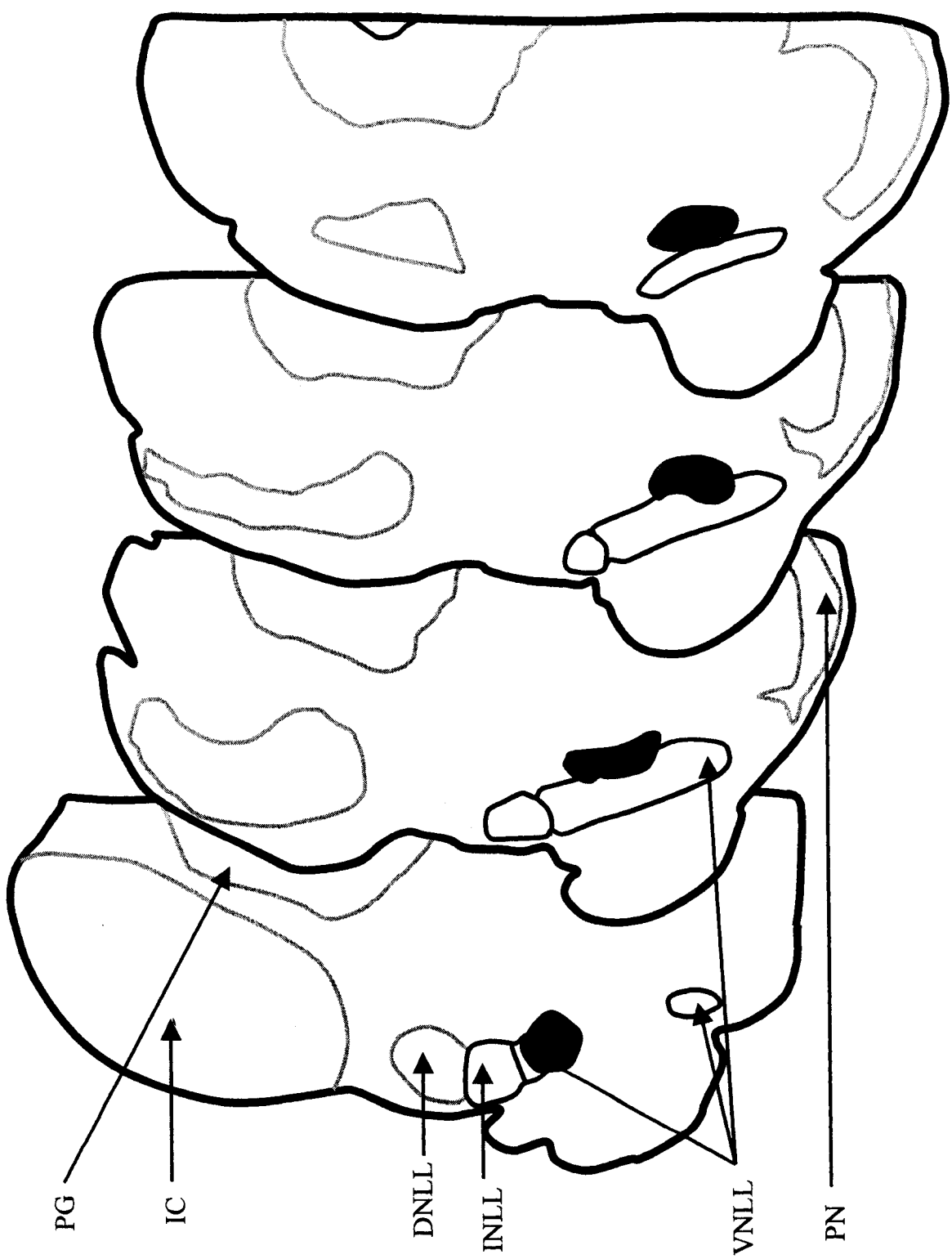
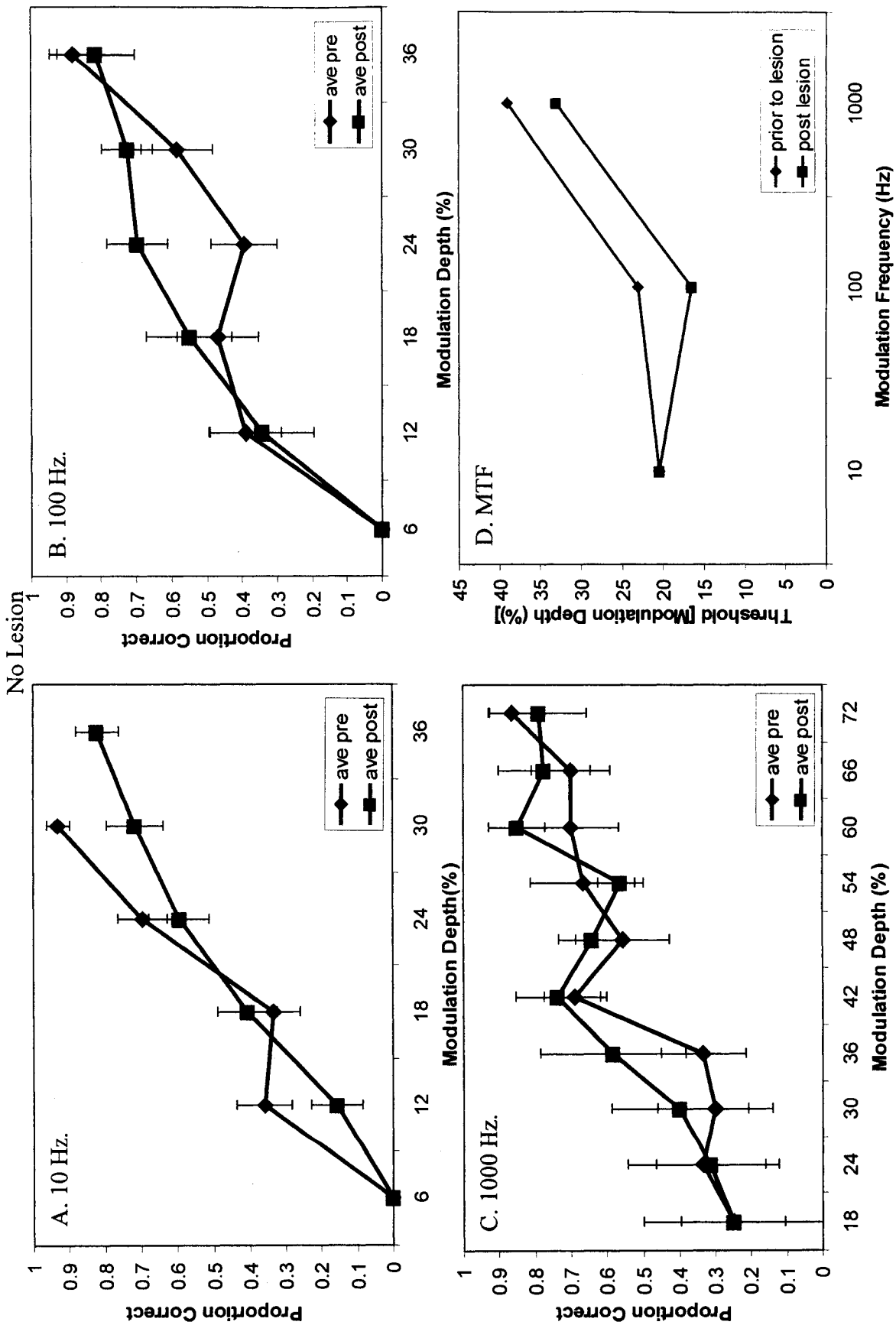

PG
IC
DNLL
INLL
VNLL
PN

Figure 12. Behavioural results of Rat 08. A: modulation sensitivity curve for Rat 08 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 08 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 08 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 08 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.


No Lesion
A. 10 Hz.
B. 100 Hz.
C. 1000 Hz.
D. MTF
Proportion Correct
Modulation Depth(%)
Modulation Depth (%)
Threshold [Modulation Depth (%)]
Modulation Frequency (Hz)
ave pre
ave post
prior to lesion
post lesion

The lesion of Rat 12 encompassed all of the VNLL and some of the reticular area medial to the VNLL. The lesion did not include the cells of other auditory areas such as the dorsal nucleus of the lateral lemniscus or the superior olive complex (see Figure 13).

The threshold of Rat 12 at a modulation frequency of 10 Hz was 11.0 % before the VNLL lesion and ear block. After the VNLL lesion and ear block, the threshold of this animal increased to 19.0 % (see Figure 14). This difference of 8 % can be seen in Table 1 and in the MTF for this animal (Figure 14).

The threshold of Rat 12 at a modulation frequency of 100 Hz was 13.5 % before the VNLL lesion and ear. After the lesion and ear block, the threshold of this animal increased to 19.0 % (see Figure 14). The difference in threshold of 5.5 % can be seen in Table 1 and in the MTF for this animal.

The threshold of Rat 12 at a modulation frequency of 1000 Hz was 28.0 % before the lesion and ear block. The threshold of this animal increased substantially to 51.0 % after the lesion and ear block (see Figure 14). The difference in the threshold of this animal after the VNLL lesion and ear block was 23 %, which can be seen in Table 1 and the MTF for this animal.

3.2.4 Rat 18

The lesion of Rat 18 was partial. There is some obvious cell loss in the medial aspect of the VNLL and in the reticular area adjacent to the VNLL. However, cells in the lateral portion of the VNLL remain intact; as are the cells in the dorsal and ventral areas of the VNLL (see Figure 15). This animal had obvious cell loss in the VNLL, while animals considered to have no lesions had no obvious loss of neurons in the VNLL.

Figure 13. Schematic image of the VNLL lesion of Rat 12. The lesion included all of the VNLL, as well as some of the reticular area medial to the VNLL and some cells of the pontine nucleus ventral to the VNLL. The lesion is shaded in gray. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

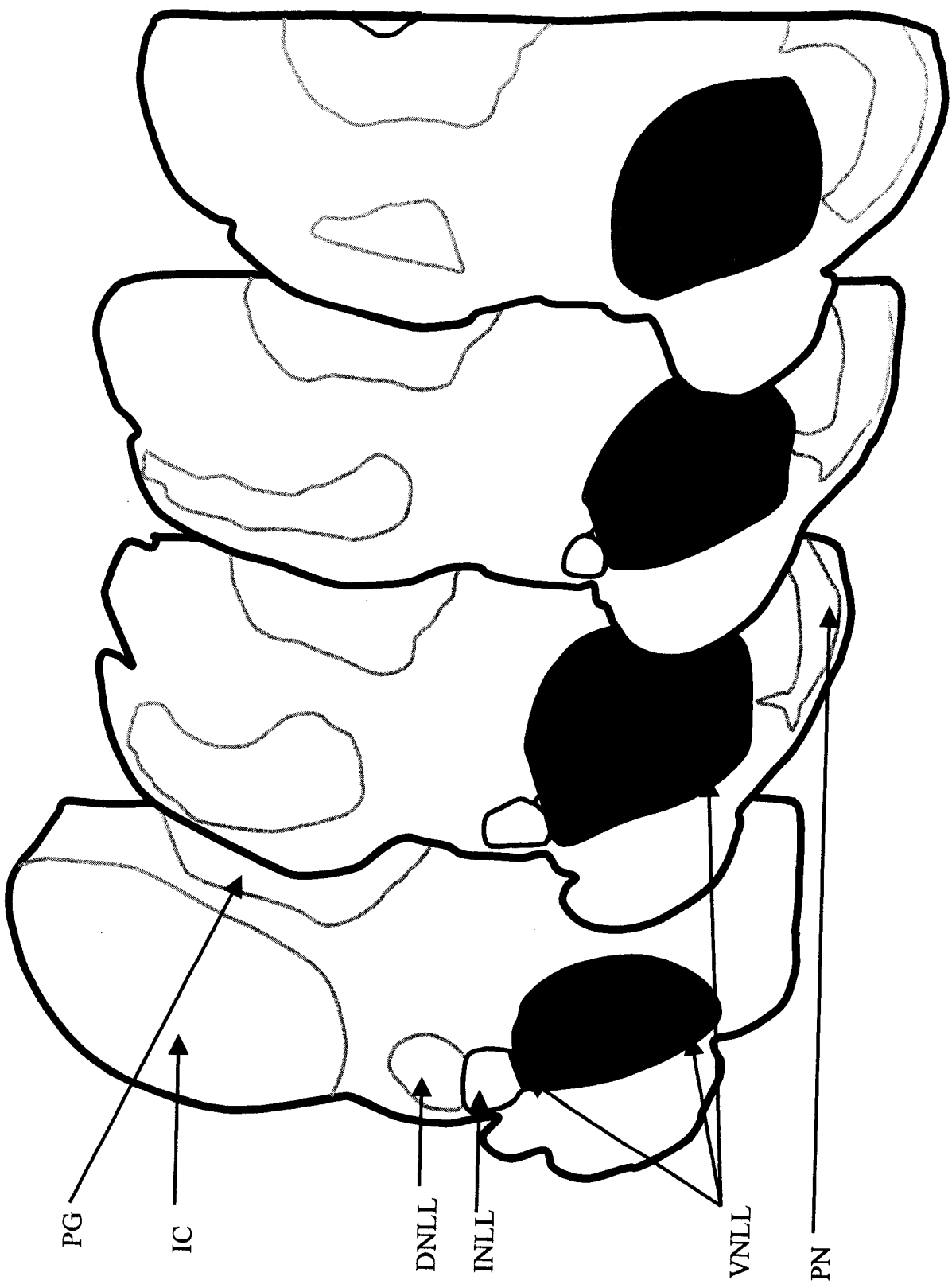
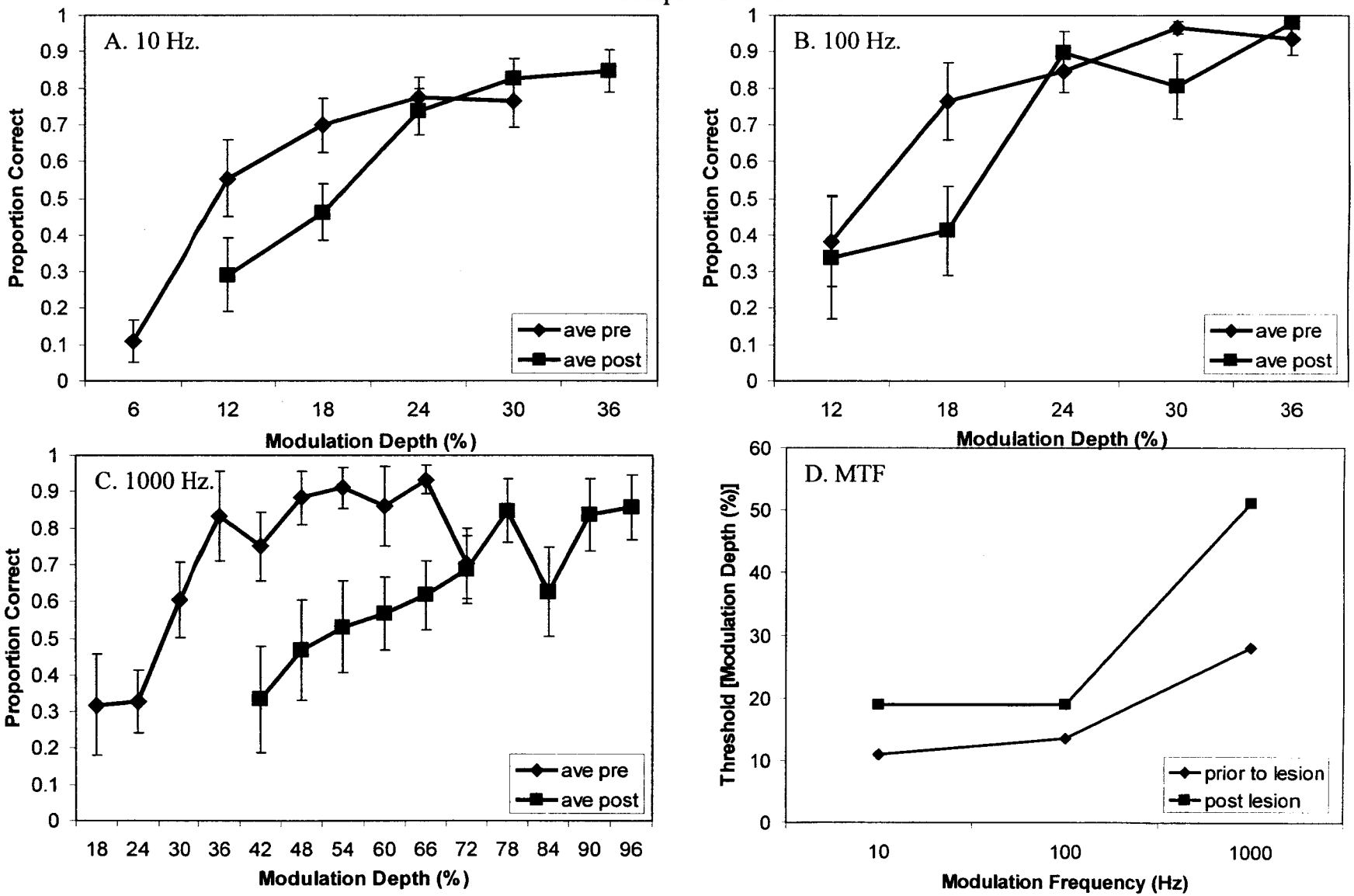

PG
IC
DNLL
INLL
VNLL
PN

Figure 14. Behavioural results of Rat 12. A: modulation sensitivity curve for Rat 12 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 12 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 12 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 12 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.

Complete Lesion



The threshold of Rat 18 prior to the VNLL lesion and ear block at a modulation frequency of 10 Hz was 8.25 %. After the lesion and ear block the threshold of Rat 18 increased to 9.75 % (see Figure 16). The difference of threshold was 1.5%, which can be seen in Table 1 and in the MTF for this animal (Figure 16).

The threshold of Rat 18 prior to the VNLL lesion and ear block at a modulation frequency of 100 Hz was 17.0 %. The threshold of this animal after the lesion and ear block decreased to 12.25 % (see Figure 16). This decrease in threshold after the lesion can be seen in Table 1 and in the MTF for Rat 18.

The threshold of Rat 18 before the VNLL lesion and ear block at a modulation frequency of 1000 Hz was 22.5 %. After the lesion and ear block the threshold of this animal increased to 32.0 % (see Figure 16). The difference of 9.5 % in threshold can be seen in Table 1 and the MTF for this animal.

3.2.5 Rat 22

The lesion of Rat 22 was partial. There was some evidence of cell loss throughout the VNLL, particularly at the caudal end, but many healthy cells remained in (Figure 17).

The threshold of Rat 22 before the VNLL lesion and ear block at a modulation frequency of 10 Hz was 17.75 %. The threshold of this animal after the lesion and ear block was 13.5 % (see Figure 18). The difference in threshold after the VNLL lesion and ear block was -4.25 %, which indicates an improvement after the lesion and ear block. This threshold difference can be seen in Table 1 and in the MTF for Rat 22 (Figure 18).

Figure 15. Schematic image of the VNLL lesion of Rat 18. The lesion was small and restricted to the medial edge of the VNLL, as well as some of the reticular area. The lesion is shaded in gray. A substantial amount of healthy neurons were visible in the area not included in the lesion. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.


PG
IC
DNLL
INLL
VNLL
PN

Figure 16. Behavioural results of Rat 18. A: modulation sensitivity curve for Rat 18 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 18 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 18 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 18 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.

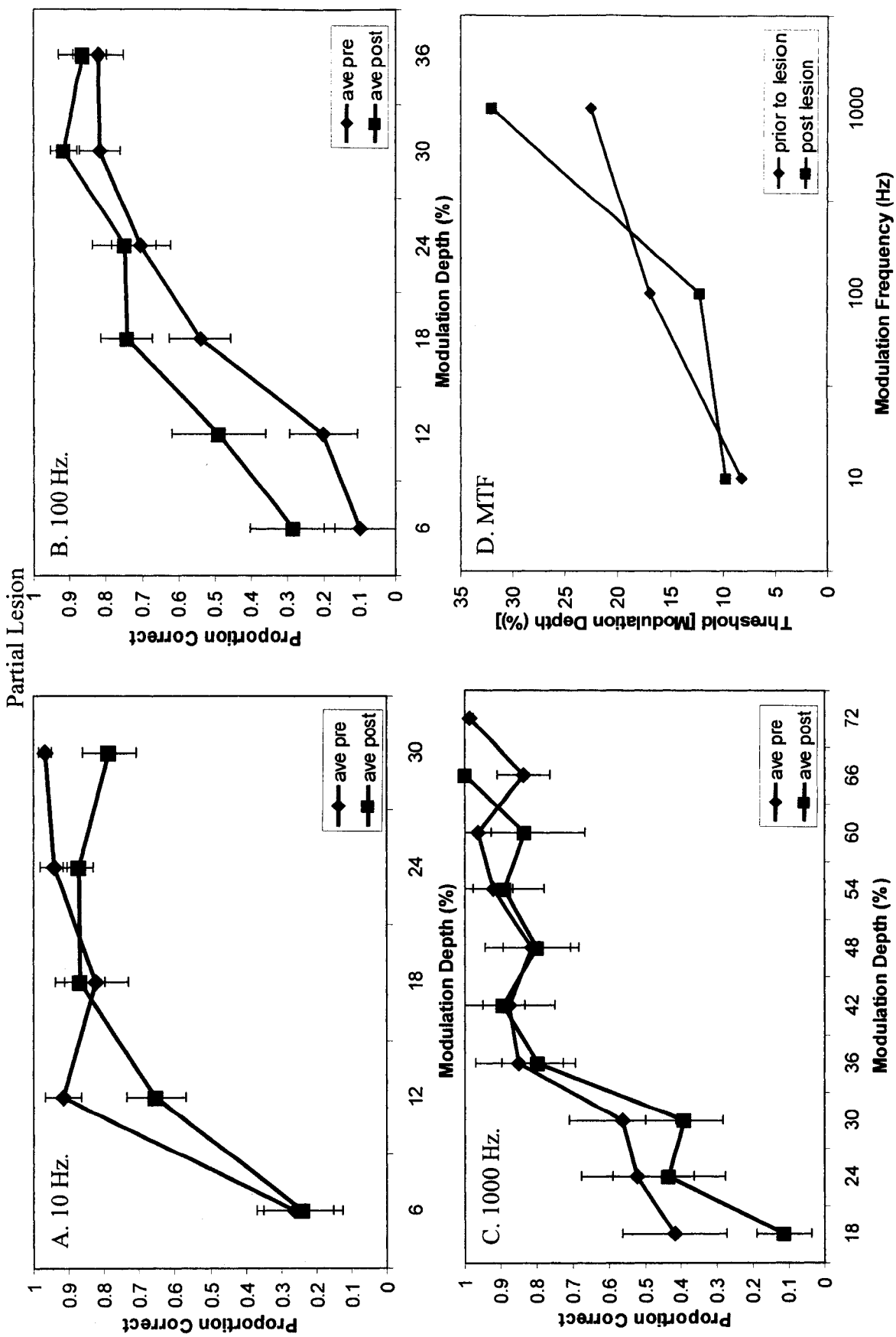

Partial Lesion
A. 10 Hz.
B. 100 Hz.
C. 1000 Hz.
D. MTF
Proportion Correct
Modulation Depth (%)
Threshold [Modulation Depth (%)]
Modulation Frequency (Hz)
ave pre
ave post
prior to lesion
post lesion

Figure 17. Schematic image of the VNLL lesion of Rat 22. The lesion was partial and included healthy cells throughout the area included in the lesion. The lesion is shaded in checker pattern. A substantial amount of healthy neurons were visible in the area not included in the lesion. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

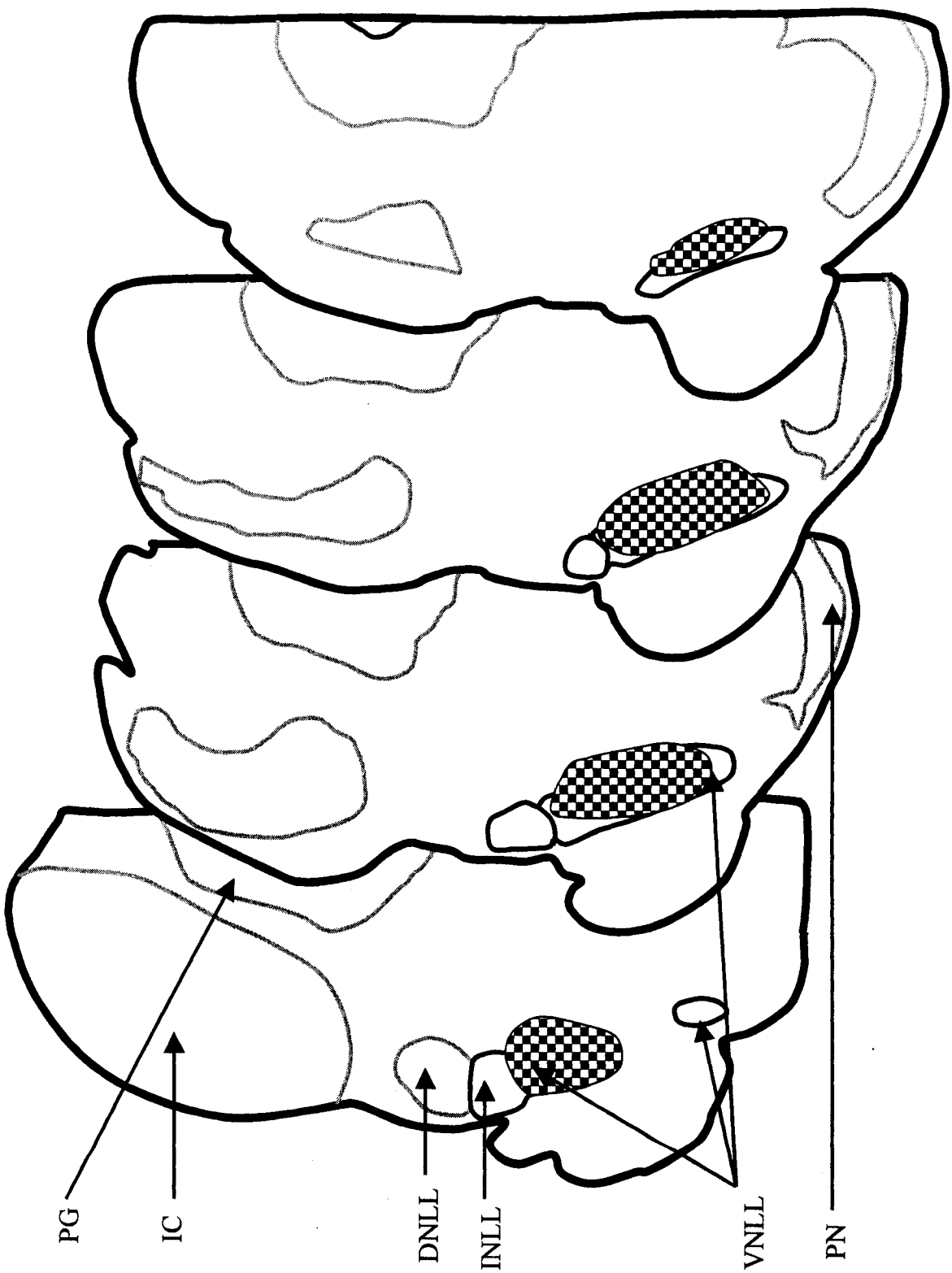
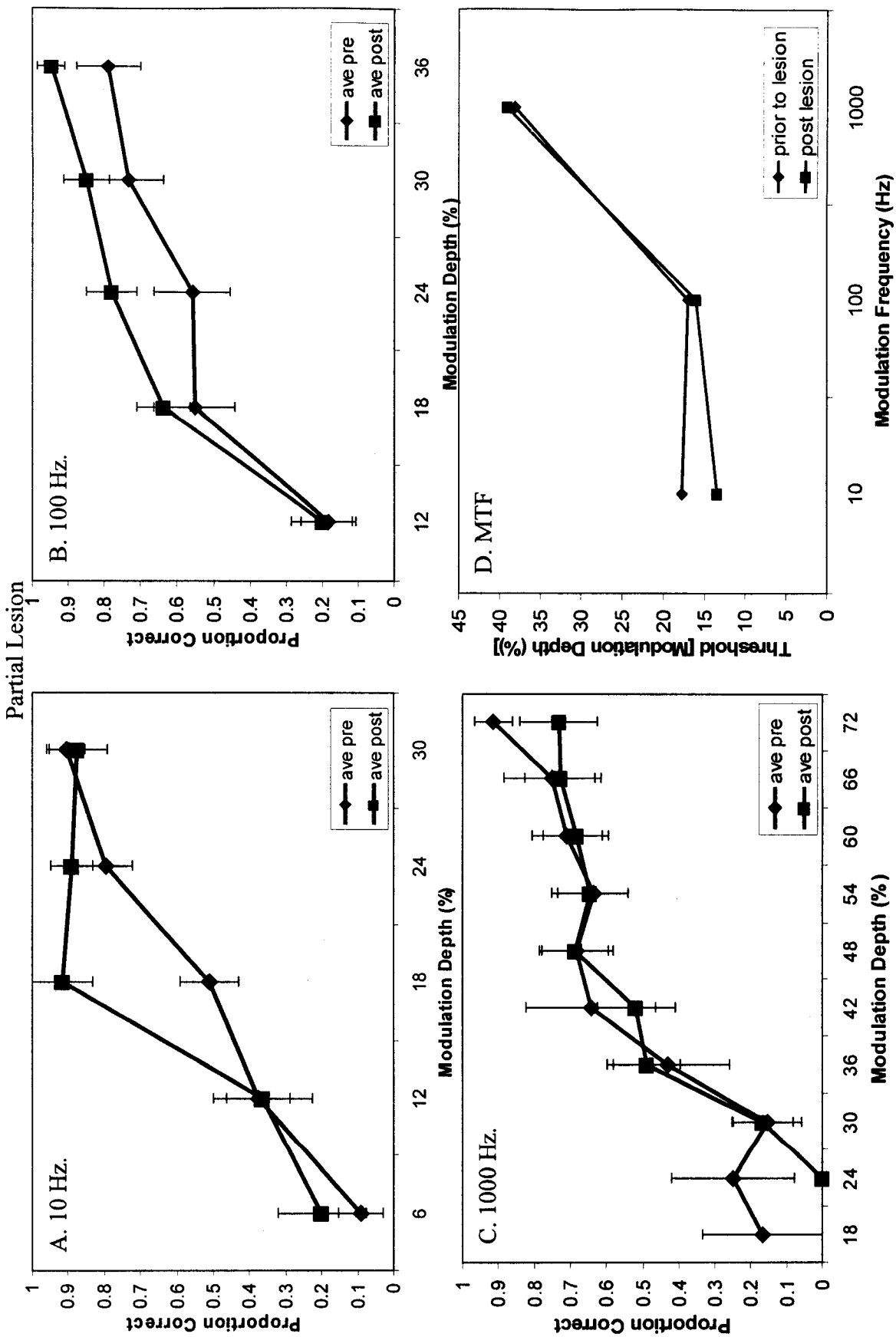

PG
IC
DNLL
INLL
VNLL
PN

Figure 18. Behavioural results of Rat 22. A: modulation sensitivity curve for Rat 22 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 22 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 22 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 22 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.


Partial Lesion
A. 10 Hz.
B. 100 Hz.
C. 1000 Hz.
D. MTF
Proportion Correct
Modulation Depth (%)
Threshold [Modulation Depth (%)]
Modulation Frequency (Hz)
ave pre
ave post
prior to lesion
post lesion

The threshold of Rat 22 before the VNLL lesion and ear block at a modulation frequency of 100 Hz was 17.0 %. After the lesion and ear block the threshold of this animal dropped to 16.0 % (see Figure 18). The difference in threshold of -1 % can be seen in Table 1 and in the MTF for Rat 22.

The threshold of Rat 22 at a modulation frequency of 1000 Hz before the VNLL lesion and ear block was 38.0 %. The threshold of the animal after the lesion and ear block increased to 39.0 % (see Figure 18). The difference in threshold of 1 % can be seen in Table 1 and in the MTF for Rat 22.

3.3 VNLL Lesions Ipsilateral to the Unblocked Ear

3.3.1 Rat 04

The lesion of Rat 04 was complete, and encompassed all of the VNLL as well as part of the reticular area. No other auditory areas were affected (Figure 19).

The threshold of Rat 04 at a modulation frequency of 10 Hz prior to the ear block and VNLL lesion ipsilateral to the unblocked ear was 16.5 %. The threshold of this animal after the VNLL lesion and ear block decreased to 15.5 % (see Figure 20). The difference of -1 % can be seen in Table 1 and in the modulation transfer function (MTF) for this animal (Figure 20).

The threshold of Rat 04 before the VNLL lesion and ear block at a modulation frequency of 100 Hz was 17 %. The threshold of this animal after the lesion and ear block decreased to 15.5 % (see Figure 20). The difference in threshold of -1.5 % is illustrated in Table 1 and in the MTF of this animal.

Figure 19. Schematic image of the VNLL lesion of Rat 04. The lesion included all of the VNLL, as well as some of the reticular area medial to the VNLL. The lesion is shaded in gray. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

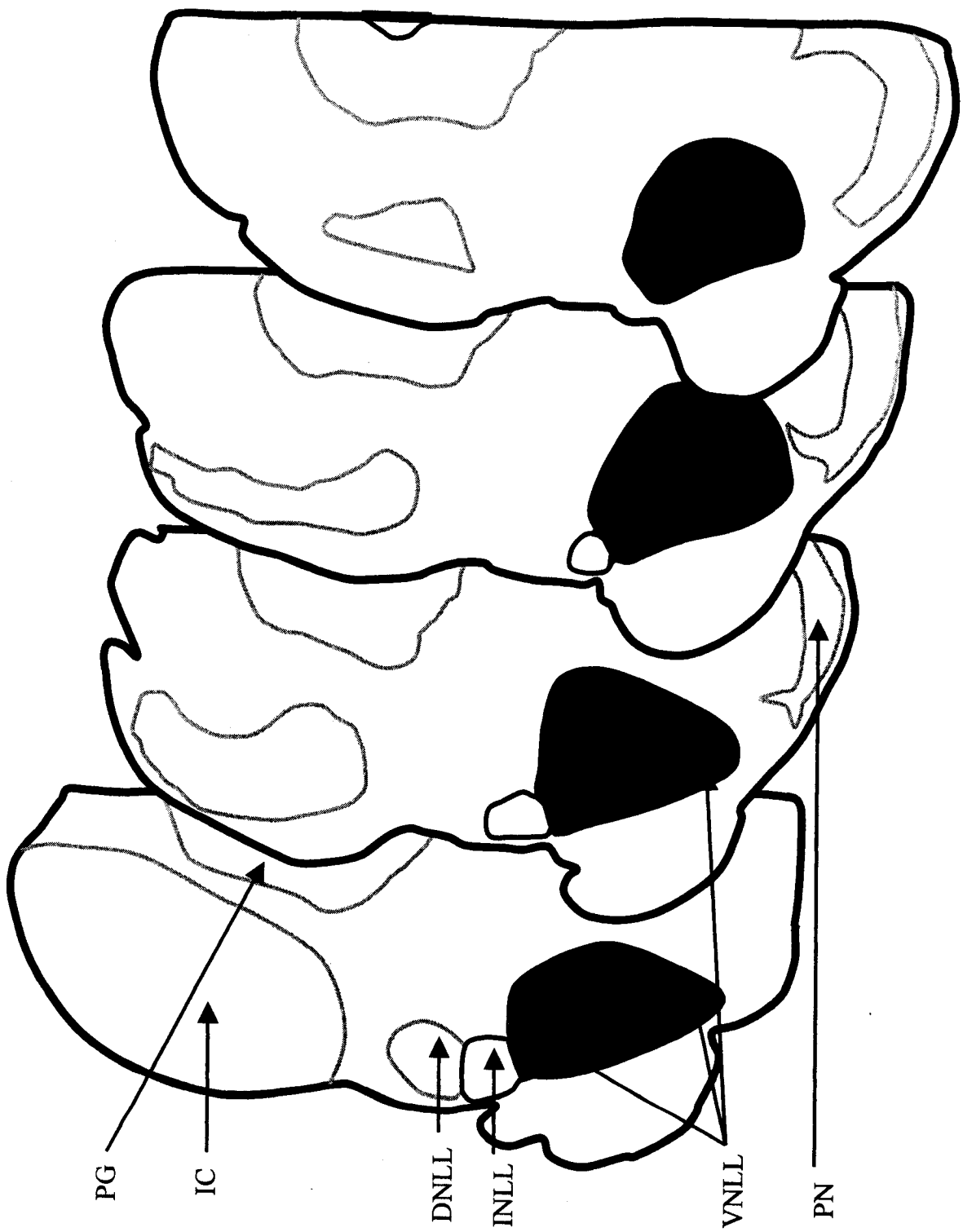
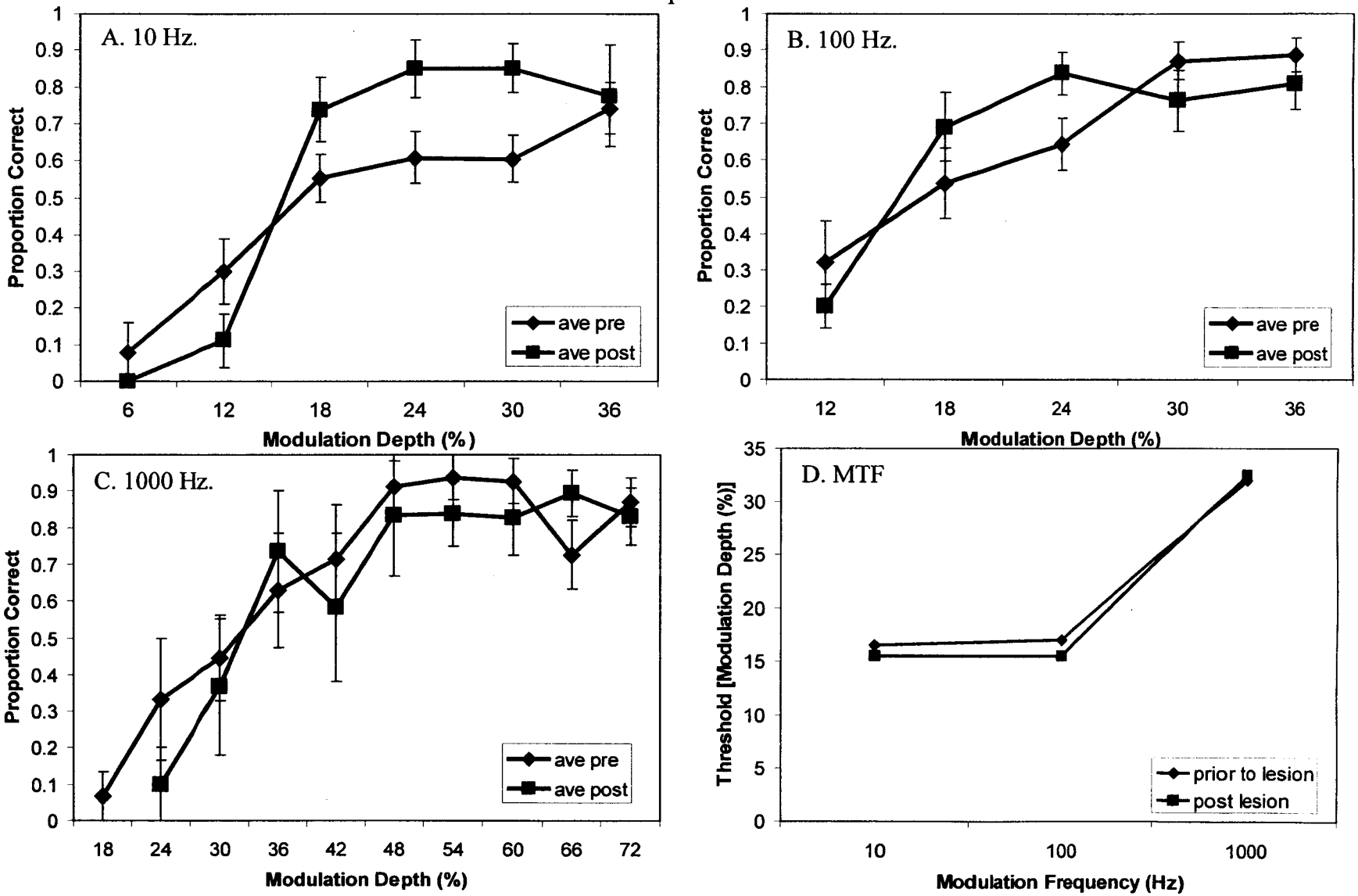

PG
IC
DNLL
INLL
VNLL
PN

Figure 20. Behavioural results of Rat 04. A: modulation sensitivity curve for Rat 04 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 04 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 04 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 04 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.

Complete Lesion



The threshold of Rat 04 before the VNLL lesion and ear block at a modulation frequency of 1000 Hz was 32.0 %. After the lesion and ear block the threshold of the animal increased slightly to 32.5 % (see Figure 20). The difference in thresholds was 0.5 %, which is illustrated in Table 1 and in the MTF for Rat 04.

3.3.2 Rat 10

There was no obvious loss of cells in the VNLL of Rat 10. There was some slight cell loss in the area medial to the VNLL, in the reticular area. However, many healthy cells remained in the VNLL (Figure 21).

The threshold of Rat 10 prior to the VNLL lesion and ear block at a modulation frequency of 10 Hz was 15.5 %. After the lesion and ear block the threshold decreased slightly to 15.0 % (see Figure 22). The difference of -0.5 % is illustrated in Table 1 and in the MTF for Rat 10 (Figure 22).

The threshold of Rat 10 prior to the VNLL lesion and ear block at a modulation frequency of 100 Hz was 24.5 %. The threshold of this animal after the lesion and ear block decreased to 17.5 % (see Figure 22). The difference in threshold of -7 % can be seen in Table 1 and in the MTF for Rat 10.

The threshold of Rat 10 at a modulation frequency of 1000 Hz prior to the VNLL lesion and ear block was 38.0 %. After the lesion and ear block the threshold of the animal decreased to 26.5 % (see Figure 22). The difference in threshold was -11.5 %, observable in Table 1 and the MTF for this animal.

3.3.3 Rat 14

Figure 21. Schematic image of the VNLL lesion of Rat 10. The lesion was restricted to the medial edge of the VNLL, as well as some of the reticular area. The lesion is shaded in gray. A substantial amount of healthy neurons were visible in the area not included in the lesion. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

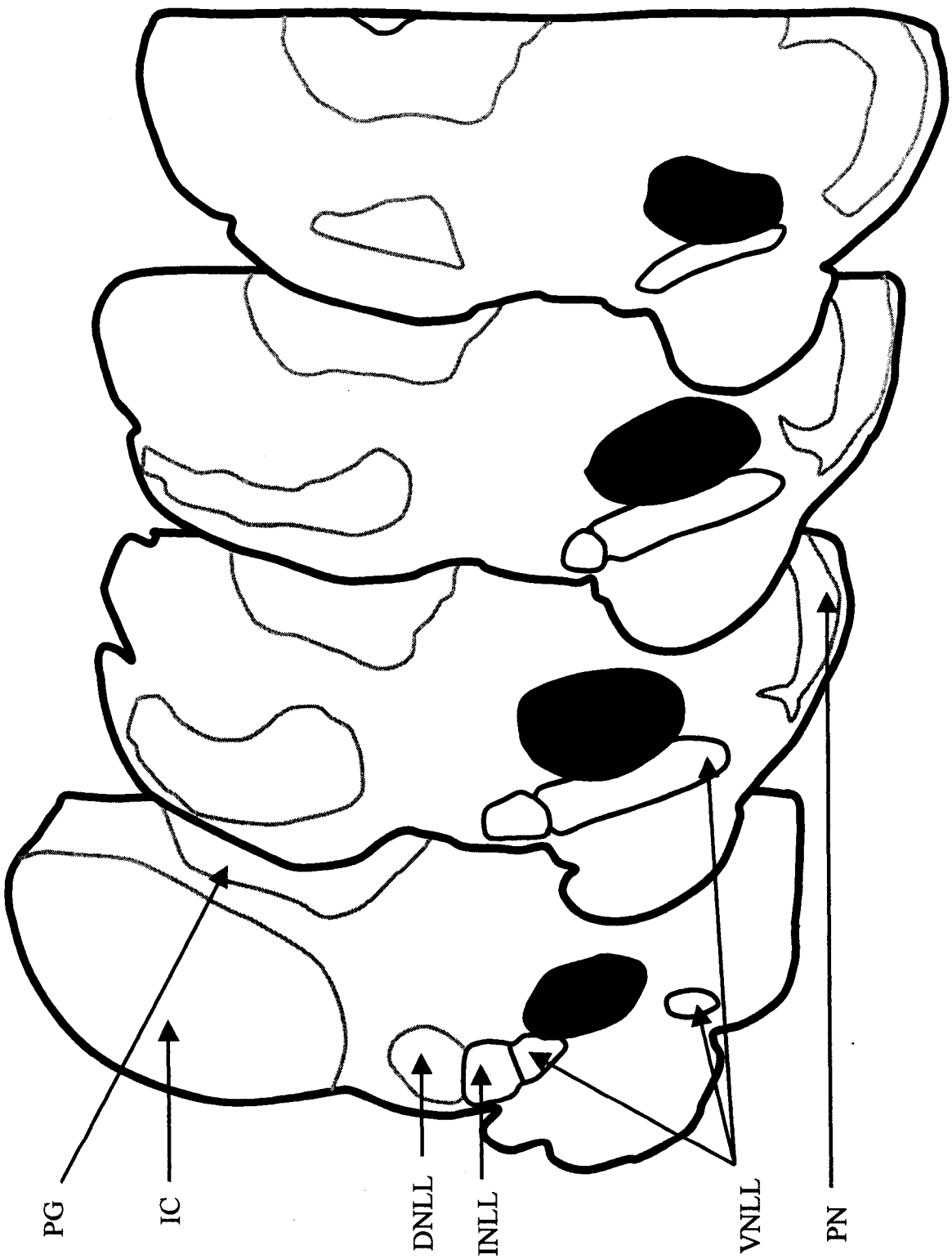
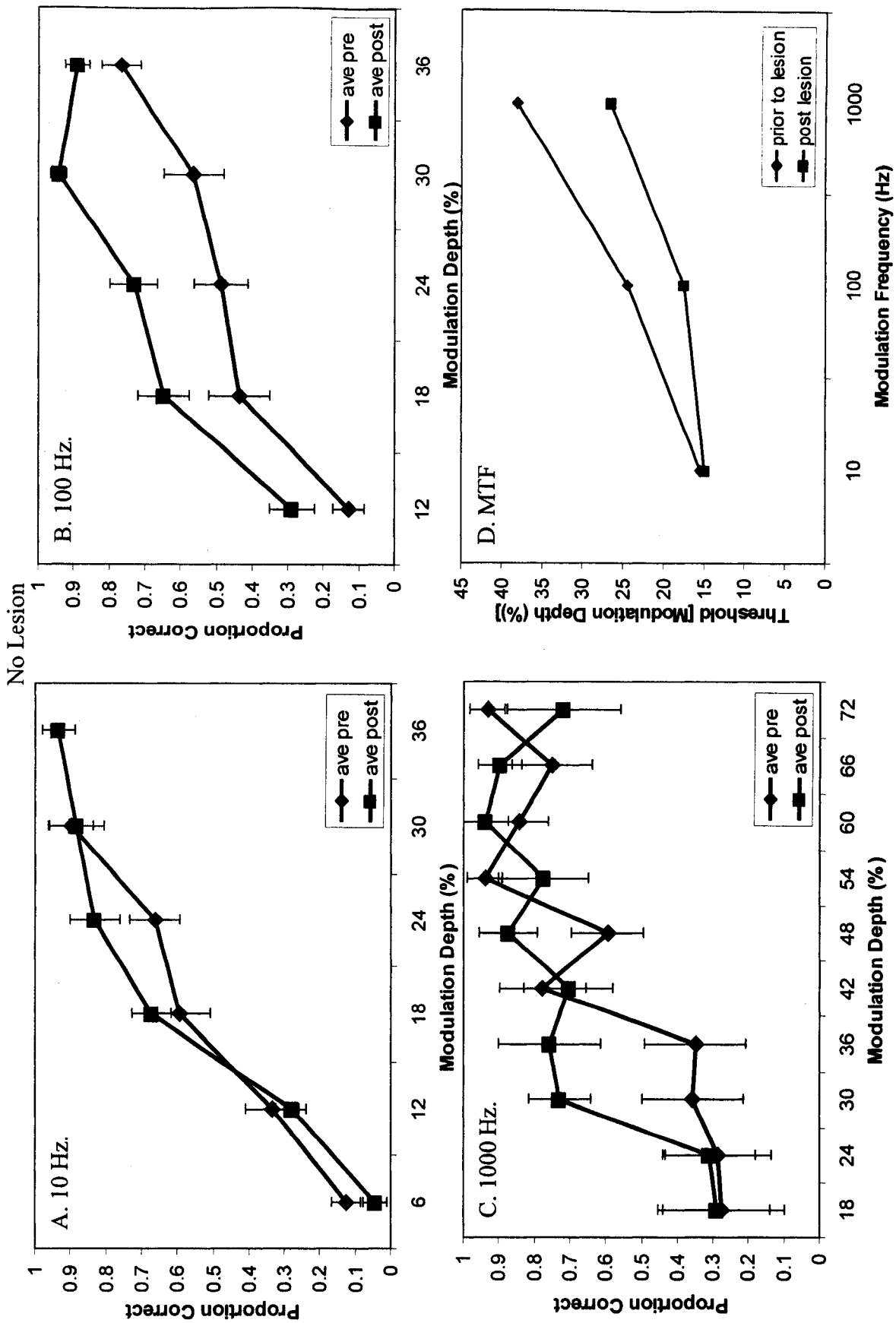

PG
IC
DNLL
INLL
VNLL
PN

Figure 22. Behavioural results of Rat 10. A: modulation sensitivity curve for Rat 10 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 10 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 10 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 10 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.


No Lesion
A. 10 Hz.
B. 100 Hz.
C. 1000 Hz.
D. MTF
Proportion Correct
Modulation Depth (%)
Threshold [Modulation Depth (%)]
Modulation Frequency (Hz)
ave pre
ave post
prior to lesion
post lesion

The lesion of the VNLL in Rat 14 was large and included all of the VNLL as well as some of the reticular area. Other auditory nuclei were not included in the lesion (see Figure 23).

The threshold of Rat 14 at a modulation frequency of 10 Hz prior to the VNLL lesion and ear block was 17.5 %. After the lesion and ear block, the threshold of this animal remained the same at 17.5 % (see Figure 24). The 0 % threshold change after the lesion and ear block can be seen in Table 1 and in the MTF for Rat 14 (Figure 24).

The threshold of Rat 14 prior to the VNLL lesion and ear block at a modulation frequency of 100 Hz was 19.0 %. The threshold of this animal decreased after the lesion and ear block to 10.0 % (see Figure 24). The –9 % threshold difference is shown in Table 1 and in the MTF for this animal.

The threshold of Rat 14 at a modulation frequency of 1000 Hz before the VNLL lesion and ear block was 33.0 %. After the lesion and ear block, the threshold of this animal increased to 41.0 % (see Figure 24). There was an 8 % decrease in the threshold of Rat 14 which is illustrated in Table 1 and in the MTF for this animal.

3.3.4 Rat 20

There was no observable lesion in Rat 20 post mortem. The cells of the VNLL appeared healthy and intact throughout. The reticular area had no visible loss of neurons. All other auditory structures remained intact (Figure 25).

The threshold of Rat 20 at a modulation frequency of 10 Hz before the VNLL lesion and ear block was 10.0 %. The threshold of Rat 20 after the lesion and ear block

Figure 23. Schematic image of the VNLL lesion of Rat 14. The lesion included all of the VNLL, as well as some of the reticular area medial to the VNLL. The lesion is shaded in gray. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

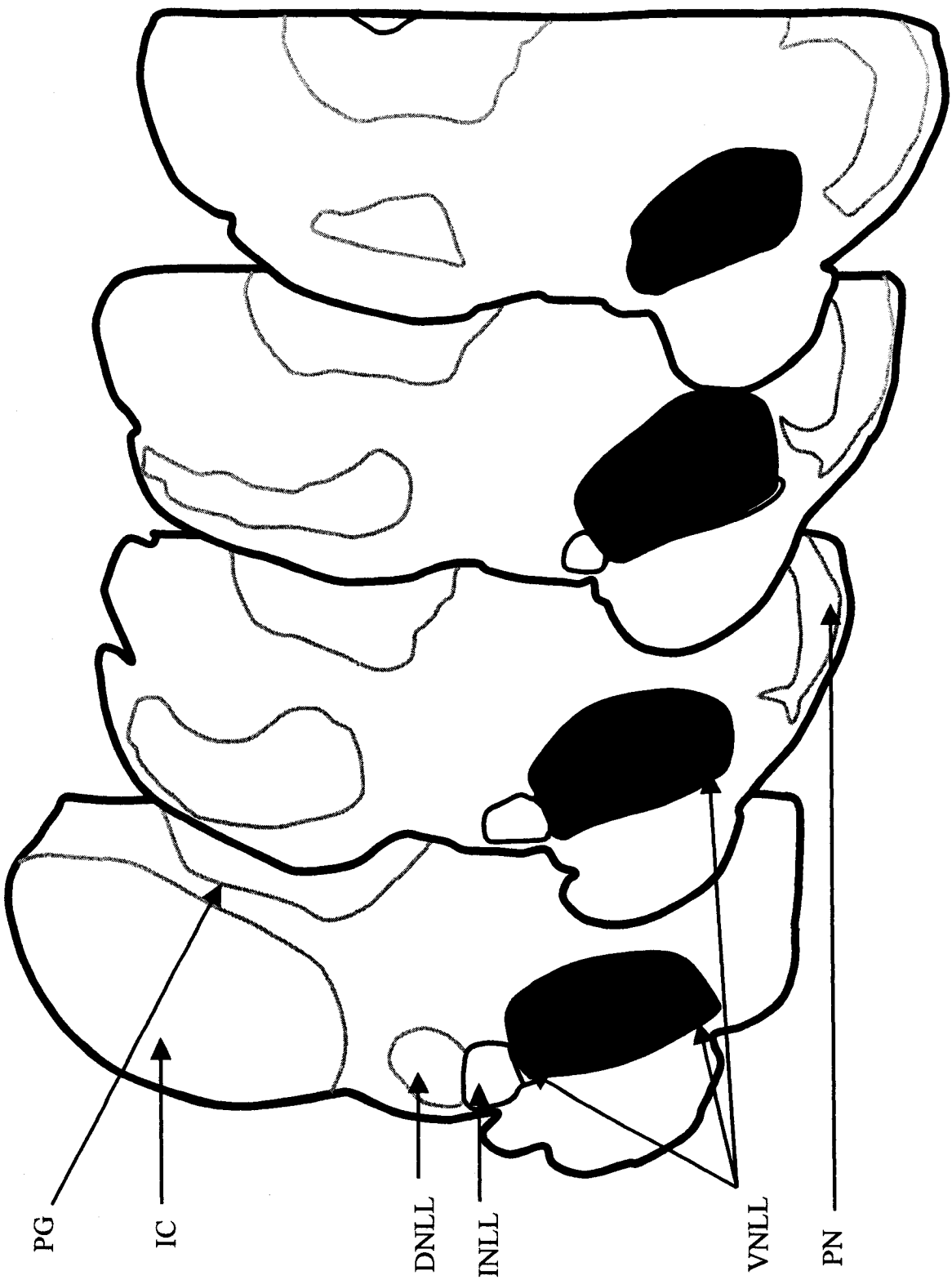
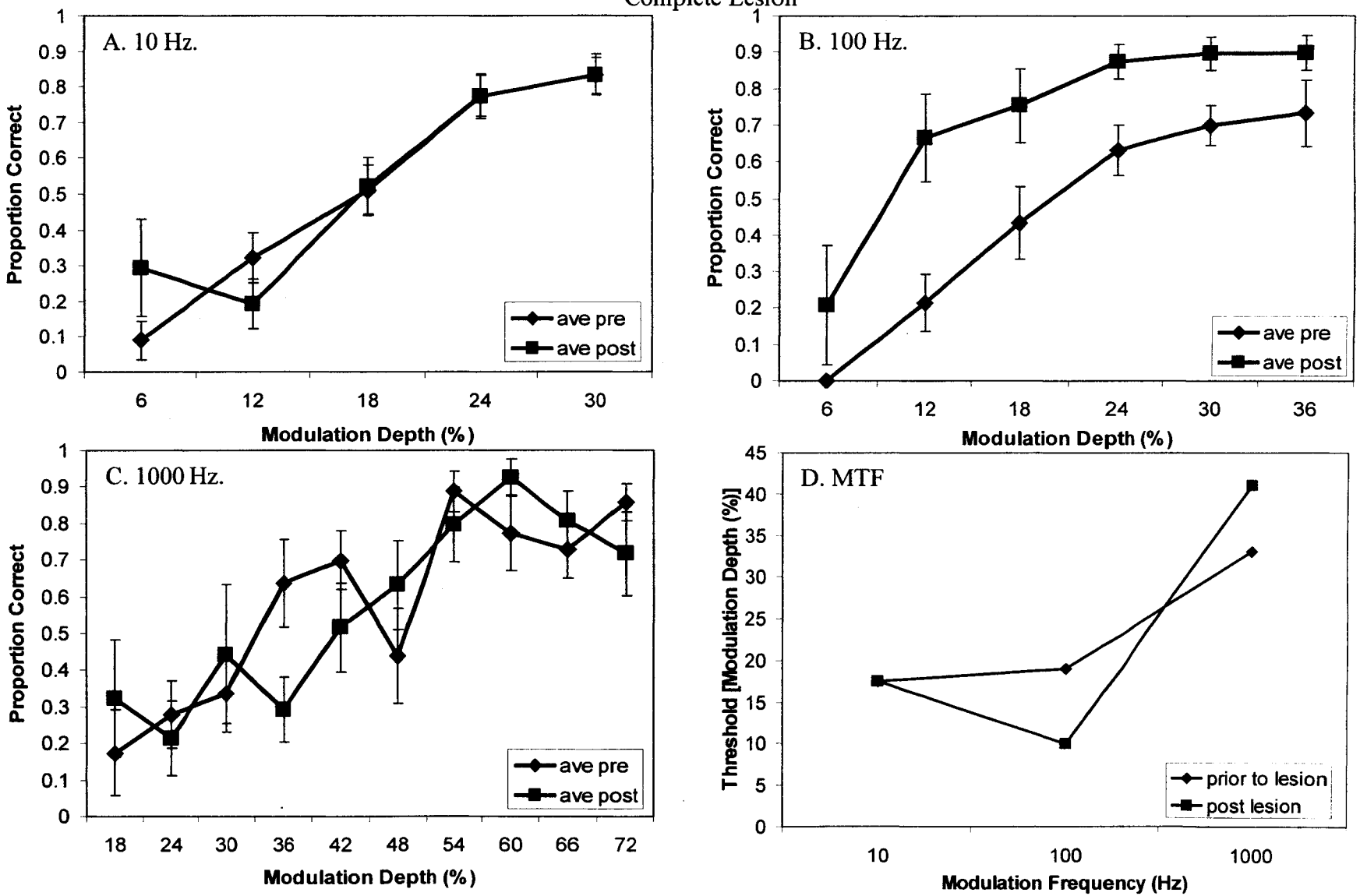

PG
IC
DNLL
INLL
VNLL
PN

Figure 24. Behavioural results of Rat 14. A: modulation sensitivity curve for Rat 14 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 14 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 14 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 14 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.

Complete Lesion



increased to 12.0 % (see Figure 26). The difference in threshold of 2 % is shown in Table 1 and the MTF for Rat 20 (Figure 26).

The threshold of Rat 20 prior to the VNLL lesion and ear block at a modulation frequency of 100 Hz was 11.25 %. After the lesion and ear block the threshold of this animal increased 14.5 % (see Figure 26). The difference in threshold of 3.25 % is illustrated in Table 1 and in the MTF for this animal.

The threshold of Rat 20 at a modulation frequency of 1000 Hz prior to the VNLL lesion and ear block was 35.5 %. After the lesion and ear block the threshold of this animal increased to 40.5 % (see Figure 26). The difference between pre and post lesion and ear block was 5 % which is shown in Table 1 and in the MTF for this animal.

3.3.5 Rat 24

The lesion of Rat 24 was partial (see Figure 27). It included the medial aspect of the VNLL as well as some of the reticular area. Many cells in the VNLL remained intact, and other auditory structures were undamaged after the lesion.

The threshold of Rat 24 at a modulation frequency of 10 Hz prior to the VNLL lesion and ear block was 21.75 %. After the lesion and ear block, the threshold of this animal decreased to 18.25 % (see Figure 28). The difference in threshold of -3.5 % is shown in Table 1 and in the MTF for Rat 24 (Figure 28).

The threshold of Rat 24 before the VNLL lesion and ear block at a modulation frequency of 100 Hz was 21.5 %. The threshold of this animal increased after the lesion and ear block to 32 % (see Figure 28). The difference in threshold after the lesion was 10.5 %, which can be seen in Table 1 and in the MTF for this animal.

Figure 25. Schematic image of the VNLL lesion of Rat 20. No lesion was apparent in Rat 20. A substantial amount of healthy neurons were visible throughout the VNLL and reticular area. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.


PG
IC
DNLL
INLL
VNLL
PN

Figure 26. Behavioural results of Rat 20. A: modulation sensitivity curve for Rat 20 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 20 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 20 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 20 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.

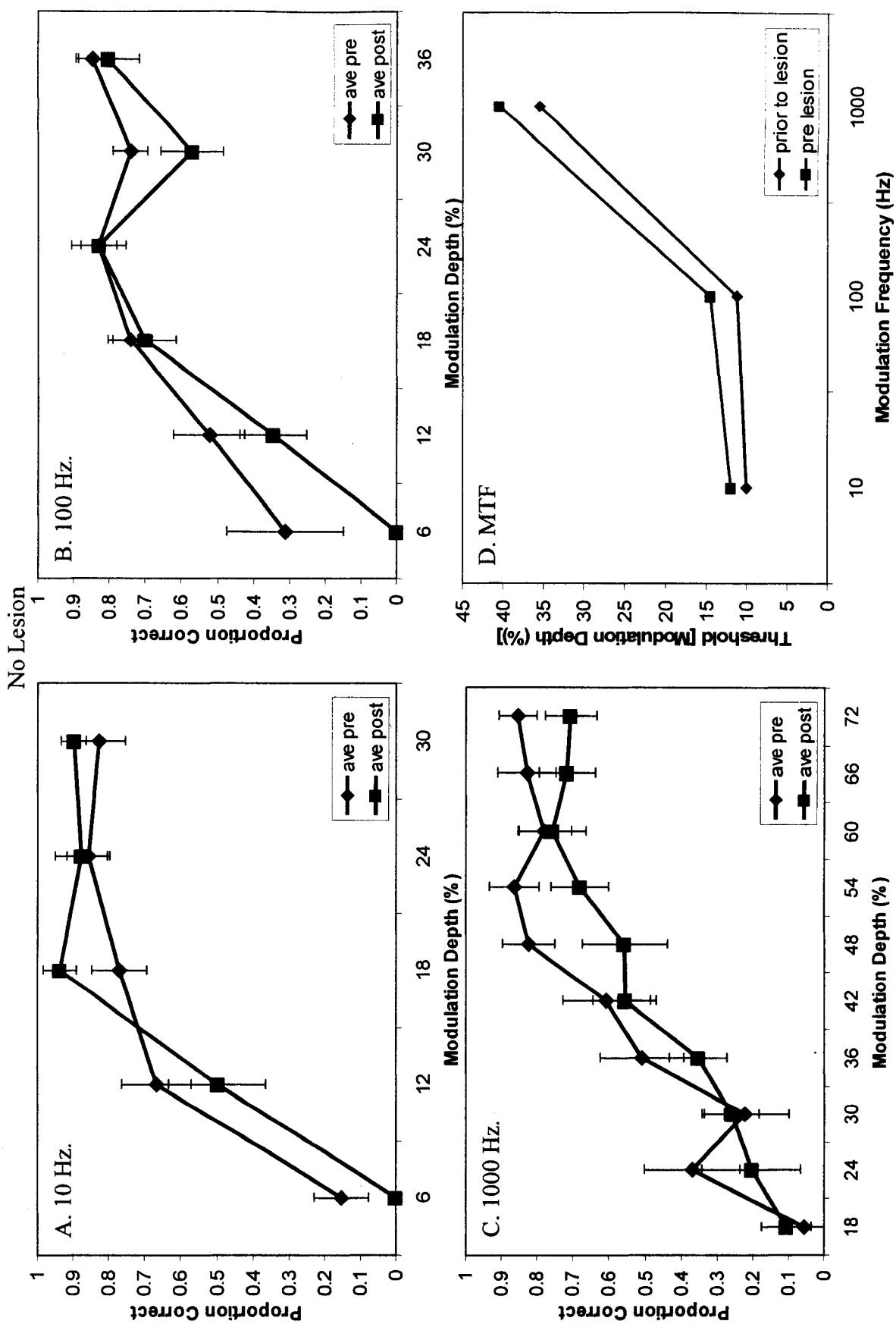

No Lesion
A. 10 Hz.
B. 100 Hz.
C. 1000 Hz.
D. MTF
Proportion Correct
Modulation Depth (%)
Threshold [Modulation Depth (%)]
Modulation Frequency (Hz)
ave pre
ave post
prior to lesion
pre lesion

Figure 27. Schematic image of the VNLL lesion of Rat 24. The lesion was partial and restricted to the ventral-medial areas of the VNLL, as well as some of the reticular area. The lesion is shaded in gray. A substantial amount of healthy neurons were visible in the area not included in the lesion. The rostral-most slide is a slice at –7.64 mm from Bregma. The second slide is at –7.80 mm from Bregma. The third slide is at –8.00 mm from Bregma. The fourth slide is at –8.30 mm from Bregma. Abbreviations in the figure represent the following: PG: periaqueductal gray; IC: inferior colliculus; DNLL: dorsal nucleus of the lateral lemniscus; INLL: intermediate nucleus of the lateral lemniscus; VNLL: ventral nucleus of the lateral lemniscus; PN: pontine nucleus.

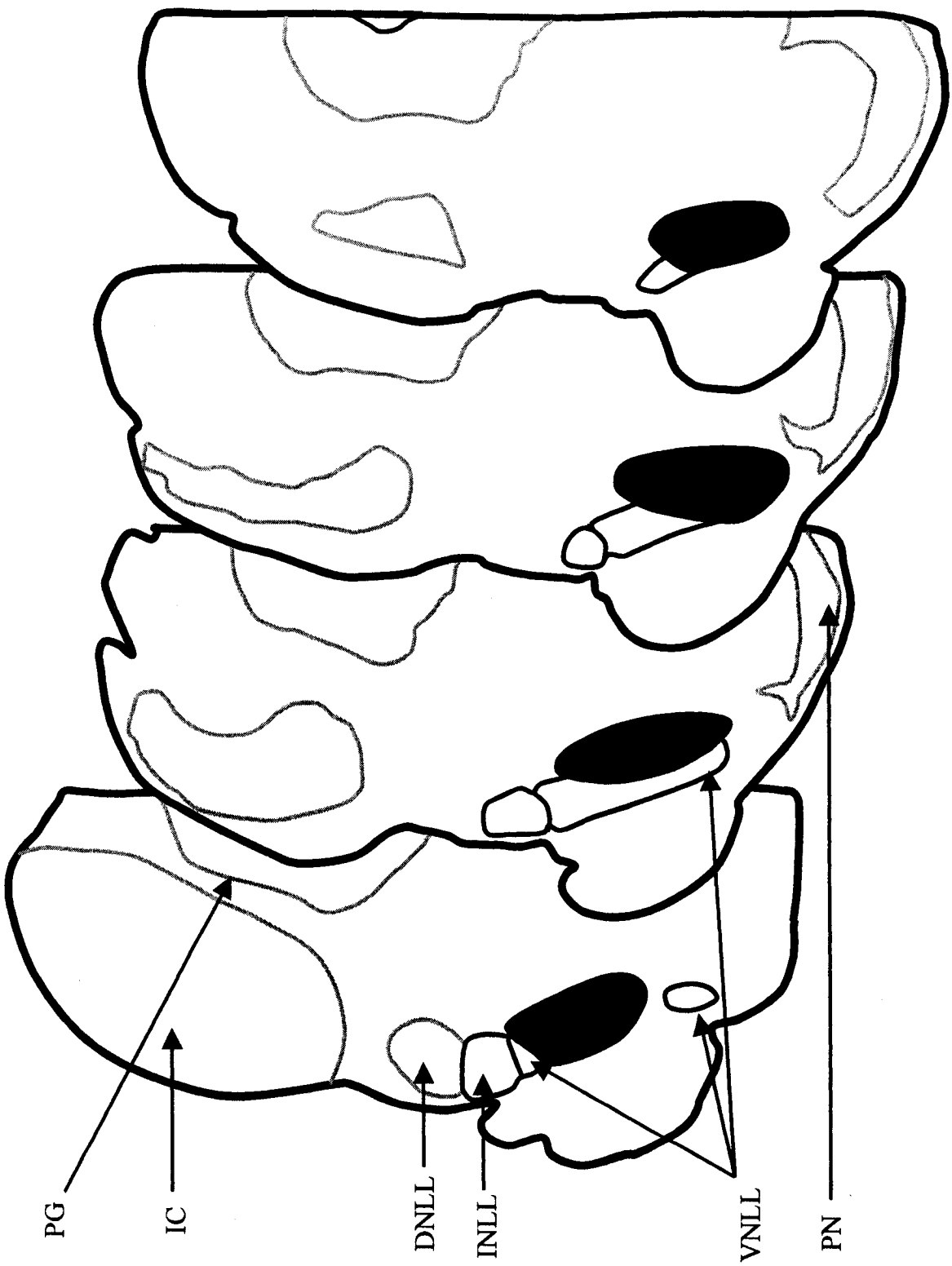
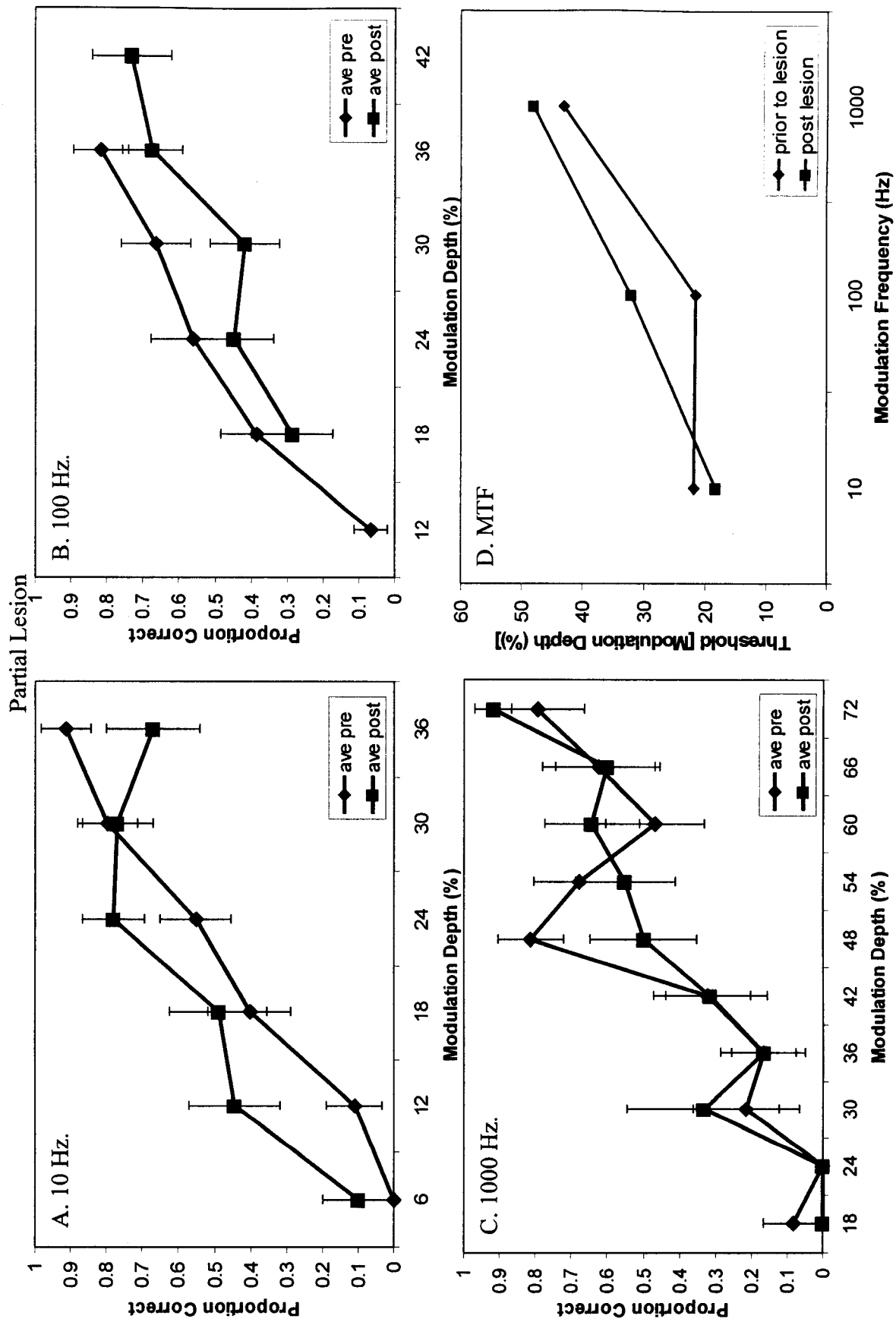

PG
IC
DNLL
INLL
VNLL
PN

Figure 28. Behavioural results of Rat 24. A: modulation sensitivity curve for Rat 24 at a modulation frequency of 10 Hz, prior to and post VNLL lesion and ear block. B: modulation sensitivity curve for Rat 24 at a modulation frequency of 100 Hz, prior to and post VNLL lesion and ear block. C: modulation sensitivity curve for Rat 24 at a modulation frequency of 1000 Hz, prior to and post VNLL lesion and ear block. D: modulation transfer function (MTF) for Rat 24 prior to and post VNLL lesion and ear block. The MTF indicates the change in threshold of the animal before and after the VNLL lesion and ear block procedure.


Partial Lesion
A. 10 Hz.
B. 100 Hz.
C. 1000 Hz.
D. MTF
Proportion Correct
Modulation Depth (%)
Threshold [Modulation Depth (%)]
Modulation Frequency (Hz)
ave pre
ave post
prior to lesion
post lesion

The threshold of Rat 24 at a modulation frequency of 1000 Hz prior to the VNLL lesion and ear block was 43.0 %. After the lesion and ear block the threshold of this animal increased to 48.0 % (see Figure 28). The difference in threshold was 5 %, which is illustrated in Table 1 and in the MTF for this animal.

A summary of the threshold shifts after the VNLL lesions of all the animals in the current experiment can be found in Figure 29. The charts along the left side of the figure (A, B, C) are animals that have had the VNLL contralateral to the unblocked ear lesioned. The charts along the right side of the figure (D, E, F) are animals that have had the VNLL ipsilateral to the unblocked ear lesioned. The top two charts, A and D, represent the threshold shifts at a modulation frequency of 10 Hz. The middle two charts, B and E, represent the threshold shifts at a modulation frequency of 100 Hz. The bottom two charts, C and F, represent threshold shifts at a modulation frequency of 1000 Hz.

3.4 Absolute Intensity Task

Three animals were tested for absolute intensity using white noise bursts against a silent background. The threshold of an animal in this task is the intensity which the animal accurately identifies 50 % of the time. This task was performed before and after double ear block surgery to ensure the ear block technique raised the threshold of the animals above 50 dB.

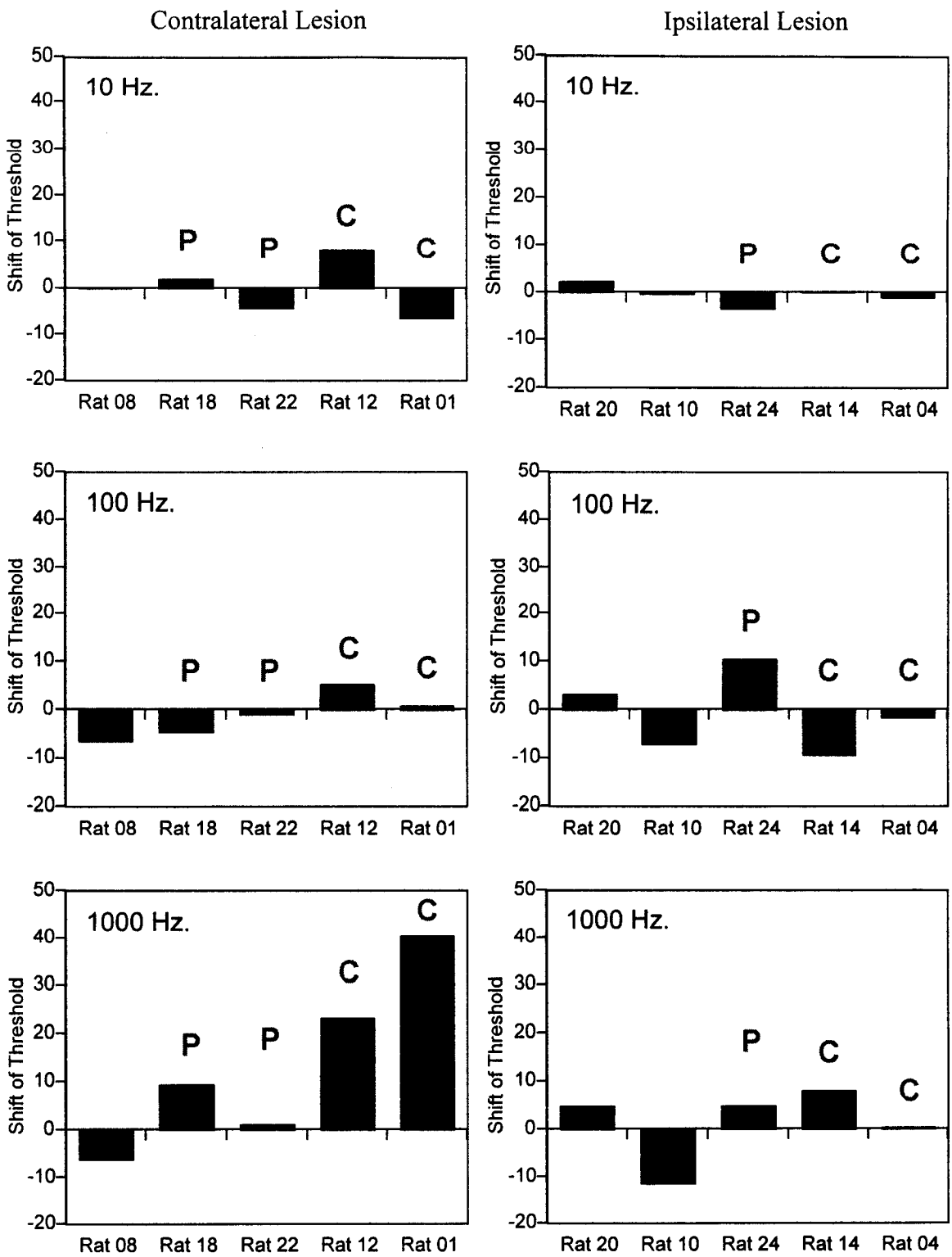
The average absolute intensity threshold of these three animals prior to the double ear block surgery was +20 dB (SPL). After the double ear block surgery, the collective threshold increased to +66 dB (SPL). This represents an average increase of 46 dB (SPL) in absolute intensity threshold with the ear blocked using the current method (see Figure

Table 1. Threshold differences after VNLL lesions and ear blocks. The group marked 'Contralateral to Unblocked Ear' consists of animals with VNLL lesions contralateral to the unblocked ear. The group marked 'Ipsilateral to Unblocked Ear' consists of animals with VNLL lesions contralateral to the unblocked ear. Negative numbers indicate that the threshold of the animal at that modulation frequency decreased after the lesion and ear block. Positive numbers indicate that threshold of the animal increased after the lesion and ear block. All numbers are threshold differences in modulation depth (%).

	Contralateral to Unblocked Ear	Ipsilateral to Unblocked Ear
10 Hz.	*Rat 1: -6.5 Rat 8: 0 *Rat 12: 8 Rat 18: 1.5 Rat 22: -4.25	*Rat 4: -1 Rat 10: -0.5 *Rat 14: 0 Rat 20: 2 Rat 24: -3.5
100 Hz	*Rat 1: 1 Rat 8: -6.5 *Rat 12: 5.5 Rat 18: -4.75 Rat 22: -1	*Rat 4: -1.5 Rat 10: -7 *Rat 14: -9 Rat 20: 3.25 Rat 24: 10.5
1000 Hz	*Rat 1: 40.5 Rat 8: -6 *Rat 12: 23 Rat 18: 9.5 Rat 22: 1	*Rat 4: 0.5 Rat 10: -11.5 *Rat 14: 8 Rat 20: 5 Rat 24: 5

Where * indicates complete VNLL lesion.

Figure 29. Behavioural summary of all animals in the VNLL experiment. Along the left side of the page are the animals with VNLL lesions contralateral to the unblocked ear, and the right side of the page are animals with VNLL lesions ipsilateral to the unblocked ear. The top two charts are the threshold changes of the animals after the VNLL lesion and ear block at a modulation frequency of 10 Hz. The middle two charts are of the threshold changes of the animals at a modulation frequency of 100 Hz. The bottom two charts are of the threshold changes of the animals at a modulation frequency of 1000 Hz. In every chart, a negative score indicates that the threshold of the animal decreased after the lesion and ear block. Conversely, a positive value indicates that the threshold of the animal increased after the VNLL lesion and ear block. The thresholds of the animals are indicated in modulation depth (%), and any change is listed as the modulation depth difference between the thresholds of the animal before and after the lesion and ear block.


Contralateral Lesion
Ipsilateral Lesion
10 Hz.
100 Hz.
1000 Hz.
Shift of Threshold
50
40
30
20
10
0
-10
-20
P
C
Rat 08
Rat 18
Rat 22
Rat 12
Rat 01
Rat 20
Rat 10
Rat 24
Rat 14
Rat 04

30). The average threshold after the double ear block technique is 66 dB (SPL), which is well above the 50 dB (SPL) intensity used in the current experiment.

To ensure that the VNLL lesion did not affect the absolute intensity of the animals, two animals, Rats 12 and 14, with complete VNLL lesions were tested for their absolute intensity threshold. Rat 12 was able to detect the 50 dB (SPL) white noise burst, with threshold just above 40 dB (SPL) (Figure 31). Rat 14 was able to detect the 50 dB (SPL) white noise burst, with threshold below 40 dB (SPL). This task was used as a control to ensure that any deficits detected were not deficits in the animal's ability to detect any sound, rather a deficit in their ability to detect sinusoidal amplitude modulation of a white noise carrier.

Figure 30: Effect of double ear block procedure on the rat's absolute threshold. The threshold for the rat to detect the presence of white noise against a silent background was 20 dB prior to the double ear block procedure and 66 dB after the double ear block procedure. This control ensures that the ear block technique impairs the hearing of the affected ear at 50 dB, which is the intensity of the stimulus used in these experiments.

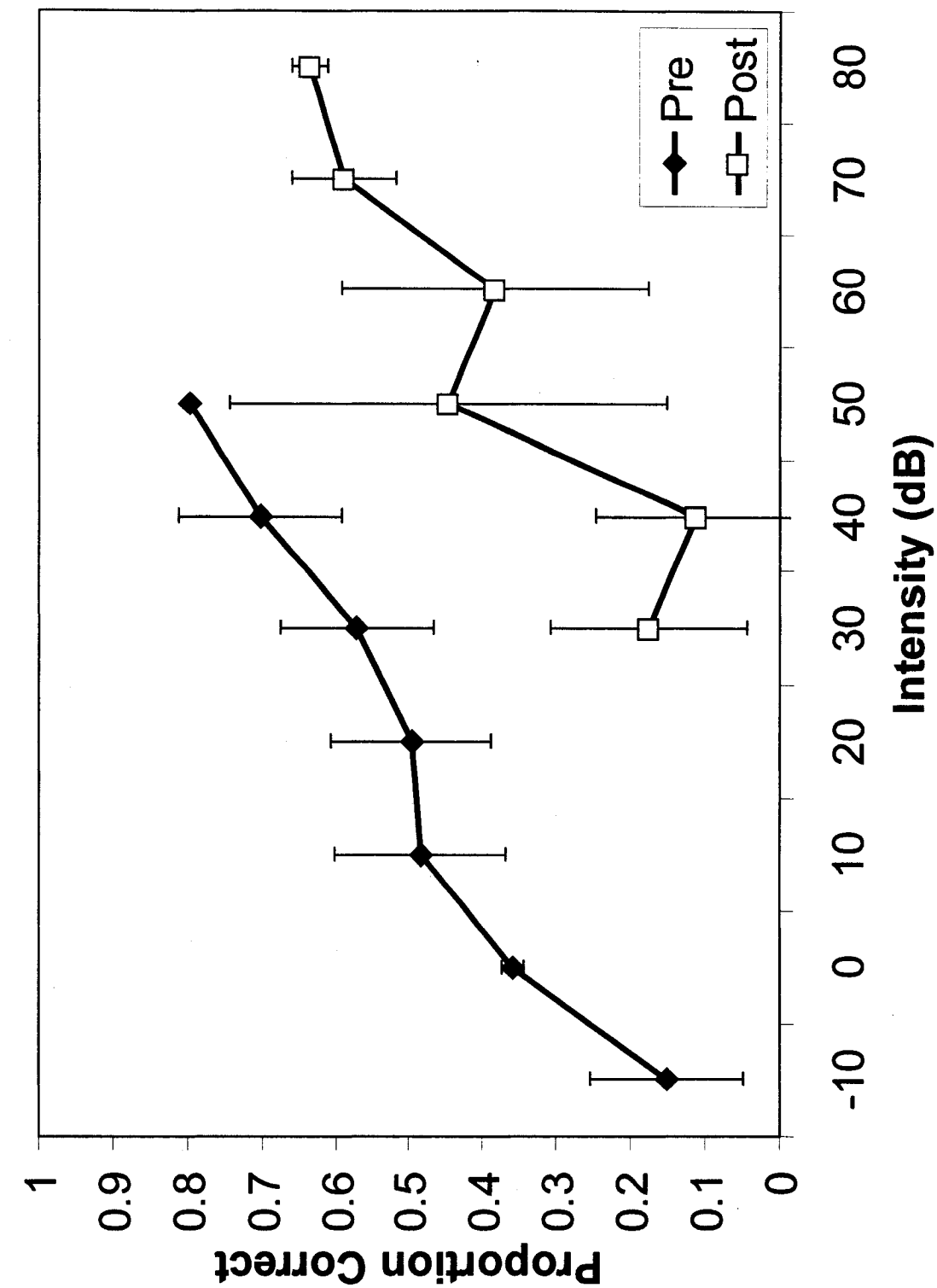
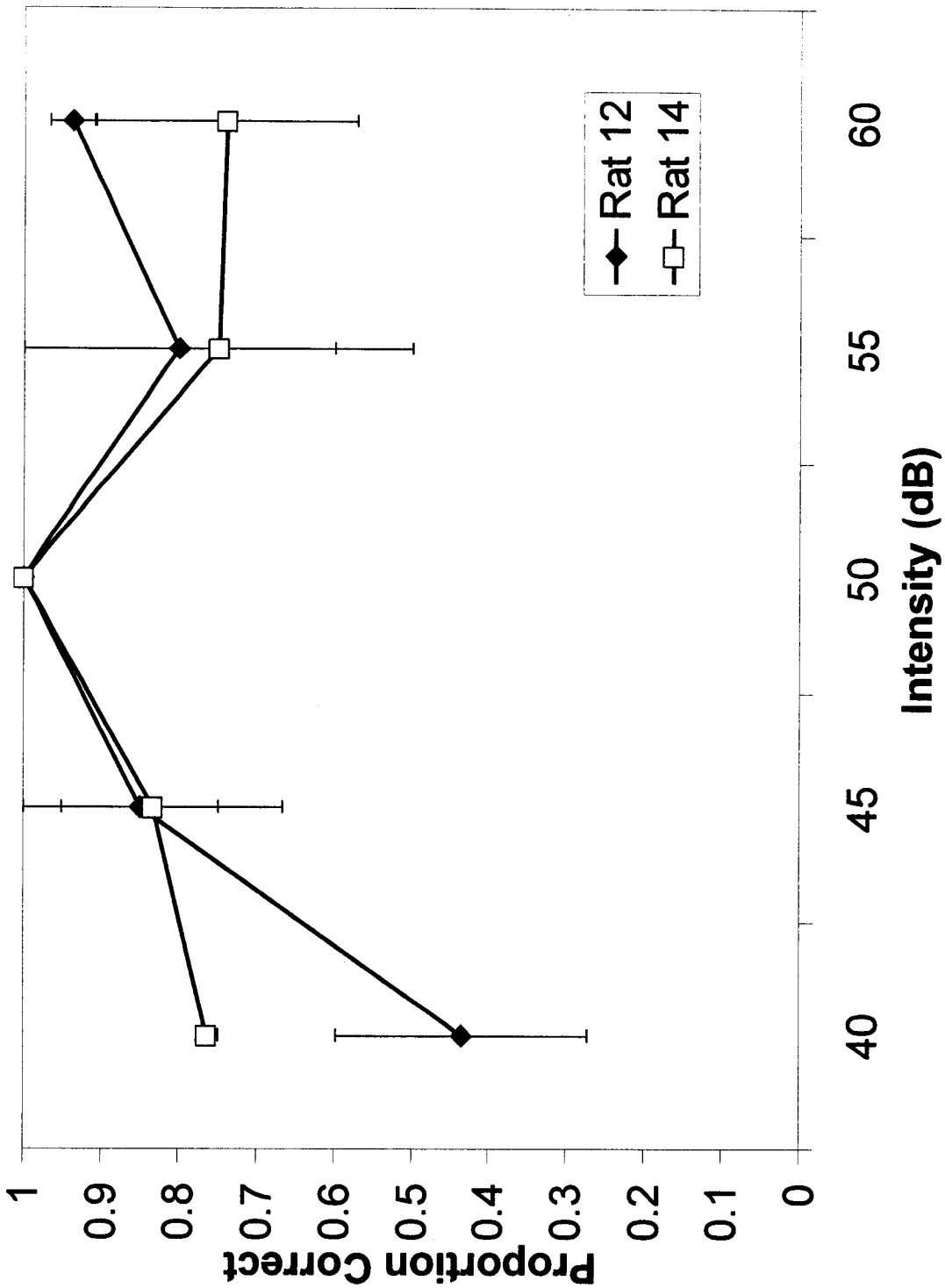

Proportion Correct
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-10
0
10
20
30
40
50
60
70
80
Intensity (dB)
Pre
Post

Figure 31. Absolute intensity curves for Rat 12 and Rat 14 after the VNLL lesion and unilateral ear block. Both animals were tested to ensure that they did not have a shift in their absolute threshold caused by the VNLL lesion. Both animals were able to detect the noise burst at 50 dB, indicating that any deficits observed were not due to large changes in absolute threshold.


Proportion Correct
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
40
45
50
55
60
Intensity (dB)
Rat 12
Rat 14

4. DISCUSSION

The current study explored the behavioural limits of the rat's ability to detect sinusoidal amplitude modulation across modulation frequencies from 5 to 2000 Hz. All animals tested required greater modulation depth to detect the presence of the amplitude modulation as modulation frequency increased. The experiment showed that the ability of the rat to discriminate sinusoidal amplitude modulation of a noise carrier is similar to that of both the chinchilla and human.

The current study also explored the contribution of the ventral nucleus of the lateral lemniscus (VNLL) to the rat's ability to detect sinusoidal amplitude modulation of a noise carrier. The results suggest that the VNLL is involved in processing sinusoidal amplitude modulated noise, specifically at higher modulation frequencies.

4.1 Normal Modulation Transfer Function

The modulation transfer function obtained from twelve normal animals across eight modulation frequencies from 5 to 2000 Hz resulted in a function with threshold that rises as a function of modulation frequency. As modulation frequency increases, the animals require greater modulation depth in order to detect the presence of the amplitude modulation.

The modulation transfer function of the rat obtained in the current experiment is very similar to those of both the chinchilla and human. It is worth noting the differences in auditory range of the species being compared. The hearing range of the human is relatively low, from 0.02 KHz to 20 KHz. The hearing range of the chinchilla is slightly higher, from 0.09 KHz to 23 KHz. The rat has high frequency hearing, from 0.20 KHz to 80 KHz (Kelly, 1990). Despite the variation of the auditory ranges of the animals

included in Figure (7), the modulation transfer functions of these animals are very similar. Due to the similarity between the results obtained with the rat and those from the chinchilla and humans, the ability to detect amplitude modulation does not appear to be affected by an animal's hearing range. This indicates that the ability to process sinusoidal amplitude modulation is independent of frequency processing.

The results of the normative amplitude modulation processing abilities are important for several reasons. It is important to understand the limits of the acoustic perceptual abilities of the rat. The current study has outlined the ability of the rat to discriminate sinusoidal amplitude modulation. Additionally, the normative results obtained in the current experiment will serve as a standard against which other amplitude modulation processing studies can be compared.

4.2 VNLL Lesion Experiment

Ten animals were tested in a second experiment that evaluated amplitude processing abilities before and after VNLL lesions at low, middle and high modulation frequencies. Half of these animals had VNLL lesions ipsilateral to an unblocked ear, while the other half of these animals had VNLL lesion contralateral to their unblocked ear. The results indicate that the VNLL contributes to the processing of amplitude modulation at high modulation frequencies. Deficits were only observed in the group with lesions contralateral to the unblocked ear, suggesting that the VNLL is a monaural structure driven from the contralateral side.

4.2.1 The VNLL Contributes to Amplitude Modulation Processing in the Rat

There were two animals (Rats 01 and 12) in the group that received complete VNLL lesions contralateral to the unblocked ear, and these animals suffered profound

deficits in their ability to detect sinusoidal amplitude modulated noise. Specifically, these animals both had large deficits at the highest modulation frequency, 1000 Hz. This result suggests that the VNLL is involved in processing sinusoidal amplitude modulated sounds. This result was expected, as several physiological studies have indicated that the neurons of the VNLL are ideally suited for processing temporal properties of sound in other mammals (Covey and Casseday, 1986; Covey and Casseday, 1991; Aitkin et al., 1970; Batra and Fitzpatrick, 1997). Physiological results *in vitro* in the rat have also revealed neuronal responses that are ideal for encoding temporal auditory information (Wu, 1999).

The VNLL provides the inferior colliculus with inhibitory input which must affect the animal's ability to either detect or react to amplitude modulation at higher frequencies. Drs. Covey and Casseday (1991) have indicated that the VNLL could provide convergent input to a single target in the inferior colliculus. The various latencies of the neurons of the VNLL could provide the target in the IC with inhibitory signals at different times, allowing the target neurons to encode specific time delays or temporal patterns of sound. As sinusoidal amplitude modulation is a temporal pattern of sound, it is feasible that the VNLL lesions produced in the current study stopped the inhibitory input to the IC, interrupting with the target neuron's ability to process the sinusoidal amplitude modulation at higher modulation frequencies.

4.2.2 Deficit Variation Across Modulation Frequencies

The ability of these animals to process amplitude modulation at higher modulation frequencies was affected by the VNLL lesions, but their ability to process lower modulation frequencies (10 and 100 Hz, specifically) remained intact. Rat 12 had

deficits at both 10 and 100 Hz, but these deficits were much less pronounced than that at 1000 Hz. Rat 01 had no deficit at 100 Hz, and actually improved after the lesion at 10 Hz. That a deficit only occurred at the highest modulation frequency suggests that the contribution of the VNLL to amplitude modulation processing may be limited to higher modulation frequencies and not to lower modulation frequencies. It is unknown whether the VNLL of the rat processes amplitude modulation at low, middle, or high modulation frequencies. The current results suggest that the rat VNLL processes amplitude modulation at high modulation frequencies, but is less involved in processing lower modulation frequencies.

No studies investigating the ability of the neurons of the rat VNLL to follow sinusoidal amplitude modulation have yet been done. A study by Huffman et al. (1998) investigating the ability of VNLL neurons to follow sinusoidal amplitude modulated pure tones revealed that neurons of the VNLLm of the insectivorous bat remained synchronized from a modulation frequency of 100 Hz up to 1000 Hz. It is difficult to infer if the neurons of the rat VNLL are capable of responding to sinusoidal amplitude modulated tones over this range, or whether these neurons have higher or lower best modulation rates.

It is possible that the neurons of the rat VNLL synchronize to all modulation frequencies tested, 10, 100 and 1000 Hz, but that lesions of the VNLL only produce behavioural deficits at 1000 Hz. The analysis of amplitude modulation likely occurs at more than one level of the central auditory system. When the VNLL is lesioned, information from lower structures such as the cochlear nucleus and superior olive complex still reach the inferior colliculus. In this way, temporal processing of signals

like sinusoidal amplitude modulation still occurs in the central auditory system. The role of the VNLL may be to contribute to the distinction of specific stimuli like high modulation rates. When the VNLL is lesioned, the inhibitory input of varying latency to the ICC is missing, and though this may not prevent the animal from detecting amplitude modulated sounds at lower modulation frequencies, it may produce a timing deficit within the ICC that is sufficient to prevent the animal from detecting the amplitude modulation at higher modulation rates.

4.2.3 Lesions Ipsilateral to the Unblocked Ear vs. Contralateral to the Unblocked Ear

While two animals with complete lesions of the VNLL contralateral to the unblocked ear had pronounced deficits in their ability to resolve sinusoidal amplitude modulation at the highest modulation frequency, 1000 Hz, the two animals with complete lesions of the VNLL ipsilateral to the unblocked ear did not have deficits at any modulation frequency. These results show that the VNLL of the rat receives input from the contralateral side.

The results of the current experiment support the information currently available regarding input to the VNLL, indicating that it receives monaural, contralateral input from the cochlear nucleus (Koch and Grothe, 2000; Covey and Casseday, 1986; Smith et al., 1991; Cant and Benson, 2003).

It has been suggested that the VNLL may be binaural in nature (Batra and Fitzpatrick, 1997; 1999; 2002). If the rat VNLL were binaural, it would be expected that some decrease in performance would occur from a unilateral lesion. However, the current study found that animals with lesions ipsilateral to the unblocked ear had no deficits in their ability to perform the task. Since no decrease in performance was

observed with lesions ipsilateral to the unblocked ear, but deficits were observed with lesions contralateral to the unblocked ear, the VNLL of the rat is likely a monaural, not binaural, structure driven from the contralateral side. It is also worth noting that the findings of Batra and Fitzpatrick were in the rabbit, so discrepancies between their studies and the current study may be due to differences across species.

It would be reasonable to argue that any deficits observed in the current study are a result of kainic acid affecting emotional response or motor response areas. Since the VNLL is in the brain stem, an area with motor control areas and emotional response areas, it is feasible that the kainic acid injections may have affected these systems. This would confound the deficit results, which may be due to auditory difficulties, motor difficulties or emotional difficulties. However, the animals with kainic acid lesions ipsilateral to the unblocked ear have no deficits. These animals received lesions nearly identical in size and location to the animals with lesions contralateral to the unblocked ear. This result suggests that animals who had deficits in their ability to perform the sinusoidal amplitude modulation task suffered from auditory deficits, not emotional or motor deficits.

4.2.4 Only Complete Lesions Produce Deficits

None of the animals in the group with lesions ipsilateral to the unblocked ear showed deficits at any modulation frequency. Two of the animals in the group with lesions contralateral to the unblocked ear had deficits at the highest modulation frequency, but the other three animals in this group did not have any deficits. The animals with deficits were the animals with complete lesions of the VNLL, while the animals with partial lesions or no lesions had no deficits. This result indicates that the

animal is still capable of processing sinusoidal amplitude modulated noise across all modulation frequencies if some healthy neurons remain in the VNLL. Only when there are no healthy neurons remaining in the VNLL is a deficit observed.

4.2.5 Comparing VNLL Lesions to Other Lesion Studies

There are no other known studies investigating the effects of lesions of the VNLL on an animal's ability to perform an auditory response task. There are studies that have investigated the effects of lesions in other central auditory nuclei on an animal's ability to detect temporally modulated stimuli.

Bilateral lesions of auditory cortex have been shown to produce deficits in the rat's ability to process sinusoidal amplitude modulation at higher modulation frequencies (Cooke and Kelly, 2004). With bilateral auditory cortex lesions, the rat was found to have significant deficits in its ability to detect the presence of sinusoidal amplitude modulation at modulation frequencies of 100 and 1000 Hz. Although deficits were seen at a modulation frequency of 10 Hz, these deficits were found to be insignificant. The current study found deficits at 1000 Hz with VNLL lesions contralateral to the unblocked ear, but ability to detect 10 and 100 Hz remained after VNLL lesions.

The difference in amplitude modulation detection after lesions of auditory cortex and VNLL indicate that amplitude modulation processing occurs at different levels of the central auditory system. The current study suggests that the VNLL is important for processing amplitude modulation at high modulation frequencies (1000 Hz), while the auditory cortex study has indicated that the cortex is important for processing amplitude modulation at higher modulation frequencies (100 and 1000 Hz). Both nuclei are important for processing amplitude modulation.

The cortical lesion study found that the severity of the deficit incurred was directly proportional to the size of the cortical lesions. Animals with more extensive lesions into the area surrounding the primary auditory cortex suffered more profound amplitude modulation detecting deficits than animals with less damage in the surrounding area. Unlike the cortex, the current study revealed that the VNLL is still capable of processing amplitude modulation with a fraction of healthy cells remaining. There is no gradient of extent of damage to severity of deficit in the VNLL; if any healthy neurons remain, the VNLL is still capable of processing amplitude modulation.

While amplitude modulation detection is an effective stimulus for testing temporal processing abilities, other stimuli like gap detection and duration discrimination are also useful for this purpose. A study investigating the contribution of the auditory cortex to duration discrimination and gap detection indicated that bilateral auditory cortex lesions produce reductions in sensitivity to both tasks (Bowen et al., 2003). Cortical lesions produced deficits in the rat's ability to discriminate duration and detect the presence of silent gaps, while lesions of the VNLL in the current study produce deficits in the rat's ability to detect sinusoidal amplitude modulation at high modulation frequencies. These experiments further illustrate that temporal processing occurs at all levels of the central auditory system.

4.2.6 Kainic Acid Lesions Do Not Produce Absolute Threshold Shifts

Eight of the ten animals were tested post-operatively to determine whether the observed deficits in sinusoidal amplitude modulation detection ability may be attributed to absolute threshold shifts as a result of the kainic acid lesion. Two of these animals, Rats 12 and 14, had complete lesions of the VNLL. All other animals had partial lesions

or no lesion at all. The two animals tested with complete lesions were reliably able to detect the presence of a 50 dB noise burst, indicating that their absolute threshold had not been significantly altered. Since the animals were able to perform the absolute threshold task after complete lesions, we can say that deficits are actually due to an inability to process sinusoidal amplitude modulation, at higher modulation frequencies.

4.3 Future Studies

While sinusoidal amplitude modulation is an effective stimulus for testing the temporal processing ability of an animal, there are others tests available that would also test temporal processing abilities in the rat. For example, a duration discrimination test, in which an animal is trained to discriminate a noise burst of longer duration than a 'standard' or 'safe' duration, is also an excellent test of temporal processing ability. A gap detection test, which requires an animal to detect the presence of a silent gap in a constant noise background, would also be useful for identifying the temporal processing abilities of the rat. An experiment using gap detection and duration discrimination in conjunction with sinusoidal amplitude modulation detection before and after lesions of the VNLL would give a more complete analysis of the contribution of the VNLL to the rat's temporal processing abilities.

An electrophysiological study investigating the *in vivo* properties of VNLL neurons would greatly enhance the understanding of the role of the rat VNLL in processing temporally modulated information. This study could include the basic response types of the cells of the rat VNLL, as well as an examination of the pharmacology of the structure. An experiment of this nature would also allow for the investigation of binaural interaction at the level of the VNLL. Most animals studied have

not shown binaural responses at the level of the VNLL, but studies involving the rabbit have. The majority of the information currently available regarding the VNLL is from *in vivo* electrophysiological studies from species like the cat, bat and rabbit. A similar study with the rat would allow for cross-species comparison, and would help establish the normal properties of the rat VNLL.

A sound localization test before and after lesions of the VNLL ipsilateral and contralateral to the unblocked ear would provide interesting insight to the spatial processing abilities of the VNLL. Most structures that process sound localization cues receive binaural input. However, it is believed that monaural structures, such as the VNLL, may be important for localizing sounds in the vertical plane. Therefore, a test involving an animal's ability to locate sound sources in the vertical plane before and after lesions of the VNLL would determine whether the VNLL contributes to this task.

The current study strongly suggests that the VNLL is involved in processing sinusoidal amplitude modulation, particularly at high modulation frequencies. This study also indicates that the VNLL of the rat is a monaural structure, receiving information from the contralateral side. Previous research has implied that the VNLL is likely part of a temporal delay line in which temporal properties of sounds are preferentially coded (Covey and Casseday, 1991). The results of the current study indicate that the VNLL is indeed part of a temporal processing system: the VNLL is required for processing sinusoidal amplitude modulation at high modulation frequencies.

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