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**THE CONNECTIVITY AND ULTRASTRUCTURE
OF THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS OF THE
ALBINO RAT**

By: Rosalie Ellen Labelle, B.Sc.

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

Master of Science

Department of Psychology

**Carleton University
Ottawa, Ontario
September, 1997**

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The Connectivity and Ultrastructure of the
Dorsal Nucleus of the Lateral Lemniscus of the
Albino Rat

submitted by Rosalie Ellen Labelle, B.Sc.
in partial fulfillment of the requirements for the degree of
Master of Science

Thesis Supervisor

Chair, Department of Psychology

Carleton University
September, 1997

Abstract

The ascending afferents to the dorsal nucleus of the lateral lemniscus (DNLL) of the albino rat were determined with retrograde tract-tracing methods via injection of the retrograde tract tracer Fluoro-Gold into the DNLL. The DNLL received projections from the contralateral DNLL and cochlear nucleus (CN), the lateral superior olivary nucleus (LSO) bilaterally and the ipsilateral : intermediate nucleus of the lateral lemniscus (INLL), ventral nucleus of the lateral lemniscus (VNLL), medial superior olivary nucleus (MSO) and superior paraolivary nucleus (SPO). Anterograde tract tracing methods were used to confirm the retrograde findings. The anterograde tract tracer Biotinylated Dextran was injected into the CN and the superior olivary complex (SOC) and evidence of terminal labelling in DNLL was found.

The efferent projections of the DNLL were determined with anterograde tract tracing methods via injection of Biotinylated Dextran into the DNLL. The main targets of the DNLL are the contralateral DNLL and bilateral inferior colliculus (IC). Smaller projections are sent to bilateral superior colliculus (SC) and periolivary nuclei of the ipsilateral SOC. The terminations of DNLL in target nuclei were studied at the ultrastructural level. The DNLL was found to make mainly symmetrical contacts with dendrites in contralateral DNLL and bilateral IC. Symmetrical contacts with dendrites were found bilaterally in SC. In periolivary nuclei of the SOC both symmetrical and asymmetrical contacts were labelled.

Observation of DNLL tissue at the ultrastructural level revealed neurons with different soma shapes and nuclear morphologies. Regardless of soma shape and nuclear morphology most DNLL neurons in this sample possessed rough endoplasmic reticulum that was dispersed throughout the cytoplasm.

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INTRODUCTION

PURPOSE OF THIS REVIEW

The general purpose of this study was to examine the connectivity and ultrastructure of DNLL of the albino rat. The afferents to the DNLL were determined by using retrograde tract-tracing methods and confirmed with anterograde tract-tracing methods. The efferent projections of the DNLL were also studied using anterograde tract-tracing methods. The terminations that the DNLL makes in target nuclei were also examined at the ultrastructural level. DNLL tissue was also examined at the electron microscope level to study the ultrastructure of cell bodies in the DNLL. To facilitate discussion of DNLL's connectivity and anatomy, previous studies of this nucleus as well as important neighbouring nuclei are outlined in the body of this report. A brief overview of the ascending auditory system will also facilitate the interpretation of the anatomical connections.

RATIONALE FOR THE PRESENT STUDY

This study was carried out because the afferent projections to the DNLL of the rat have not been thoroughly studied. The afferent projections to DNLL have been studied extensively in the cat (as will be outlined below), but little data is available on the rat. Although the efferent projections of the DNLL of the rat have been studied at the light level (as will be outlined below), there have not been any previous studies of the ultrastructure of the terminations of DNLL in target nuclei in the rat. The ultrastructure of DNLL's terminations in contralateral DNLL and bilateral IC have been determined in the cat but there has not been a previous study of this nature done in the rat. The ultrastructure of neurons in the DNLL of the cat has been studied, but again there has been no investigation in the rat.

WHY STUDY THE ANATOMY OF THE DNLL OF THE RAT?

The DNLL is an important nucleus in the central auditory system. The DNLL exhibits binaural physiological response properties (Aitkin et. al., 1970, Brugge et. al., 1970, Buckthought et. al., 1993, Covey, 1993, Markovitz and Pollak, 1993 and 1994). The DNLL plays a fundamental role in shaping the binaural physiological responses in contralateral IC (Li and Kelly, 1992; Li, 1994; and Kidd and Kelly, 1996) and it is involved in sound localization (Kelly et. al., 1996 and Ito et. al., 1996). Studies of the connectivity and anatomy of the DNLL help to explain how its physiological responses are generated and what mechanisms underlie its involvement in shaping responses in contralateral IC, and its role in sound localization. Previous studies of DNLL connectivity have been done mainly in the cat. Our lab studies the physiology of the auditory system of the rat. Hence more information on the rat is beneficial to our research.

OVERVIEW OF THE ASCENDING AUDITORY SYSTEM

The auditory system consists of ascending and descending divisions. The ascending division processes and transmits information from the ear toward the auditory cortex while the descending division processes and transmits information from the auditory cortex to lower centers and the ear. This report focuses on the ascending auditory system.

Central auditory system neurons are tonotopically organized. They are arranged in a regular order of best frequency along one dimension across the nucleus (Pickles, 1988). Each subdivision of auditory nuclei, which are defined as distinct groups of neurons, may have specific combinations of inputs. The pattern of inputs to each subdivision may be termed its nucleotopic organization (Oliver and Shneiderman, 1991).

A review of the connectivity of the DNLL will be facilitated by a brief description of the nuclei of the central auditory system (See **Figure 1**). The auditory nerve (AN) on each side projects from the cochlea to the cochlear nucleus (CN) on the same side of the brain. The CN is located in the brainstem near the boundary between the medulla and pons. The CN is divided into the dorsal cochlear nucleus (DCN) and the ventral cochlear nucleus (VCN). The VCN is subdivided into anterior and posterior divisions. The axons of CN neurons that project centrally stream out along three pathways: the dorsal acoustic stria, the trapezoid body (TB) and the intermediate acoustic stria. Some CN neurons project along these pathways directly to the inferior colliculus (IC) in the midbrain whereas others project to various subdivisions of the superior olivary complex (SOC) and the nuclei of the lateral lemniscus, which in turn project to the IC (Irvine, 1986). A dorsal-to-ventral, high-to-low frequency organization exists in each of the three CN subdivisions (Irvine, 1986).

The SOC is comprised of a number of closely associated nuclei located in the lateral part of the ventral pons (Irvine, 1986). The major nuclei of the SOC are the lateral superior olivary nucleus (LSO), the medial superior olivary nucleus (MSO) and the medial nucleus of the trapezoid body (MNTB). In coronal section the LSO appears as an N-shaped nucleus. The LSO is comprised of two limbs: the medial limb which is directed dorsomedially and the lateral limb which is directed ventrolaterally. The LSO is tonotopically organized with high frequencies represented in the ventro-medial limb and low frequencies in the dorsolateral limb. There is a disproportionate representation of high frequencies in LSO (Irvine, 1986). The MSO is a column of cells, with their long axes oriented horizontally, located medial to the LSO. In the cat the MSO is tonotopically organized with high frequencies ventrally and low frequencies dorsally. In the cat there is a disproportionate representation of low frequencies in MSO (Irvine, 1986). The MSO of the rat is relatively small when compared to that of the cat (Irving and Harrison, 1967). The MNTB lies within the fibers of the TB ventromedial to the MSO (Irvine, 1986). The

MNTB is tonotopically organized with high-to-low best frequencies arranged in a ventromedial-to-dorsolateral direction (Irvine, 1986). There is a disproportionate representation of high frequencies in the MNTB of the cat (Irvine, 1986). In addition to the major nuclei of the SOC, the SOC of the rat and other rodents contains a prominent nucleus termed the superior paraolivary nucleus (SPO) which is situated dorsomedial to the MSO. The SPO is tonotopically organized with high frequencies represented medially and low frequencies represented laterally. A number of smaller and less clearly defined nuclei are also present within the SOC. Ventral to the MNTB and the LSO respectively are nuclei termed ventral and lateral nuclei of the trapezoid body (VTB and LTB).

All major nuclei of the SOC project either directly to the inferior colliculus (IC) via fibers of the lateral lemniscus (LL), or indirectly by synapsing on cells of the nuclei of the lateral lemniscus (NLL) which in turn project to the IC (Irvine, 1986). The inferior colliculi appear as paired rounded protuberances on the dorsal surface of the caudal midbrain. The IC is the site of convergence of numerous ascending and descending auditory pathways. The IC is tonotopically organized with high frequencies represented ventrally and low frequencies represented dorsally (Flammino and Clopton, 1975; Kelly et al., 1991). The lateral lemniscus is a large fiber tract which enters the ventral base of the IC. The three nuclei of the lateral lemniscus (dorsal, intermediate and ventral) can be distinguished as groups of cells embedded within the fibers of the LL. It is unclear to what extent the innervation of NLL neurons is provided by collaterals of fibers proceeding to IC as opposed to fibers terminating solely in NLL (Irvine, 1986).

From the IC, axons ascending via the brachium of the inferior colliculus (BIC) (a tract coursing rostroventrally from the lateral side of the IC (Faye-Lund and Osen, 1985)), terminate in the medial geniculate body (MGB) in the thalamus. The medial geniculate in turn projects to various subdivisions of the auditory cortex.

Auditory information is also transmitted from higher to lower levels. The auditory cortex sends a descending projection to the medial geniculate body and to the inferior colliculus. These nuclei in turn give rise to projections that descend further down the auditory system.

A Schematic Representation Giving An Overview of the Ascending Auditory System

Figure 1. Generalized schematic summary of ascending auditory system projections, excluding minor pathways. AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus; MSO, medial nucleus of the superior olive; LSO, lateral nucleus of the superior olive; MTB, medial nucleus of the trapezoid body; IC, inferior colliculus; MGB, medial geniculate body; NLL, nuclei of the lateral lemniscus (taken from Pickles, 1988).

DORSAL NUCLEUS OF THE LATERAL LEMNISCUS (DNLL) DEFINED

The lateral lemniscus (LL) is a fiber bundle containing axons from both ascending and descending auditory pathways that courses laterally on both sides of the brain through the pons, medulla and midbrain. The DNLL is one of three prominent groups of neurons embedded within the fibers of the lateral lemniscus, the other groups are the intermediate (INLL) and the ventral (VNLL) nuclei of the lateral lemniscus. The DNLL lies ventral to the inferior colliculus (IC), is bordered laterally by the nucleus sagulum, ventrally by the INLL, and medially and rostrally by the paralemniscal zone (Caicedo and Herbert, 1993).

The DNLL is located in the lateral part of the midbrain. It is demonstrated in cresyl-violet-stained coronal sections as an ovoid nucleus made up of intensely stained, fairly large somata. The somata form dorsoventrally oriented columns of cells which are aligned in parallel to the fibers of the LL (Caicedo and Herbert, 1993 and Merchan et al., 1994). The IC is situated dorsal to the DNLL and is separated from DNLL by a region of low neuronal density which is considered to be part of the subcollicular tegmentum (Merchan et al., 1994). The DNLL is related medially to the commissure of Probst (CP), which is a fiber bundle connecting the DNLL with the contralateral DNLL and IC. The DNLL is bordered ventrally by the INLL. The INLL consists of darkly stained, medium-sized neurons whose oval somata are also grouped in clusters that are formed by fibers of the lateral lemniscus. The nucleus sagulum (Sag), situated just lateral to DNLL, can be distinguished from DNLL because it contains smaller neurons that are lighter-stained. The paralemniscal zone (PL) borders the nuclei of the lateral lemniscus medially. This zone is composed of lightly stained small neurons that appear as a string of pearls that is aligned along the dorsoventrally running fibers of the lateral lemniscus (Caicedo and Herbert, 1993).

DNLL CYTOARCHITECTURE AND ULTRASTRUCTURE

Cytoarchitecture

Researchers have classified the neurons of the DNLL in various animals on the basis of cell shape and dendritic morphology using several techniques (Nissl stain, Golgi impregnation, and labelling via tract tracers) (cat: Adams, 1979, Kane and Barone, 1980, and Glendenning et. al., 1981; mouse: Iwahori, 1986; rat: Bajo et. al., 1993 and Wu and Kelly, 1995; bat: Covey and Casseday, 1986). All of the above studies have revealed that the DNLL contains a diversity of cell types but the classification nomenclature has varied between authors. This report will focus on the classification schemes proposed by Bajo et. al. (1993) and Wu and Kelly (1995) for the rat.

Following intracellular injection of biocytin into DNLL, Wu and Kelly (1995) reported that there were five main groups of neurons: multipolar, elongate 1, 2, 3 and round. Multipolar cells had several large dendrites with multiple branches while elongate cells had extended cell bodies with polar dendrites. The dendrites of elongate 1 and 2 cells were mainly oriented in the horizontal plane with their dendritic branches extended across the DNLL from its medial to lateral borders. Elongate 2 neurons also displayed profuse dendritic branching that spread out dorsoventrally along the margins of DNLL. Elongate 3 cells had extended cell bodies but their dendrites did not extend across the DNLL and did not show preferential orientation (Wu and Kelly, 1995). Round neurons were common. They had small round cell bodies and radial dendrites that extended over large areas within the DNLL (Wu and Kelly, 1995).

Nissl stains (basic cationic dyes such as methylene blue, toluidine blue and cresyl violet-acetate) used for light microscope preparations display the basophilic nucleic acid constituents of the cell. The nuclei and the RNA component in the cytoplasm referred to as Nissl substance or Nissl bodies (rough endoplasmic reticulum) are stained. Different

neuronal types display distinctive and consistent Nissl staining patterns (Peters et al, 1976).

A study in the rat using Nissl-stained material (Bajo et. al., 1993) only reported four neuronal types in DNLL. Cells classified as **type 1** neurons were large and multipolar. They had triangular somata that possessed abundant cytoplasm containing darkly stained Nissl-substance. They possessed a heterochromatin ring around the inner face of the nucleus. The nucleus, which was large and positioned centrally, contained a large nucleolus. These cells were the largest neurons observed in the DNLL and they were mainly found in the most dorsal and caudal parts of DNLL. **Type 2** neurons were large bipolar cells with medio-laterally oriented somata. These cells were longer than Type 1 cells. Their nuclei were large and centrally placed. The cytoplasm of these cells contained darkly stained Nissl substance that was condensed at the two poles. These cells were found intermingled with CP fibers entering and exiting the DNLL and they had the same orientation as the commissural fibers. **Type 3** neurons were medium in size (they were smaller than types 1 and 2) and their somata were ovoid. They possessed a prominent nucleus which was eccentrically placed. Their Nissl-substance was abundant and darkly stained. The dendrites of these cells showed differential orientation with respect to the lemniscal tract (somata were oriented parallel to lemniscal fibers (especially in lateral DNLL) or positioned perpendicularly or obliquely). These cells were the most obvious and most commonly observed cell type in the DNLL. They were homogeneously distributed throughout DNLL although lateral (rostral) DNLL was observed to have the highest numbers of these neurons. **Type 4** neurons were small round cells (they were the smallest neurons observed in the DNLL). They possessed a prominent centrally placed nucleus and clear scant cytoplasm. These cells were reported to be found throughout the DNLL, but to be the least common neuronal type.

Ultrastructure

Researchers have studied the ultrastructure of the cell bodies of neurons in order to classify them. Features such as nuclear morphology and distribution of rough endoplasmic reticulum in the cytoplasm of neurons are related to soma size and shape in order to distinguish different cell types. Nerve cells in the cochlear nucleus and the inferior colliculus have been distinguished on this basis (Cant and Morest, 1979; Cant, 1981; Oliver, 1984).

There has only been one previous electron microscope study of the DNLL. Oliver and Shneiderman (1989) identified two possible cell types in the DNLL of the cat based on the ultrastructure of their nuclei and the position of the rough endoplasmic reticulum within their cytoplasms. One cell type possessed irregularly shaped nuclei and perinuclear rough endoplasmic reticulum. These cells had fusiform shaped cell bodies. The other type had smooth nuclei and did not possess perinuclear rough endoplasmic reticulum. The authors reported that the proportion of these cell types was difficult to determine since cell bodies were infrequent in their sample.

DNLL PARCELLATION

The DNLL has been subdivided by various authors using different criteria. Some of the features that have been used to distinguish different divisions of the DNLL are: cellular architecture, tonotopicity and patterns of connectivity. Some authors have proposed subdivisions of the DNLL of the cat based on differential distribution of cell types (Shneiderman et al., 1988). Other investigators believe that the DNLL of the rat cannot be subdivided on the basis of cell type segregation (Bajo et al., 1993). As mentioned above Bajo et al. (1993) reported four neuronal types in the DNLL of the rat. These cell types differed in their spatial distributions within the DNLL (Type 1-mainly in dorsal and caudal parts, Type 2-intermingled with CP fibers, Type 3-highest numbers in

lateral DNLL, Type 4-uncommon but evenly distributed). Nevertheless Bajo et al. (1993) concluded that there were no clear subdivisions in the DNLL of the rat since all neuronal types could be found to some degree throughout the entire nucleus. Alternately Shneiderman et al. (1988) distinguished three subdivisions in the DNLL of the cat: dorsal, ventral and lateral parts (the lateral group being the only division remaining at rostral levels of the DNLL). These subdivisions were reported to differ in size, shape and orientation of their neurons and in the proportion of cell types. In the dorsal part of the DNLL the cells were large and spherical, while in the ventral part the cells were horizontally oriented, flattened or fusiform. The main cell type of the lateral DNLL was smaller and more rounded than the neurons in the dorsal and ventral parts (Shneiderman, et al., 1988).

Based on physiological recording and tonotopic patterns of projections of DNLL to IC (see **physiology section**), other authors propose a parcellation of DNLL into dorsal regions (where low frequencies are represented) and ventral regions (where high frequencies are represented) (cat: Aitkin et al., 1970; gerbil: Ryan et al., 1982; mustache bat: Markovitz and Pollak, 1993). A rostral versus caudal parcellation of the DNLL of the rat has also been proposed on the basis of differential sources of efferent projections from the two areas (rostral parts project to superior colliculus while caudal parts project to IC) (see **DNLL efferent section**) (Tanaka et al., 1985). Recently the DNLL of the mustache bat has been subdivided into anterior and posterior divisions on the basis of their connectivity and their physiological response properties (anterior-receives VNLL projections and responds to low frequencies; posterior-receives MSO projections and responds to high frequencies) (see **DNLL afferent section**) (Yang et al., 1996).

PREVIOUS STUDIES OF DNLL'S AFFERENT CONNECTIONS

DNLL ASCENDING AFFERENTS

The sources of ascending projections to the DNLL have been most thoroughly investigated in the cat (See Figure 2). There are two studies in the cat that are the main sources of reference. One of these studies employed retrograde tract-tracing with HRP injections directly into the DNLL of the cat (Glendenning et. al., 1981). The other study employed anterograde tract-tracing methods to trace connections from the SOC and CN to the DNLL of the cat (Shneiderman et. al., 1988).

CN

Several investigations have led various authors to conclude that the projection from the cochlear nucleus (CN) to the DNLL is small and comes from the contralateral CN. For example Glendenning et. al. (1981) found that the majority of projections from the contralateral CN of the cat passed through the DNLL, en route to IC, rather than terminated within it. It has been further suggested that the few existing direct projections from the CN to the DNLL originate almost entirely in the ventral division of the contralateral CN (VCN). One study of the projection of the CN to the nuclei of the lateral lemniscus of the rat led the authors to conclude that there was no projection from the contralateral VCN to the DNLL (Friauf and Ostwald, 1988). The projection from the DCN to the DNLL is believed to be minor (Glendenning et. al., 1981). Shneiderman et. al., 1988 explicitly reported that there was no projection from the DCN to the DNLL of the cat following anterograde tract-tracing studies. Distinctions have been made regarding the relative contribution of projections from the anterior and posterior divisions of the cochlear nucleus (AVCN and PVCN respectively). Glendenning et. al. 1981 found that the projection from AVCN was larger than that from the PVCN. The projection from contralateral CN to DNLL of the bat has not been reported by earlier authors

(Covey and Casseday, 1994). Recently a small projection from contralateral VCN and DCN has been reported in the bat (Yang et al, 1996).

While it is agreed that the projection from the CN to the DNLL originates mainly from the contralateral VCN, a slight projection from the ipsilateral CN to the DNLL has also been reported. Glendenning et. al. 1981, showed that there was a sparse projection from the CN to ipsilateral DNLL using both anterograde degeneration and retrograde tracing. More specifically, Shneiderman et. al. (1988) reported a bilateral projection from AVCN to DNLL. The high frequency (dorsomedial) part of the AVCN was found to project exclusively to the contralateral ventral DNLL, while the ventrolateral (low frequency) part of the AVCN was found to project bilaterally to dorsal DNLL. PVCN projections to the DNLL were not studied by Shneiderman et. al. 1988.

SOC

It is accepted that the superior olivary complex is a major source of input to the DNLL. A retrograde tract-tracing study in the cat (Glendenning et. al., 1981) found that the lateral superior olivary complex (**LSO**) had a bilateral projection to the DNLL. Glendenning and Masterton (1983) employed anterograde degeneration techniques (they lesioned LSO and looked for degenerated terminals in DNLL) and confirmed the dense bilateral LSO projection to DNLL. They reported that they had no evidence to conclude that the bilateral projection was symmetrical in density or in the locus of termination within the DNLL.

Another study in the cat that employed anterograde tract-tracing methods (Shneiderman et. al., 1988) also confirmed the bilateral projection of the LSO to the DNLL. This study also described a topography of the projection. The lateral limb of the LSO (the low frequency part) was found to project to the dorsal DNLL while the more medial limb (the high frequency part) projected to the ventral DNLL. The projection to dorsal DNLL was found to be evenly distributed while the projection to the ventral DNLL displayed a mediolateral gradient. The ipsilateral LSO was found to project more

heavily to lateral parts of the DNLL while the contralateral LSO was found to project more heavily to medial parts of the DNLL (Shneiderman et. al., 1988). This report is similar to the report by Glendenning et. al. (1981). They reported that there was a bilateral projection to DNLL via medial lateral lemniscal fibers and an ipsilateral projection via lateral lateral lemniscal fibers.

An ipsilateral projection from the medial superior olivary nucleus (**MSO**) to the DNLL is well established. Following tritiated leucine injections into the cat MSO Elverland (1978) reported ipsilateral projections from MSO to the DNLL. Zook and Casseday (1979) reported that the bat MSO projected to the DNLL. Glendenning et. al. 1981 found twice as many labelled cells in the ipsilateral cat MSO than the ipsilateral LSO following injections of HRP into the DNLL. This led them to conclude that the projection from the MSO to the DNLL was substantial in the cat. Henkel and Spangler (1983) reported that label was found in ventral DNLL following anterograde tract tracer injections into ventral MSO. Dorsal MSO injections resulted in dorsal DNLL labelling.

The anterograde tract-tracing study by Shneiderman et. al. 1988 also reported an ipsilateral projection of MSO to DNLL but a different description of the topography of the projection was given. The dorsal part of the cat MSO (low frequency part) was found to project to dorsal DNLL, but the ventral MSO was found to project to the middle of the DNLL rather than to the ventral DNLL. They also found that the MSO did not project to ventral DNLL. These reports are consistent with previous reports. The MSO is a low frequency nucleus (Irvine, 1986). Ventral DNLL mainly represents high frequencies (Merchan et al., 1984). Thus it follows that a low frequency nucleus would have few projections to a high frequency area (**see physiology section**) (Merchan et al., 1994). The MSO projection was not found to exhibit a mediolateral gradient within the DNLL unlike the LSO, yet it was observed that there was little labelling in lateral DNLL following MSO injections (Shneiderman et. al., 1988).

Following injections of anterograde tract tracer into low frequency regions of the LSO and MSO of the mustached bat, Vater et. al. (1995) found that the projections from these two nuclei overlapped in their site of termination within the DNLL. Following injections into low frequency areas, labelling was found in the most dorsal and lateral corner of DNLL. Following injections into high frequency areas within the LSO and MSO they found that the projections from these two nuclei also overlapped but that projections from LSO extended more rostrally within the DNLL. Injections into high frequency regions of MSO and LSO labelled fibers in a broad horizontal band in the middle to ventral part of the DNLL (Vater et. al., 1995).

Another anterograde tract-tracing study in the cat showed that the MSO sends axons to the IC that pass along the medial side of the DNLL, where they give rise to thin right-angle collaterals before entering the IC (Henkel, 1989 and 1997). The collateral branches ended in DNLL in thin, horizontal bands forming laminae that extended throughout DNLL from rostral to caudal. A dorsal-ventral topography was apparent in the position of the laminae in DNLL with respect to the injection site in MSO (dorsal MSO injections labelled laminae in dorsal DNLL while ventral MSO injections labelled laminae in ventral DNLL), but no relation between the rostral-caudal location of labeled endings and the injection site was observed. The terminal branches of the collateral axons exhibited round or oval boutons en passant and terminaux. Individual arbors distributed boutons in restricted domains which were only a subcomponent of the area of the laminae in which they were distributed. The author suggested that the horizontal tiers of afferent fibers in DNLL are a mosaic of restricted axonal arbors rather than uniform sheets from divergent axonal arbors (Henkel, 1989; Henkel, 1997).

The superior paraolivary nucleus (SPO) is a prominent nucleus of the SOC in the rat. Recently it has been reported that terminals were observed in the DNLL following injections of anterograde tract tracer into the SPO of the rat (Colombo, et. al., 1996). There have also been a few reports of projections from periolivary nuclei to the DNLL in

the cat. Glendenning et. al. 1981 reported labelling of isolated cells of the lateral and ventral nuclei of the trapezoid body, and in the dorsomedial, ventromedial, dorsal and dorsolateral periolivary nuclei of the cat following injection of HRP into the DNLL of the cat.

The medial nucleus of trapezoid body (**MNTB**) has been reported to send few or no projections to the DNLL. Glendenning et. al. 1981 reported a few labelled cells in the ipsilateral MNTB following HRP injections into the DNLL. Spangler et. al. 1985 and Shneiderman et. al. 1988 reported an absence of a projection from the MNTB to the DNLL. Shneiderman suggested that the results of Glendenning may be due to uptake by damaged fibers of passage.

NLL

The connection of the **DNLL** with its contralateral counterpart has been known since the time of Probst (1902), and confirmed many times (see Glendenning et. al., 1981). Shneiderman et. al. 1988 reported a symmetrical organization of the projection (dorsal injections labelled dorsal contralateral DNLL while ventral injections labelled ventral contralateral DNLL). They also reported that both dorsal and ventral DNLL injections labelled the lateral part of the DNLL and adjacent sagulum. Projections from **INLL** to DNLL have been documented in the bat (Yang et al., 1996). Retrograde tract-tracing studies in the cat (Glendenning et. al., 1981) and the bat (Yang et al., 1996) have revealed an ipsilateral projection from the VNLL to the DNLL.

DNLL DESCENDING AFFERENTS

Studies on descending afferents to DNLL report an afferent projection from the IC to the DNLL (guinea pig: Syka et al., 1988; rat: Caicedo and Herbert, 1993). Following injection of anterograde tract-tracers into the IC both authors reported terminal labelling in the DNLL. Caicedo and Herbert (1993) reported differential projections from different subdivisions of the IC to the DNLL of the rat (ipsilateral DNLL received strong

projections from the ICC, the intercollicular zone and the EC while the DC was reported not to project to the DNLL).

PREVIOUS STUDIES OF DNLL'S EFFERENT PROJECTIONS

ASCENDING EFFERENTS

Major Targets

The DNLL has strong projections to **ipsilateral ICC** and to **contralateral DNLL and ICC** via the commissure of Probst (see Shneiderman, 1988; Hutson et al., 1991; Bajo et al., 1993). The commissure of Probst is an obligatory avenue for decussating fibers from the DNLL to the contralateral DNLL and IC, rather than a general avenue for decussating fibers of the LL (Hutson et al., 1981). The projection of DNLL to its counterpart on the opposite side is topographically arranged in that there is symmetry between the location of the injection site and that of the corresponding labeled terminal field in the opposite DNLL (Shneiderman et al., 1988; Bajo et al., 1993).

The projection of DNLL to ICC is tonotopically organized (**for a more detailed description see physiology section**). The IC itself is tonotopically organized with high frequencies represented ventrally and low frequencies represented dorsally (Clopton and Winfield, 1973; Ryan et al., 1988; Kelly et al., 1991). Injection of retrograde tracers into physiologically characterized sites in the ICC results in patterns of labelling that suggest mirror-imaged frequency representation in DNLL (from high to low to high) (Preuß, 1991). Similar results were reported using anterograde methods (Caicedo and Herbert, 1993). Others have proposed an onion-like tonotopic organization of DNLL. In this model, low frequencies are represented centrally within the DNLL, and progressively higher frequencies are represented in surrounding outer rings (Saldana, 1993; Merchan et al., 1994; Kelly et al., 1995). This type of labelling is more evident in the DNLL contralateral to the injection site. The rings were thicker ventrally within DNLL

suggesting that a larger proportion of the volume of this nucleus is devoted to processing higher frequency sounds (Merchan, et al., 1994).

Most studies report that DNLL's ICC projection is contralaterally biased with the crossed projection being greater than the ipsilateral (rat: Tokunaga et al., 1984 and Tanaka et al., 1985 and Merchan et al., 1994; chinchilla and guinea pig: Saint Marie and Baker, 1990; cat: Brunso-Bechtold et al., 1981; mole: Kudo et al., 1990). In the cat the crossed projection from the DNLL to the opposite ICC has been reported to be banded while the uncrossed projection to the ipsilateral ICC was reported to be more diffuse (Shneiderman et al., 1988).

It is in general agreement that many of the same DNLL neurons that innervate the contralateral IC also innervate the opposite DNLL via axon collaterals (the same DNLL neurons that project to the opposite DNLL also project to the contralateral IC) (Merchan et al., 1994). Recently in the rat the crossed projection to ICC was found to arise from at least two types of axons: the first type gave rise to collaterals in the opposite DNLL and then arborized profusely as small boutons terminaux and en passant in contralateral IC. The second type bypassed the opposite DNLL and terminated directly in the contralateral IC with simpler axonal arborization (Bajo et al., 1993). The uncrossed projection to the ipsilateral ICC consists of fibers with profuse axonal arborization showing abundant large boutons terminaux and en passant (Bajo, 1993).

Double labelling studies following IC injections have revealed that DNLL cells projecting to ipsilateral and contralateral IC are segregated populations (few cells were labelled with both tracers) (Willard and Martin, 1984; Tanaka et al., 1985, and Hutson, 1988). In the rat these two cell populations are believed to coexist in the same concentric layers of the DNLL (Merchan et al., 1994).

There is also a bilateral projection from DNLL to the external cortex (EC) of the IC in the rat (Bajo et al., 1993). An ipsilateral projection to the EC was previously reported in the rat (Coleman and Clerici, 1987). This projection has been reported by several

investigators for the cat (Kudo, 1981; Shneiderman et al., 1988; Hutson et al., 1991).

Some authors in the cat have reported a projection to the dorsal cortex (DC) of the IC from the DNLL yet no evidence for such a projection has been found in the rat (Bajo et al., 1993).

Other Targets

In addition to these major projections the DNLL has been reported to have ascending projections via extralemniscal pathways that bypass the IC. In the cat Kudo (1981) obtained evidence of projections from DNLL to the caudal half of the deep layers of SC. He reported a bilateral projection to deep layers with the ipsilateral projection being greater than the contralateral projection. In the rat Druga and Syka, 1984; Bajo et al., 1993 and Tanaka et al. 1985 also reported bilateral projections to the SC. Tanaka et al. (1985) specified that DNLL neurons projecting to the SC were located rostrally whereas those projecting to the IC were located caudally. With double-labelling retrograde tract tracing techniques they showed that these two cell populations were segregated (no double labelled neurons projecting to both the IC and SC were observed following injection of two optically distinct tracers into the IC and the SC of the same animal) (Tanaka et al., 1985). Projections from DNLL to SC have also been described in the tree shrew (Casseday et al, 1979). The DNLL has also been reported to project to the medial and dorsal divisions of the medial geniculate body (Oliver and Hall, 1978; Henkel, 1983; Kudo, 1981, Bajo, 1993). The projection is mainly to ipsilateral MGB and it is not dense; it is limited to a few axons (Bajo, 1993). These observations have been disputed in a study of the cat which concluded that the DNLL does not project beyond the IC (Hutson et al., 1991).

DESCENDING EFFERENTS

In the cat (Kudo et al., 1981) reported descending projections to the ipsilateral SOC (to NTB and marginal regions of SOC). In the rat Bajo et al., (1993) also reported a projection to the ipsilateral SOC. Specifically they found sparsely labelled terminals in all ipsilateral periolivary regions following injections of anterograde tracer into the DNLL. Labelling was dense in the lateral part of the rostral periolivary (RPO) region and in the SPO. Lateromedial labelled fibers were found in MNTB, parallel to TB fibers, and they gave rise to single boutons on the somata of principle neurons. Some labelled fibers were also seen in the VTB and in dorsal periolivary regions surrounding the LSO. The LSO and MSO were never labelled in their study (Bajo et al., 1993). Following intracellular injections of biocytin into gerbil and mouse DNLL tissue slices, Zook and Kuwabara (1995) found that descending fibers formed collateral branches within INLL and VNLL. Some axons descended to ipsilateral SOC where they branched repeatedly. Terminal boutons were only seen within the ventral nucleus of the trapezoid body (VTB) (Zook and Kuwabara, 1995). Kudo (1981) also reported that some fibers from DNLL terminated in ipsilateral VNLL but that the majority proceeded further to the SOC. A descending projection from DNLL to the CN has also been reported (Kane and Conlee, 1979; Kane and Finn, 1977).

ULTRASTRUCTURE OF DNLL's EFFERENT PROJECTIONS

There is general agreement in the literature that terminals forming asymmetrical contacts, (those possessing an accumulation of dense material on the cytoplasmic face of the postsynaptic membrane which gives the junctions asymmetry) and possessing spherical vesicles are associated with excitatory input. These types of synapses are referred to as Type 1 synapses (Gray, 1959). They have wide synaptic clefts (30 nm)

and normally occur on dendritic spines and the shafts of smaller dendrites. Terminals forming symmetrical contacts (the dense regions of the junction are short and thin, giving the junction a symmetrical appearance) and possessing flattened or pleomorphic vesicles are associated with inhibition. These types of synapses are referred to as Type 2 (Gray, 1959). The synaptic cleft is about 20 nm wide. Symmetrical synapses normally occur on both the dendritic trunks and the cell bodies of neurons (Peters et. al., 1976).

The ultrastructure of the terminations of DNLL in contralateral DNLL and bilateral IC have been studied previously only in the cat. Oliver and Shneiderman (1989) found that labelled terminals in contralateral DNLL, following DNLL injections, possessed pleomorphic vesicles and formed symmetrical contacts mainly with dendrites. They also found a significant number of symmetrical contacts on the somata of DNLL neurons. A few endings with round synaptic vesicles appeared labelled in one sample (Oliver, Shneiderman and Henkel, 1987). An EM study of the terminations of DNLL in bilateral IC in the cat revealed that labelled terminals in contralateral IC contained pleomorphic vesicles and made symmetrical contacts with dendrites (Shneiderman and Oliver, 1989). The majority of labelled terminals in ipsilateral IC were also found to contain pleomorphic vesicles and to make symmetrical contacts with dendrites but a significant proportion (approximately 30%) of labelled endings contained round synaptic vesicles. The ultrastructure of DNLL terminations in SC and SOC have not yet been studied.

PHYSIOLOGY AND TONOTOPIC ORGANIZATION OF THE DNLL

Extracellular recordings indicate that the majority of neurons in the DNLL have binaural response characteristics (cat: Aitkin et al., 1970 and Brugge et. al., 1970; rat: Buckthought et. al., 1993; big brown bat: Covey, 1993; mustache bat: Markovitz and Pollak, 1993 and 1994). In the rat most neurons (74%) were found excited by contralateral stimulation and inhibited by ipsilateral stimulation (most displayed EI responses) (Buckthought et. al., 1993). This study reported that the DNLL of the rat

showed narrow frequency tuning. Whether or not there is an orderly tonotopic representation in the DNLL, and how best frequencies are arranged, has yet to be resolved since physiological and anatomical reports on the matter are not in complete agreement.

Physiological studies in the bat (Covey, 1993) and the cat (Aitkin et. al., 1970) have found some evidence of an orderly frequency progression in DNLL (with low frequencies represented dorsally and high frequencies represented ventrally). Yet another study in the cat employing uptake of ^{14}C -2-deoxyglucose following low or high frequency stimulation failed to find evidence of tonotopy in DNLL (Nudo and Masterton, 1984). A study of the physiology of the rat DNLL (Buckthorpe, 1993) also failed to find evidence of a clear linear frequency progression within the DNLL.

Non-linear arrangements of best frequencies in DNLL have been reported. In a study looking at c-fos labelling following pure-tone acoustic stimulation, an orderly tonotopic organization was observed in the DNLL of the rat: higher frequencies were represented ventrally and dorsally within the DNLL, while lower frequencies were represented centrally within the DNLL (Friauf, 1992). Preuß (1991) found that separate injections of two retrograde fluorescent tracers into distinct electrophysiologically characterized sites in the ICC resulted in a pattern of labelling that suggested a mirror-imaged frequency representation in DNLL (from high to low to high). Caicedo and Herbert (1993) reported that descending inputs from the IC to the DNLL appear to have a tonotopic arrangement which is compatible to the reports by Friauf (1992) and Preuß (1991). Injection of the anterograde tracer PHAL into the ICC resulted in mediolaterally oriented bands of terminal labelling found dorsally and ventrally within DNLL. A shift of the injection sites along the dorsoventral axis of the ICC, to higher frequency regions, resulted in a shift in labelled terminal bands within DNLL towards the dorsal and ventral boundaries of the DNLL (Caicedo and Herbert, 1993).

Another group of studies (rat and cat: Saldana, 1993; rat: Merchan et. al., 1994 and Kelly et. al., 1995) which have studied labelling in the DNLL following injection of tract tracers into the ICC, have proposed an "onion-like" tonotopic organization of DNLL. In this model low frequencies are represented centrally within DNLL, and progressively higher frequencies are represented in surrounding outer rings. The labelled rings are more evident in the DNLL contralateral to the IC injection, are thicker ventrally, incomplete medially and laterally, and vary along the rostral to caudal axis. Thus the dorsoventral high-to-low-to-high tonotopic organization proposed by Prueß (1991) and Friauf (1992) may be extended without incongruity into the peripheral-to-central high-to-low frequency organization suggested by the above authors. It has been suggested that a larger proportion of the volume of this nucleus is devoted to processing higher frequency sounds since high-frequency layers surround lower-frequency layers (Merchan et al., 1994).

A study of the DNLL of the mustache bat led the authors to conclude that the DNLL consists of two divisions which are distinguished based on their physiological responses and connectivity (Yang et. al., 1996). The anterior division responded to tone bursts with an onset discharge pattern and only phase-locked to sinusoidal amplitude modulated (SAM) signals with low modulation frequencies (< 300 Hz). The posterior division responded to tone bursts with sustained discharge patterns and phase-locked to SAM signals with higher modulation frequencies (400-800 Hz). Retrograde tract tracing revealed that the ipsilateral VNLL projected almost exclusively to the anterior division while the MSO projected to the posterior division. Other nuclei such as bilateral LSO, ipsilateral INLL and contralateral DNLL were found to project to both divisions but the cells projecting to each division were separate cell populations.

DNLL PHARMACOLOGY

Inhibitory Projection from DNLL

The DNLL is an important source of ascending inhibitory projections. As mentioned above the DNLL projects to its counterpart on the opposite side and to the IC bilaterally via the CP. Immunohistochemical studies have shown that neurons in the DNLL react positively to antibodies for both GABA and GAD (the enzyme responsible for the conversion of L-glutamic acid to GABA- a transmitter producing inhibition) (cat: Adams and Mugaini, 1984; gerbil: Roberts and Ribak, 1987; guinea pig: Thompson et al., 1985; rat: Moore and Moore, 1987, Mugaini and Oertel, 1985, Gonzalez-Hernandez et al, 1996). The fact that DNLL reacts positively for GABA and that labelled terminals forming symmetrical contacts and containing pleomorphic vesicles (morphology associated with inhibition) are found in contralateral DNLL and bilateral IC following DNLL injections (cat: Oliver and Shneiderman, 1989; Shneiderman and Oliver, 1989) has been taken as evidence that the DNLL is a prominent GABAergic nucleus that is a source of inhibitory influence on contralateral DNLL and IC. A study that combined retrograde tract-tracing and immunohistochemistry for GABA has found that most of the GABAergic input to the IC comes from the contralateral DNLL and the IC (Gonzalez-Hernandez et al, 1996).

Physiological studies support the idea that DNLL exerts an inhibitory influence on contralateral DNLL and IC. Extracellular physiological studies have shown that interaural intensity difference (IID) functions of single EI (contralateral stimulation excites and ipsilateral stimulation inhibits) units in the rat ICC, are partially based on an inhibitory projection from the contralateral DNLL (Li and Kelly, 1992b). Blockade of excitatory activity in DNLL (with kynurenic acid a non-selective excitatory amino acid

antagonist) reduces the inhibitory influence of DNLL on contralateral IC, resulting in a release of the inhibition imposed by ipsilateral stimulation, in the contralateral but not the ipsilateral IC (Li, 1994). Specifically, the binaural inhibition produced in IC by varying the interaural intensity difference (IID) of tones delivered simultaneously to the two ears is reduced by injection of antagonists into the DNLL contralateral to the recording site.

The DNLL also plays an important role in shaping the responses of neurons in IC to interaural time differences (ITDs). Single-unit action potentials recorded from the contralateral IC after injection of kynurenic acid into the DNLL showed a reduced extent of inhibition from paired clicks with contralateral stimulation delayed relative to ipsilateral stimulation (Kidd and Kelly, 1996). Surgical transection of the commissure of Probst, the fiber bundle connecting the DNLL to contralateral DNLL and IC, had the effect of decreasing the extent of inhibition in IC produced by acoustic stimulation of the ipsilateral ear. This result also indicates that DNLL's projection to the IC shapes binaural responses in the IC (van Adel et al., 1997).

Intracellular recordings in brain slice preparations revealed that stimulation of the CP elicits IPSP's in DNLL (yet numerous EPSP's were also generated) (Wu and Kelly, 1995). These IPSP's are believed to be mediated by GABA A receptors since they can be blocked by the application of the GABA A antagonist bicuculline (Wu and Kelly, 1996). This finding supports the immunolabelling data presented above that reports that the crossed projection of DNLL to contralateral DNLL through the CP is inhibitory and GABAergic (Kelly, 1997). Recent physiological studies also indicate that the DNLL shapes the binaural response properties of single EI units in contralateral DNLL. When the DNLL is pharmacologically blocked the effect in contralateral DNLL is a decrease in the extent of inhibition that is produced by acoustical stimulation of the ipsilateral ear (Kelly and Kidd, 1997).

Inhibitory and Excitatory Projections to DNLL

The DNLL receives inhibitory and excitatory ascending projections. Intracellular recording studies in the rat (Wu and Kelly, 1995) revealed that stimulation of the ipsilateral LL produces IPSP's and EPSP's in the DNLL. Wu and Kelly (1996) found that the glycine antagonist, strychnine, blocked these ipsilaterally generated IPSP's. As mentioned above, the DNLL of the cat receives projections from ipsilateral MSO and VNLL, bilateral LSO, and contralateral CN. It is thought that a source of this lemniscal inhibition is from glycinergic neurons in ipsilateral LSO. In the cat the ICC receives a heavy glycinergic projection from the ipsilateral LSO (Glendenning et al., 1992; Saint Marie et al., 1989; Saint Marie and Baker, 1990). It is assumed that the LSO sends parallel or collateralized projections to the DNLL on its way to the IC. Hence, it is believed that the DNLL receives the same glycinergic projection from ipsilateral LSO as IC neurons. Cells in the VNLL of the rat are reported to react positively for glycine (Aoki et al., 1988), and are thought to be another source of inhibition in the LL projection to the DNLL. Yet recently the VNLL has been reported to be an important source of GABAergic projections to the IC (Gonzalez-Hernandez et al., 1996). Thus the VNLL may also provide a non-glycinergic source of inhibition to the DNLL as well.

The EPSP's evoked in DNLL by ipsilateral LL stimulation can be blocked by the non-NMDA glutamate receptor antagonist, CNQX (Wu and Kelly, 1996). The lemniscally generated EPSP's are believed to be derived from various sources including contralateral LSO, ipsilateral MSO, and contralateral CN (Kelly, 1997). Ipsilateral LSO sends glycinergic projections to IC (as mentioned above), but contralateral LSO does not (Saint Marie and Baker, 1990 and Glendenning et al., 1992). Glendenning et al., 1992 reported that the projection from the contralateral LSO to the IC was glutamate immunoreactive

and may provide glutamatergic excitation to the IC. Since LSO is believed to send collateral projections to DNLL on its way to IC, it is believed that LSO is a source of excitatory projections to DNLL as well. The MSO sends ipsilateral projections to the DNLL (Henkel and Spangler, 1983 and Henkel, 1997). The MSO is glutamate immunoreactive and is also believed to provide an excitatory projection to the DNLL (Glendenning et al., 1992).

The cochlear nucleus sends projections to the inferior colliculus that have a morphology (round vesicles and asymmetrical contacts) typically associated with excitation (Oliver, 1987, Oliver, 1984). Hence it is accepted that there is an excitatory projection from CN to the IC. It is also believed that there is an excitatory projection from the CN to the DNLL as well. Oliver et al. (1983) and Glendenning et al. (1992) failed to show tritiated aspartate labeling of CN projections to the IC. Lesions of cochlear nuclear projections failed to affect glutamate levels or release of glutamate in the IC (Adams and Wenthold, 1979; Suneja et al., 1995). A recent study using transport of tritiated aspartate, a selective marker for glutamatergic synapses, failed to find labelling of SOC or CN following IC injections (Saint Marie, 1996). From the results of his study this author suggests that the excitatory pathways to the IC (and presumably to DNLL as well) use a non-glutamatergic transmitter (Saint Marie, 1996). Additional studies need to be carried out to confirm the identity of the transmitters subserving the ascending projections to the IC and DNLL.

CONNECTIVITY OF IMPORTANT NEIGHBOURING NUCLEI

Inferior Colliculus

The mammalian IC is considered to be an important structure for the processing of auditory information because it is the site of convergence of numerous ascending and descending auditory pathways (Saldana and Merchan, 1992). The inferior colliculi appear

as paired rounded protuberances on the dorsal surface of the caudal midbrain (Irvine, 1986). The IC of the rat consists of a core central nucleus (ICC) and surrounding (DC) and external cortices (EC) (Faye-Lund and Osen, 1985). The ICC is confined to the caudomedial part of the IC and is flattened rostrocaudally. The ICC is a laminar structure whose sheets are formed by layering of the afferent axons and the dendrites of densely packed intrinsic neurons. The laminar structure of the cortex is a product of the axonal network rather than the arrangement of cell bodies and dendrites (Oliver and Shneiderman, 1991). The EC is located lateral, rostral, ventral and ventrocaudal to the ICC while the DC is located dorsocaudal and dorsomedial to the ICC (Faye-Lund and Osen, 1985). The commissure of the inferior colliculus (CoIC) is a fiber tract adjoining the two colliculi that is most conspicuous in the rostral one third of the IC (Faye-Lund and Osen, 1985).

As mentioned above, the IC is tonotopically organized with low frequencies represented dorsolaterally and high frequencies represented ventrally (Kelly et al., 1991). Neurons in the IC are binaurally sensitive with the majority being EI units (contralateral stimulation excites and ipsilateral stimulation inhibits) (Kelly et al., 1991).

Retrograde tract tracing studies have revealed the ascending **afferents** to the ICC of the rat (**See Figure 3**). The ICC receives projections from all three divisions of the contralateral CN (AVCN, PVCN and DCN). The DNLL, LSO and LTB project bilaterally. The INLL, VNLL, MSO, SPO and VTB project ipsilaterally (Beyerl, 1978; Druga and Syka, 1984; Tokunaga et al., 1984; Coleman and Clerici, 1987 and Fiauf and Kandler, 1990). Similar projection patterns to the IC have been seen in a variety of other mammals (cat: Adams, 1979, and Brunso-Bechtold et al, 1981; opossum: Willard and Martin, 1983 and 1984; brush-tailed possum: Aitkin and Kenyon, 1981; guinea pig: Saint Marie and Baker, 1990 and Schofield and Cant, 1992; gerbil: Nordeen et al, 1983; ferret: Moore, 1988; mole: Kudo et al 1988 and 1990; chinchilla: Saint Marie and Baker, 1990; mouse: Ryugo et al, 1981 and Gonzalez-Hernandez et al, 1987; mustache bat: Zook and

Casseday, 1982 and Schweitzer, 1981). These nuclei project in a tonotopic fashion to the IC. Each IC receives substantial input from the contralateral IC via the commissure of the IC. The subdivisions of the IC are associated via intrinsic connections (Saldana and Merchan, 1992).

The EC of the rat also receives ascending auditory information. The ipsilateral DNLL and the contralateral DCN and PVCN (Coleman and Clerici, 1987, Bajo et al., 1993) project to EC. The EC receives intrinsic and commissural connections with the IC itself (Coleman and Clerici, 1987). The DC of the rat receives fewer projections from lower auditory brainstem nuclei (Coleman and Clerici, 1987, and Tokunaga et al, 1984). The DCN was found to be the most significant lower brainstem input. The DC is distinct in that it receives substantial sets of descending afferents from the auditory cortex (Faye-Lund, 1985 and Coleman and Clerici, 1987). The DC also receives input from its counterpart on the opposite side and intrinsic input (Coleman and Clerici, 1987).

The ICC sends **efferent** projections in a topographic order to the ipsilateral DNLL, the ipsilateral rostral periolivary nucleus (RPO), the ipsilateral VTB and the DCN bilaterally (Caicedo and Herbert, 1993). The EC projects to the ipsilateral nucleus sagulum, RPO, VTB and the DCN bilaterally. The DC projects mainly to the ipsilateral sagulum.

Intermediate Nucleus of the Lateral Lemniscus

As mentioned above, the INLL is one of three prominent groups of neurons embedded within the fibers of the lateral lemniscus. The INLL borders the DNLL ventrally. The INLL is defined as a region of sparsely distributed multipolar cells (Adams, 1979; Glendenning et al, 1981). Neurons in the INLL are mainly monaural (EO-contralateral stimulation excites) but there are binaural responses as well (EI units-contralateral stimulation excites and ipsilateral stimulation inhibits) (Buckthout, 1993; Aitkin and Brugge, 1970; Covey and Casseday, 1986 and 1991). Physiological studies suggest that

frequency progressions in INLL may not lie in the dorsal-ventral direction (Buckthout, 1993; Covey and Casseday, 1991), yet the exact arrangement of best frequencies in INLL has yet to be determined.

The INLL of the cat receives **afferents** from the CN and the SOC (Glendenning et al, 1981) (See Figure 2). It receives substantial projections from the contralateral VCN (about three quarters from AVCN and one quarter from PVCN) (Glendenning et al, 1981). In the bat most of the input to the INLL arises from the contralateral CN (AVCN and PVCN) (Zook and Casseday, 1982 and 1985; Covey and Casseday, 1986). A small projection is received from the ipsilateral MSO and bilateral LSO (Glendenning et al, 1981). A heavy projection from the LTB was reported in the bat (Zook and Casseday, 1979), while only a small ipsilateral projection was reported in the cat (Glendenning et al, 1981). The INLL is thought to be distinct from other NLL because only it receives a substantial projection from the ipsilateral MNTB (Glendenning et al, 1981). The INLL of the cat was also reported to receive a projection from the ipsilateral VNLL (Glendenning et al, 1981).

The main differences between the ascending afferents to the DNLL and INLL are: 1) the INLL receives a larger input from the contralateral VCN than the DNLL. 2) the INLL receives a substantial input from ipsilateral MNTB while DNLL does not. 3) the DNLL receives substantial projections from bilateral LSO and ipsilateral MSO while INLL receives minor projections from these nuclei. 4) the DNLL receives input from contralateral DNLL while INLL does not.

INLL neurons send **efferents** to ipsilateral IC (rat: Friauf and Kandler, 1990; cat: Whitley and Henkel, 1984; bat: Zook and Casseday, 1982 and Covey and Casseday, 1986). It also sends projections to the ipsilateral DNLL (Yang et al., 1997). The INLL of the cat has also been reported to project bilaterally to the medial division of the MGB (Hutson et al, 1991). The main difference between the efferent projections of DNLL and

that of INLL is that the INLL projects ipsilaterally to the IC while the DNLL projects bilaterally to the IC.

Ventral Nucleus of the Lateral Lemniscus

As mentioned above, the VNLL is one of three prominent groups of cells embedded within the fibers of the lateral lemniscus. The VNLL is the ventral- most group of cells in the LL. In the cat and rodents the VNLL is recognized as a single group of cells of mixed character. In the bat two regions are recognized: a lateral region containing columns of spherical cells parallel to LL fibers and a medial region of multipolar cells (Covey and Casseday 1986 and 1991). In contrast to the binaural sensitivity of DNLL neurons, most VNLL units are monaural and respond only to contralateral stimulation (are EO-contralateral stimulation excites) (Aitkin et al., 1970). A concentric frequency arrangement has recently been proposed for the VNLL based on its tonotopic projection patterns to the IC (Merchan, 1996).

The major **afferent** to the VNLL is the contralateral VCN (Glendenning et al, 1981; Covey and Casseday, 1994) (**See Figure 2**). Three times as many cells from AVCN project to the VNLL than do cells from PVCN (Glendenning et al, 1981). Degeneration and tracer studies show a weak projection from contralateral DCN (Adams and Warr, 1976; Fernandez and Karapas, 1967; Glendenning et al, 1981). A weak projection from the ipsilateral SOC has been reported. The projection seems to be derived from the MNTB and periolivary cell groups (Glendenning and Masterton, 1983; Glendenning et al, 1981).

The main differences between ascending afferents to the VNLL and the DNLL are : 1) the VNLL receives a larger source of input from contralateral VCN than DNLL. 2) the DNLL receives stronger projections from SOC than VNLL. 3) the DNLL receives input from contralateral DNLL while VNLL does not.

The VNLL sends **efferents** to ipsilateral ICC and DNLL (Whitley and Henkel; 1984, Glendenning et al., 1981). A small descending projection to the SOC (to the dorsomedial periolivary region and the VTB) has also been reported (Whitley and Henkel, 1984). A small projection to the ipsilateral MGB had also been observed (Whitley and Henkel, 1984).

The main differences between the efferent projections of DNLL compared to VNLL is that the DNLL has strong projections to contralateral DNLL and bilateral IC while VNLL projects to ipsilateral DNLL and IC.

Nucleus Sagulum

The nucleus sagulum borders the DNLL laterally. The sagulum can be distinguished from DNLL because it contains small neurons that stain lightly (Merchan et al., 1994).

The nucleus sagulum **projects to** its counterpart on the opposite side via the commissure of Probst (CP) (Hutson et al., 1991 and Henkel and Shneiderman, 1988). The CP is a specific avenue for decussating fibers from the sagulum and DNLL (Hutson et al., 1991). The nucleus sagulum is characterized by its projection to the superficial layers of the ipsilateral dorsal cortex of the inferior colliculus (Henkel and Shneiderman, 1988). The nucleus sagulum also projects to the dorsomedial nucleus and the rostral pole nucleus of the IC with some bilateral distribution and the ventral intercollicular tegmentum bilaterally.

Hutson et al, (1991) reported that the largest projection of nucleus sagulum is its projection to the dorsal division of the homolateral medial geniculate body. It may also project to the superior colliculus (Hutson et al., 1991 and Bajo et al., 1993).

As mentioned above the sagulum receives an **afferent** projection from its counterpart on the opposite side. A descending afferent projection from the ipsilateral EC and DC of the IC to the nucleus sagulum has been reported in the rat (Caicedo and Herbert, 1993).

Thus the sagulum can be distinguished from the DNLL in that it does not share the same sources of ascending input from SOC and CN.

Schematic Representations of the Afferent Projections to the Nuclei of the Lateral Lemniscus

Figure 2. (A) and (B) Schematic diagrams summarizing ascending projections to the nuclei of the lateral lemniscus from ipsilateral and contralateral (shown to the left of the broken vertical line) subdivisions of (A) the cochlear nucleus and (B) the superior olivary complex. Major and minor projections are shown by full and broken lines, respectively. Cross-hatched area in ventral nucleus of the lateral lemniscus (VNLL) indicates dorsomedial region receiving input from ipsilateral ventral cochlear nucleus (VCN). Although all projections have been shown as terminating in the lemniscal nuclei, it is uncertain as to what extent they are derived from collaterals of fibers proceeding to the inferior colliculus (taken from Irvine, 1986). (C) A schematic diagram emphasizing the main afferents (contralateral CN and DNLL, bilateral LSO, and ipsilateral MSO) to the DNLL in the cat. The main efferent projections from DNLL are also indicated (contralateral DNLL and bilateral IC) (taken from Oliver and Shneiderman, 1989 and modified).

A Schematic Representation of the Afferent Projections to the Central Nucleus of the Inferior Colliculus

Figure 3. **A-C**. Schematic summaries of major (full lines) and minor (broken lines) ascending to ICC from ipsilateral and contralateral (shown to the left of bold vertical broken line) nuclei of **A** the lateral lemniscus, **B** superior olivary complex, and **C** cochlear nucleus. ICC also receives sparse projections from a number of other brainstem structures, as described in text. Note also that each of the major nuclei depicted schematically here contains neurons of different morphological characteristics, and that these diagrams therefore present an extremely simplified picture of the ascending auditory pathways (taken from Irvine, 1986).

PURPOSE

One purpose of this study was to determine the afferent projections to the DNLL of the albino rat. This was accomplished by injecting the retrograde tract tracer Fluoro-Gold directly into the DNLL. Labelling in nuclei was taken as evidence that these cell groups project to the DNLL. These retrograde results were confirmed by injecting anterograde tract tracer into nuclei that were labelled with Fluoro-Gold to ensure that these areas had terminations within the DNLL.

Another purpose of this study was to determine the efferent projections of the DNLL of the albino rat. This was accomplished by injecting the anterograde tract-tracer Biotinylated Dextran into the DNLL. Terminal labelling in nuclei was taken as evidence that these cell groups receive projections from the DNLL. Target nuclei were also examined at the ultrastructural level to gain some information about the synaptic contact that DNLL makes with bilateral IC and contralateral DNLL. In particular the site of termination (cell body or dendrite) and the symmetry of the contact (symmetrical vs asymmetrical) were documented.

Normal DNLL tissue was also examined at the ultrastructural level. As mentioned earlier a previous study of the DNLL of the cat at the electron microscope level revealed that there might be two different cell types in the DNLL of the cat (one type had irregularly shaped nuclei and perinuclear rough endoplasmic reticulum (and had mainly fusiform shaped cell bodies), the other type had rounded nuclei and lacked perinuclear reticulum) (Oliver and Shneiderman, 1989). A study of normal DNLL tissue was carried out to see if a similar morphological classification could be applied to DNLL neurons in the rat.

METHODS

Subjects

Experiments were conducted on adult male Wistar albino rats (300-450 g) obtained from Charles River Canada Inc., St. Constant, Quebec.

Animal Preparation

Animals were anesthetized with sodium pentobarbitol (60 mg/kg, i.p.) and placed in a stereotaxic headholder frame. A midline incision was made in the scalp and the skin and muscle were retracted laterally. For DNLL injections a craniotomy was made along the anterior bank of the lateral skull fissure. For IC injections a small hole was made near midline just rostral to lambda. For SOC and CN injections craniotomies were made approximately 3mm and 1mm caudal to lambda, respectively.

The right **DNLL** was located using the coordinates of the DNLL in three dimensions with reference to true lambda (Li and Kelly, 1991). A glass pipette was tilted 30 degrees against the sagittal plane. The coordinate was referenced to a point 6.7 mm lateral and 0.40 -0.50 mm rostral to true lambda. The pipette was lowered to a depth of 7.8 mm from the reference point.

The right **ICC** was located stereotaxically with the electrode in a vertical orientation. The coordinate was 1.7- 2.0 mm lateral, 0.3-0.5 mm rostral, and 3.0 -5.0 mm ventral to lambda.

For **SOC** placement the pipette was tilted backward 11 degrees relative to the frontal plane (Li, 1994). The coordinate was referenced to a point 2.5 - 3.0 mm caudal and 1.7 - 2.0 mm lateral to lambda and then lowered to depth of 10.7 mm from the reference point.

For CN placement the pipette was inserted stereotaxically in a vertical orientation. For AVCN placement the coordinate was 0.40 cm lateral, 0.11 cm caudal and 0.82 cm ventral to true lambda.

Retrograde Tract Tracing Experiments

Tracer Injection

Retrograde tract tracer was ejected into the right DNLL via a glass pipette with an outer diameter of 20-35 μm . Fluoro-Gold (Fluoro-Chrome Inc, USA) was dissolved in 0.9 % saline or distilled water to obtain a 2% weight per volume solution. The tracer was ejected iontophoretically (positive, continuous current, 1-2 μA for 1-2 minutes). Following ejection, the pipette was left in place for 10 minutes.

Histology

Following five to seven day survival times, the animals were deeply anesthetized with sodium pentobarbital and perfused through the left cardiac ventricle with 0.9% saline followed by 10% formalin. The brains were immediately removed from the skulls. The brains were then stored in 20% sucrose in 0.1 M phosphate buffered saline (PBS) at 5 degrees Celsius for one to two days. If necessary the brains were post-fixed for several hours. The brains were then sectioned at 40 μm in the coronal plane into an A and B series using a freezing microtome. The left side of the brains were marked. The A series was collected into distilled water and the B series was collected into cryoprotectant (30% sucrose, 1% polyvinylpyrrolidone (PVP) and 30% ethylene glycol). The B series was stored at -20 degrees Celsius. Sections collected in distilled water were mounted immediately onto gelatin coated slides. Slides were allowed to air dry in the dark at least overnight. The sections were then cleared in xylene for 10 minutes and coverslipped using DPX. The sections were then stored in the dark at room temperature.

Visualization of Tracer and Data Analysis

The A series of each case was examined using a Zeiss Axioplan microscope equipped with a mercury lamp for fluorescence microscopy. For visualization of Fluoro-Gold, UV excitation was used along with a longwave pass (420nm) barrier filter (Fluoro-Gold shows ice-blue). For each case the site of injection of tracer within the DNLL (or surrounding structures) and the zone of diffusion of the tracer were determined. The centre of the injection site was taken as the area of densest tracer deposit (and/ or the area where the electrode track could be seen). The zone of diffusion was taken as the area where tracer was visible but not as concentrated as it was in the centre. The distribution of labelled cells in auditory brainstem structures was noted. For Fluoro-Gold cases cells were considered to be labelled if they fluoresced brightly with regard to the background on low power (10 times objective). Exemplary sections of the A series of certain cases were then photographed. Black and white photographs were taken using T-Max 100 or Ilford 400 film.

Anterograde Tract Tracing Experiments

Tracer Injection

Biocytin or Biotinylated Dextran (a 3% weight per volume solution in 0.5 sodium chloride) was ejected into the right DNLL, SOC, CN, or the ICC using the stereotaxic coordinates given above. Glass pipettes (outer diameter 20-35 μm) were filled with the tracer and a tungsten electrode was inserted into the pipette. The tracer was delivered iontophoretically (positive current, 7s on/7s off for 30 minutes) or by pressure using a syringe pump. Following deposition the pipette was left in place for 10 minutes. In cases involving CN injections multiple unit physiological recording was done to determine the characteristic frequency of the area. The tungsten electrode was connected to recording equipment. Physiological potentials were amplified by Dagan EX4-400 amplifiers, displayed on an oscilloscope and monitored acoustically by a loudspeaker. Neural

responses were digitized and processed by MALab 881, a data acquisition system designed by Steve Kaiser for use on a Macintosh (a Quadra 700 computer). Tones were presented separately to the two ears through headphone drivers (Pioneer SE-50D) mounted in sealed housings fitted to hollow couplers inserted into the external meati of the rat. Tones (100 msec with 10 msec rise and fall times) were generated digitally by a Kaiser Instruments DA interface controlled by MALab 881. The characteristic frequency was determined for a tone presented to the ipsilateral ear for CN recording. The characteristic frequency was defined as that frequency for which the lowest sound pressure level of ipsilateral stimulation would elicit spikes.

Histology

Following 36 hour survival times for animals injected with Biocytin and five to seven day survival times for animals injected with Biotinylated Dextran, animals were deeply anesthetized with sodium pentobarbitol and perfused through the left cardiac ventricle with room temperature saline (0.9%) followed by ice-cold fixative (2% glutaraldehyde plus 2% paraformaldehyde in 0.1 M phosphate buffer). This was immediately followed by a rinse with ice-cold 10% sucrose in 0.1 M phosphate buffer. The brains were immediately removed from the skulls and stored in 10% sucrose at 5 degrees Celcius and post-fixed (for 2-4 hours) if necessary in the same fixative. The brains were sliced in the frontal plane on a freezing microtome into an A, B and C series (the A and B series were cut at 40 μ m while the C series was cut at 80 μ m). The A series was cut into 0.1 M phosphate buffered saline on ice for immediate reaction as outlined below. The B and C series were collected into cryoprotectant (30% sucrose, 1% PVP (polyvinylpyrrolidone) and 30% ethylene glycol) and stored at -20 degrees Celcius. Representative sections of the C series, for cases in which Biotinylated Dextran was injected into the DNLL and CN, were rinsed three times in 0.1 M PBS, reacted for the presence of tracer and then processed for electron microscopy as outlined below.

Histochemistry for Visualization of Biocytin

For reaction, the tissue was incubated with avidin biotin peroxidase complex for peroxidase reaction with DAB as chromagen and nickel-cobalt intensification (the method used by Wu and Kelly, 1995 for single slices was modified to react large batches of tissue for tract-tracing).

For reaction, the sections were rinsed three times with 0.1 M PBS, incubated for 20 minutes in 0.5% hydrogen peroxide in 0.1 M PBS, and then rinsed three times for 15 minutes each in 0.1 M PBS. The sections were then treated with Triton-X 100 (0.5% in PBS) for 1 hour. Half an hour before use the avidin biotin peroxidase reagent (ABC reagent Vector stain) was prepared (4 drops of A plus 4 drops of B for 20 ml of PBS). The sections were allowed to incubate in the ABC reagent at room temperature with gentle agitation overnight. The following day the sections were rinsed with 0.1 M PB three times for 15 minutes each. Diaminobenzidine (DAB) from Sigma was prepared (30 mg in 50 ml of 0.1 M PB) and filtered. The sections were incubated in the DAB solution alone for 20 minutes. A mixture of 2ml of 1% aqueous cobalt chloride plus 1.6 ml of 1% aqueous nickel ammonium sulfate (3.3 ml total delivery) was added to the sections in the 50 ml of DAB. The sections were then allowed to incubate for 20 minutes. Hydrogen peroxide (0.03%, 3.23 ml) was carefully added to the reaction vessel and the reaction was allowed to take place at 5 degrees Celcius on ice for 20 minutes. The sections were then rinsed twice in 0.1 M PB for 5 minutes and once in 0.01 M PB for 5 minutes. The tissue was mounted on gelatin coated slides, allowed to air dry overnight, dehydrated for 1 minute with 95% ethanol followed by 2 minutes in 100% ethanol, cleared in fresh xylene for 10 minutes, and coverslipped using DPX.

Visualization of Tracer and Data Analysis

The A series of each case was examined using bright field microscopy. For each case the site of injection and the zone of diffusion of the tracer were noted. The distribution of labelled axon terminals was documented for each case. Exemplary sections were photographed using T-MAX 100 or Ilford 400 black and white film..

ULTRASTRUCTURAL STUDIES

Normal DNLL Tissue

Tissue Processing:

Pefusion of rats and histology was performed as outlined above for visualization of biocytin. The C series was processed for electron mircoscopy as outined below. The DNLL was identified in the embedded slices with the assistance of the A series which was stained with Cresyl violet-acetate (for method see Humason, 1960) The sections were heated on a hot plate and the left DNLL of each section (from caudal to rostral) (and laterally surrounding sagulum) was extracted with a razor blade (the tissue was marked to indicate orientation-dorsoventral and mediolateral). The pieces of DNLL were mounted onto trimmed Epon Araldite blocks using cyanoacrylate. The blocks were trimmed prior to slicing so that the block face was positioned in the middle of the DNLL (except in one block in which the block face was positioned medially in DNLL near the commissure of Probst). The block face was cut so that the small face was dorsal and the large face was ventral to maintain correct orientation.

Using a Leica ultramicrotome and glass knives, thick 1.0-0.5 μm sections were cut from each block face to confirm that the tissue was DNLL. The sections were placed on glass slides, stained with 1% Methylene blue in 1% Borax while heating on a hot plate, and rinsed with water. Representative thin (80-120 nm) sections were cut from each

block and collected onto 300 mesh grids coated with formvar. These grids were stained with 4% aqueous uranyl acetate followed by Reynolds lead citrate.

Data Analysis:

The A series, that was stained with Cresyl violet acetate, was examined using the light microscope (Zeiss Axioplan) to confirm that the left DNLL was correctly dissected from caudal to rostral.

The thick sections cut from each block face from caudal to rostral were examined under the light microscope to verify that the tissue cut for EM analysis was the DNLL. The presence of bundles of fibers forming the lateral lemniscus (running dorsal to ventral within the tissue) was taken as an indication that the tissue was DNLL.

The thin sections from each block from caudal to rostral were examined using a Phillips 201 Transmission Electron Microscope. The tissue was examined qualitatively to determine if different neurons could be found. The main features that were observed were soma shape, dendritic branching pattern (if possible), nuclear shape (irregular or smooth) and organization of the distribution of rough endoplasmic reticulum in the cytoplasm (dispersed, perinuclear or stacked). Neuronal cell bodies were identified in the tissue by the presence of a nucleus. Care was taken to avoid erroneously identifying glial cells as neurons (see Peters et al., 1976 and Peters et al., 1970). Large glial such as fibrous astrocytes were distinguished from neurons because they were irregular in shape and possessed scant cytoplasm that contained few organelles and many fibrils. Protoplasmic astrocytes were distinguished by their irregular shapes, their scant cytoplasm and the astrocytic processes that they extended from the cell body. Oligodendrocytes were distinguished on the basis of their dense cytoplasm containing few organelles and dark staining. Representative plates were taken using Kodak Electron Image Film.

Tissue Examined Following Anterograde Transport of Biotinylated Dextran from the
DNLL and the CN

Tissue Processing

Following examination of the axon terminal labelling patterns in the A series, sections from the C series were rinsed in PBS and were reacted for localization of biocytin as outlined above and then prepared for EM embedding according to the procedure of Bolam (1992) with slight modification. Following removal of the phosphate buffer the sections were post-fixed with 1% osmium tetroxide (in 0.1 M phosphate buffer pH 7.3-7.4) for 35 minutes. Upon removal of the osmium the sections were washed (three times for 15 minutes each) with 0.1 M phosphate buffer. They were dehydrated with a large volume of 50% ethanol for 15 minutes and stained en bloc with 1% uranyl acetate in 70% ethanol for 30 minutes. They were dehydrated further with 95% ethanol (for 15 minutes) followed subsequently by two 15 minute treatments with 100% ethanol. The sections were transferred to separate glass vials and treated twice for 15 minutes with propylene oxide. The tissue was infiltrated with a mixture of resin (Epon/Araldite from J.B. EM services) and propylene oxide for two hours.

The sections were transferred to individual tin foil boats, covered with resin and left overnight. The following day they were transferred to a plastic mold (with shallow square impregnations) rolled flat with a brush and covered with fresh resin. A glass coverslip was placed on each section to keep it flat. The tissue was baked for 2 days at 62 degrees Celcius to harden it.

The coverslips were removed from the sections by heating them on a hot plate and carefully working them off. The nuclei of interest (those in which labelled terminals were observed in the A series) were identified in the slices with the assistance of the A series, which was reacted as outlined above for Biocytin visualization. Sections were heated on a hot plate and the nuclei of interest were extracted with a razor blade. The

tissue was marked to indicate type of tissue and orientation-dorsoventral and mediolateral. The pieces were mounted onto trimmed Epon/Araldite blocks using cyanoacrylate. The blocks were trimmed prior to slicing so that the block face was positioned where most labelled terminals were located according to observation of the A series. The block face was cut so that the small face was dorsal and the large face ventral, so that correct orientation could be maintained.

Using a Leica ultramicrotome and glass knives, thick 1-0.5 μm sections were cut from each block face. The sections were placed on glass slides and stained with 1% Methylene blue in 1% Borax with heating on a hot plate and rinsed with water. Representative thin (80-120 nm) sections were cut from each block and collected onto 300 mesh grids coated with formvar. The grids were stained with 4% aqueous uranyl acetate followed by Reynolds lead citrate.

Data Analysis

Thick Sections

The thick sections cut from each block were examined using the light microscope. Thick sections helped to ensure that the area cut was that intended for cutting. The presence of bundles of fibers forming the lateral lemniscus (running dorsal to ventral within the tissue) was taken as an indication that the tissue was the DNLL.

Thin Sections

The thin sections from each block were examined using a Transmission Electron Microscope (Phillips 201). Labelled terminals were studied at the ultrastructural level to gain qualitative information regarding the nature of the contacts axons from DNLL neurons make with target nuclei. The tissue was examined for the presence of labelled axon terminals, which were identified because they were darker than the surrounding tissue (since they were filled with black reaction product). Representative plates were taken and the type of contact that the terminals made (asymmetrical or symmetrical) and

the site of contact (small or large dendrites or cell bodies) were documented for each labelled terminal. The type of contact (asymmetric or symmetric) and the site of contact (dendrite or cell body) were the criteria used to describe the contact. If vesicle morphology was not obscured by the reaction product in the terminal, vesicle shape (round or flat) was also noted.

RESULTS

DNLL AFFERENTS

RETROGRADE EXPERIMENTS

Fluoro-Gold injections were made in twelve animals (cases RL12, 14, 15, 16, 17, 19, 20, 22, 54, 37, 75 and 83). In case 22, the injection site was located in INLL, in case 14 the injection was in sagulum, and in case 54 the injection was just dorsal to DNLL and just ventral to IC, in subcollicular tegmentum. In all other cases the injections were located in DNLL. All DNLL injections were located in mid DNLL (with reference to rostral to caudal position) except for one case, RL15, in which the injection was located extremely rostrally (this case will be considered separately). The DNLL injections varied with respect to their medial -to- lateral position within DNLL. These results have appeared in abstract form (Labelle and Kelly, 1996).

DNLL INJECTIONS

Nuclei of the Lateral Lemniscus

Injection Sites

The DNLL FG injection sites varied with respect to their medial -to- lateral positions within the DNLL. The injections were restricted to the DNLL except for an extreme lateral injection with spread into nucleus sagulum (case 16) and a very large, laterally concentrated injection which spread to the INLL and sagulum (case 83). **See Figure 4** which gives a schematic of DNLL injection sites.

Pattern of Labelling

Following each of the DNLL FG injections, the contralateral DNLL showed prominent labelling (see **Figures 5, 9 and 12, 17 19**). The contralateral DNLL was labelled from its rostral (**Figures 5A, 9A and 12A**), middle (**Figures 5B, 9B and 12B**) through to caudal (**Figures 5C, 9C and 12C**) extent. In contralateral DNLL, Fluoro-Gold filled neurons with different shaped somata and orientations could be distinguished. In caudal DNLL, large multipolar neurons were observed (**Figures 6A and Fig 13A**). Elongate neurons were observed in all parts of the DNLL that were oriented dorsoventrally or mediolaterally and which extended dendritic processes that sometimes passed across the entire DNLL (**Figures 6B, 13B and 19C**). Neurons with round somata were common and were observed throughout the DNLL (**Figures 19D**). The ipsilateral VNLL also showed dense labelling while the INLL showed light labelling following middle DNLL injections (see **Figures 5D**). Injections positioned more laterally resulted in less dense labelling of VNLL and INLL (see **Figures 9D and 12D**).

Inferior Colliculus

Following DNLL injections situated more laterally in DNLL, there was heavy labelling of the ipsilateral ICC (see **Figure 16** for case RL16). Following DNLL injections located more centrally little labelling of the ICC was observed, but some labelling of the EC and the subcollicular area was observed (see **Figure 16** for case RL17).

Superior Olivary Complex

The LSO was labelled bilaterally following most DNLL injections (**Figures 7, 10, 14, 18, 20**). Labelled cells were dispersed throughout the ipsilateral LSO while mainly the medial limb of the contralateral LSO was labelled (**Figures 7, 10, 18 and 20**). Only following extremely lateral injections was an absence of contralateral LSO labelling

observed (**Figure 14**). The ipsilateral **MSO** was also labelled (**FIGS 7, 10, 18, 20**). Only following extremely lateral injections was an absence of ipsilateral **MSO** labelling observed (**Figure 14**). Following a large **DNLL** injection that spread to **INLL**, but was also concentrated laterally, little **MSO** labelling was also observed (**Figure 20**). Following middle to mid-lateral **DNLL** injections, labelling of the medial part of the ipsilateral **SPO** was observed (**Figures 7 and 10**). In one case with a middle injection, no ipsilateral **SPO** labelling was observed (**Figure 18**). Following extremely lateral injections stronger labelling of the medial part of the ipsilateral **SPO** was observed (**Figures 14 and 15**). In most cases the ipsilateral **LTB** and **VTB** showed sporadic labelling, while in one case following a large **DNLL** injection that spread to **INLL**, the ipsilateral **LTB** (RL83) showed more prominent labelling (**Figure 20**) and in another case (RL 37) the ipsilateral **VTB** showed more prominent labelling (not shown). The contralateral **LTB** also showed sporadic labelling in all cases. The ipsilateral **MNTB** was not labelled following **DNLL** injections that did not spread to the **INLL** (**Figures 7, 14, 18**) but was labelled following an injection that spread to **INLL** (**Figure 20**).

Cochlear Nucleus

The contralateral cochlear nucleus was labelled following all **DNLL** injections (**Figure 8, 11, 21**). Almost no ipsilateral labelling was observed (not shown). The contralateral **AVCN** showed relatively the most prominent labelling (**Figures 8, 11, 21**). The contralateral **PVCN** also showed consistent labelling (**Figures 8, 11, 21**). The labelling in the contralateral **AVCN** and **PVCN** was observed mainly in the most dorsal parts and medial and lateral edges, and consisted of cells with small round somata. The ventral tip showed little labelling. In most cases the contralateral **DCN** contained very few labelled cells that were observed in the most dorsal parts (**Figures 8 and 11**). More labelling of dorsal parts of contralateral **DCN** was observed in a case in which a large

injection was made that was concentrated laterally in DNLL (and which spread to INLL) (**Figure 21**).

Summary of Labelling Patterns

Following injection into DNLL the main nuclei to show retrograde labelling were the contralateral DNLL and CN, bilateral LSO and ipsilateral INLL, VNLL, MSO, and SPO (see **Figure 28**). The ipsilateral VTB, LTB were also labelled sporadically. The ipsilateral IC was also labelled depending upon the injection site.

ROSTRAL DNLL INJECTION CASE

In one case (RL15) the injection site of FG was located at the rostral extreme of DNLL. The contralateral DNLL was labelled only at rostral levels. Labelling was found in the ipsilateral SC, MGB, sagulum, subcollicular area and EC of IC (not shown). There was no labelling of ascending auditory nuclei in this case (i.e. no labelling of CN or SOC) (not shown).

INLL INJECTION

Following an INLL injection (**Figure 22A**) there was no labelling of the ipsilateral or the contralateral DNLL (not shown). The ipsilateral VNLL showed sparse labelling (**Figure 22A**). In the superior olivary complex, the ipsilateral MNTB showed the strongest labelling (**Figure 22B**). The ipsilateral LSO showed sparse labelling (**Figure 22C**). The contralateral CN also showed labelling following an INLL injection. Some labelling was observed in dorsal parts of the AVCN and the PVCN (**Figure 23**). No labelling was observed in contralateral DCN (not shown).

Subcollicular Tegmentum

Following an injection in subcollicular tegmentum (case 54), just dorsal to DNLL and just ventral to IC, labelling was prominent in contralateral DNLL (**Figure 24**). The ipsilateral INLL and VNLL were also labelled (not shown) while ipsilateral DNLL was not. Labelling was prominent in bilateral LSO and ipsilateral MSO (**Figure 25**). Labelling was also observed in ipsilateral SPO (**Figure 25**). All three divisions of the contralateral CN were labelled (**Figure 26**) while the ipsilateral CN was not (not shown). The most dorsal parts of the contralateral AVCN, PVCN and DCN were labelled. Little labelling was observed in the ventral tip of each CN division.

Sagulum Injection

Following a very small injection into nucleus sagulum (case 14) labelling was observed in contralateral sagulum (**Figure 27**). No labelling in ascending auditory nuclei was observed but some labelling in ipsilateral MGB was observed (not shown).

ANTEROGRADE EXPERIMENTS

Cochlear Nucleus Injections

Following an injection into a **high frequency region of CN** (22,000 Hz) (the dorsal part of the AVCN) (**Figure 29**), dense terminal labelling was observed in ventral (the high frequency part) contralateral ICC (**Figure 31**). Labelling in contralateral DNLL was arranged in a ring but was most dense ventrally (see **Figures 29 and 30**). The contralateral INLL and VNLL were also labelled (**Figure 30**). Thick and thin labelled fibers were observed. Labelling in contralateral INLL was most dense ventromedially. Terminal labelling in contralateral VNLL was arranged in a column of horizontally

oriented patches. The terminal labelling in contralateral ICC and VNLL was more prominent than in contralateral INLL and DNLL. The ipsilateral ICC, DNLL, INLL and VNLL also were labelled (not shown) but the contralateral labelling was much more prominent (see **Figure 37**, which shows sparse labelling in ipsilateral DNLL). The SOC was also labelled bilaterally. The medial limb of the ipsilateral LSO was labelled (**Figure 32**). In contralateral SOC, periolivary nuclei were labelled (SPO and VTB and the area just lateral to MNTB) (**Figure 33**).

Following injection into the high frequency part of AVCN, preliminary analysis of contralateral DNLL labelling at the ultrastructural level has thus far revealed that labelled terminals make asymmetric contacts with small dendrites and dendritic spines (**Figure 34**).

Following an injection into a **low frequency area of CN** (1,300 Hz) (into the ventral tip of the AVCN) (**Figure 35**), terminal labelling in the dorsolateral contralateral ICC was observed (**Figure 38**). A few labelled terminals were also observed in the middle of the contralateral DNLL (**Figures 35 and 36**). The contralateral INLL and VNLL were also labelled (**Figure 36**). The terminal labelling observed in contralateral ICC and VNLL was more prominent than in contralateral DNLL and INLL. The ipsilateral ICC, DNLL, INLL and VNLL also showed some labelling (not shown) but the contralateral labelling was much greater. The SOC was also labelled bilaterally. The lateral limb of the ipsilateral LSO was labelled, as was the ipsilateral MSO (**Figure 39**). The contralateral MSO was labelled (**Figure 40**).

ICC CASES

Following an injection into ventromedial ICC, fiber labelling was found in ipsilateral DNLL. Some labelled terminals and fibers were observed laterally in DNLL and surrounding nucleus sagulum (**Figure 41**).

SOC CASES

LSO CASE

Following an injection into the lateral limb of the LSO (low frequency limb), in the dorsal-most (or top-most) part, terminal labelling was observed in ipsilateral DNLL (**Figures 42A and B**). The labelling ring shaped and located centrally not peripherally. The ring was incomplete medially and laterally and consisted of patches of terminal labelling that appeared between fibers of the lateral lemniscus (in areas where cell bodies are located) (**Figure 42C**). Ventrally the terminals were oriented horizontally while more medially they were oriented in a vertical column (**Figures 42D and E**).

Little terminal labelling was observed in contralateral DNLL (**Figure 43**). Terminal labelling was prominent in contralateral ICC and was located laterally (**Figure 44**). Little labelling was observed in ipsilateral ICC (not shown).

MNTB CASE

Following injection into the MNTB, labelling was observed in ipsilateral SPO and LSO (especially in the medial parts of both nuclei) (**Figure 45**). Sparse terminal labelling was also observed in ipsilateral INLL and VNLL (**Figure 46**). No labelling was observed in DNLL (**Figure 46**) and IC (not shown).

VTB CASE

Following injection into the VTB (**Figure 47**), labelled fibers passed through the ipsilateral DNLL en route to the ipsilateral ICC. A few collaterals were seen to branch and terminate in DNLL (**Figure 47**). Labelled terminals and fibers were more evident ventrally in ipsilateral ICC (**Figure 48**).

SPO CASE

Following injection into the lateral part of the SPO (**Figure 49**) terminal labelling was mainly observed in the middle of the ipsilateral ICC (**Figure 51**). Labelling was also observed to a lesser extent in ipsilateral nuclei of the lateral lemniscus (**Figure 50**). There was little terminal labelling in DNLL and that which was found was located laterally (**Figure 50A, B, C, F**). Labelled fibers mainly passed through the DNLL medially en route to the IC. Labelling in the INLL was concentrated ventrally and was more prominent than DNLL labelling (**Figure 50 D, F**). Labelling in INLL consisted of tiny boutons terminaux. Labelling was most obvious in ipsilateral VNLL (**Figure 50 E, F**). No labelling was seen in contralateral ICC, DNLL or INLL but some labelled fibers were seen in contralateral VNLL (not shown). Labelling was also observed in the ipsilateral LSO and MSO (**Figure 49**). The CN was labelled bilaterally (VCN, PVCN and DCN) and was more pronounced contralaterally (**Figure 52** shows contralateral labelling).

DNLL EFFERENTS

Light Microscopy

Injection Sites

Injections of Biotinylated Dextran were made in ten animals (cases 36, 43, 62, 78, 51, 67, 64, 49, 53 and 68). The injections varied in their locations with reference to rostral- to caudal- position within the DNLL. Some injections were located caudally (RL 64) while others were located in the middle parts of DNLL (RL43 and 62) while still others were

located in rostral DNLL (RL 78 and 51). Most injections spread to the surrounding nucleus sagulum except cases RL64 and RL 51. Two injections were confined to the nucleus sagulum (RL49 and 67). These results have been presented in abstract form (Labelle and Kelly, 1997). **Figure 55** gives an overview of the labelling results at the light level and depicts the areas that were extracted for EM analysis.

Efferent Fiber Trajectory

Injections resulted in labelling of fiber paths so that their route to target nuclei could be visualized. Fibers exited the DNLL via 3 distinct bundles (medial, dorsal and ventral bundles). Fibers ran medially through the commissure of Probst to contralateral DNLL. Once in DNLL, fibers collateralized in DNLL and then continued dorsally to contralateral ICC. Other medially running fibers did not enter the DNLL but turned dorsally after the midline and headed directly to contralateral IC (bypassing contralateral DNLL). Labelled fibers also ran dorsally from the DNLL to the ipsilateral ICC. Labelled fibers also ran ventrally through the lateral lemniscus to the SOC. Fibers running dorsally to approach the ipsilateral IC, entered the IC in one of two paths: one that ran laterally from DNLL laterally into IC and the other that ran medially from DNLL medially into IC (**Figure 53B**). In contralateral IC two fiber paths were also observed: one that ran medially through the IC and one that ran laterally (**Figure 53A**). The medially running path was observed to continue to deep layers of SC. The commissure of Probst was clearly labelled following Biotinylated Dextran injections into DNLL. In coronal section, at caudal levels the CP did not pass directly across to contralateral DNLL. In middle levels the CP ran directly across to the contralateral DNLL. At rostral levels the CP ran down further ventrally than at mid levels (see **Figure 54**).

Terminal Labelling Patterns

Figure 55 gives an overview of the labelling at the light level and depicts the areas extracted for EM analysis.

Contralateral DNLL

Dense terminal labelling was observed in contralateral DNLL following injections of Biotinylated Dextran into the DNLL. The heaviest labelling in DNLL occurred in contralateral DNLL at the same level as the injection site and was located in symmetry to the injection site. Following a ventral DNLL injection (case RL43) (**Figure 55**), labelling was observed in contralateral DNLL (**Figure 56**) that was most dense ventrally. Even following minute injections (RL64) or very small injections (RL 51), sparse labelling was found in a symmetrical location in contralateral DNLL (**Figure 59**). When injections were in middle parts of DNLL with reference to rostral to caudal axis, the labelling in contralateral DNLL was arranged in vertical columns (where cell bodies are located) between fibers of the lateral lemniscus (**Figure 57A and B**). Following a very lateral DNLL injection into rostral DNLL (but including nucleus sagulum), labelling in contralateral DNLL was concentrated laterally at caudal levels (**Figure 58**), yet at rostral levels labelling was spread throughout rostral contralateral DNLL. In contralateral rostral DNLL terminal fiber labelling was not arranged tightly in vertical columns, fine fibers ran mediolaterally and dorsoventrally (**Figures 57C and D**). Injections confined to surrounding nucleus sagulum (cases RL67 and 49) resulted in little or no labelling of contralateral DNLL (**Figure 60**).

Bilateral IC

An abundance of labelled terminals were observed bilaterally in the ICC following large middle DNLL injections (**Figures 66 and 72**). In general terminal labelling was denser and less diffuse in the contralateral ICC. The location of labelled terminals in bilateral ICC varied with regard to the injection site within the DNLL. For example

ventral DNLL injections (**Figure 55 A**) resulted in ventral ICC labelling. Even following a minute injection in caudal DNLL (case RL64) labelled terminals were found bilaterally in ICC (see the injection site shown in **Figure 59B**). Following a small injection in rostral DNLL (case RL 51) (see the injection site shown in **Figure 59 D**) few terminals were observed in the IC bilaterally at the level of the injection site but not at more caudal levels. Following an injection (case RL 67) into sagulum (as shown in **Figure 60B**) no labelling of the ICC was observed but the ipsilateral EC was labelled even at caudal levels. In most cases the EC was labelled bilaterally at middle and rostral levels. Following an injection into sagulum at rostral levels (case RL 49) (**Figure 60 C**) no terminal labelling in ICC was observed.

Superior Colliculus

Less dense terminal labelling (compared to contralateral DNLL and bilateral IC labelling) was also found bilaterally in deep and intermediate layers of the SC (**Figures 77 and 81**) following large DNLL injections that may have spread to sagulum. Labelled fibers ran through the deep and intermediate parts of the SC. Following a minute injection in the middle of caudal DNLL (**Figure 59A**) that did not spread to nucleus sagulum, no terminal labelling in SC was observed (not shown). Following a small injection in the middle of rostral DNLL that did not spread to nucleus sagulum (**Figure 59 D**), sparse labelling was found in the SC bilaterally. Following an injection into nucleus sagulum (RL67) labelling of the ipsilateral SC was observed. Following an injection into sagulum at rostral levels (RL 49), dense bilateral labelling of the SC was observed.

Other Areas

Labelling was observed bilaterally in the intercollicular area (see Paxinos and Watson, 1986) (just medial to rostral levels of the EC of the IC) (**Figures 86 and 87**). Labelling

was denser contralaterally and more diffuse ipsilaterally. In a few cases sparse labelling of ipsilateral MGB was observed (cases RL78, 62, and 51) (not shown).

Superior Olivary Complex

Fibers that exited the DNLL ventrally through the ipsilateral lateral lemniscus were labelled and in two cases (RL62 and 43) some terminal labelling was observed in the VNLL and rostral periolivary nucleus (not shown). Fibers continued to the ipsilateral SOC where sparse terminal labelling was observed. Labelling was mainly found in SPO, MNTB and VTB (**Figure 88**). In one case (RL62) some terminal labelling was observed in the ipsilateral LSO.

Electron Microscopy

Selected areas that contained labelled endings at the light level were prepared for electron microscopy (cases 43, 62 and 78). Labelled endings in the areas selected for analysis were identified by the presence of dark reaction product. The site of contact (dendrite or cell body) and the type of contact (symmetrical or asymmetrical) were noted for all documented labelled terminals. Qualitative observations are presented below.

Contralateral DNLL

Labelled axon terminals in contralateral DNLL mainly formed symmetrical contacts with large to medium sized dendrites (see **Figures 62 and 63**). Some endings also formed symmetrical contacts with the cell bodies of DNLL neurons (**Figure 61**). In some cases labelled terminals formed asymmetric contacts with small to medium sized dendrites (**Figures 64 and 65**).

Bilateral IC

Labelled terminals observed in contralateral IC formed symmetrical contacts mainly with large to medium sized dendrites (**Figures 67-70**). Fewer contacts were observed on smaller dendrites (**Figures 71**). Labelled terminals in ipsilateral IC mainly formed symmetrical contacts with medium to large sized dendrites (**Figures 73-75**). No labelled terminals forming clear asymmetric contacts have been observed in ipsilateral IC, yet unlabelled asymmetric contacts were observed (Figure 76). Some of the labelled terminals observed in IC were quite large (approximately 1 μm in width).

Bilateral SC

Labelled terminals were observed in contralateral SC that formed symmetrical contacts with large and medium sized dendrites (**Figures 78-80**). Labelled terminal boutons observed in ipsilateral SC formed symmetrical contacts with medium to small sized dendrites (**Figures 82-84**). In some instances labelled boutons and their axons formed several contacts terminaux and en passant with dendrites (**Figure 85**).

Ipsilateral SOC

When extracting tissue from periolivary nuclei of the SOC for EM analysis it was difficult to isolate only one nucleus. An attempt was made to isolate medial parts of SPO and lateral parts of MNTB. Labelled terminals in these samples formed symmetrical and asymmetrical synapses with dendrites (**Figures 89 to 92**). A few labelled terminals were observed that formed contacts on cell bodies (**Figures 93 and 94**).

Summary of Terminal Labelling

Terminal labelling following injection of anterograde tracer into DNLL showed that the DNLL forms symmetrical contacts on dendrites in its main target nuclei (contralateral DNLL and bilateral IC). The DNLL also forms symmetrical contacts on dendrites in

bilateral SC. The DNLL forms symmetrical and asymmetrical contacts with dendrites and cell bodies in periolivary nuclei of the SOC.

DNLL CELLULAR ULTRASTRUCTURE

Neurons

A sample of 38 neurons was observed that exhibited diverse somatic morphologies. Neurons had somas that were round (n=17 or 45.0 %) (see **Figure 95**), elongate (n=16 or 42.0 %) (see **Figure 96**) or triangular (n=5 or 13.0 %) (see **Figure 98**). Neurons with irregular and smooth nuclear contours were observed. Regardless of soma shape or nuclear contour most neurons possessed rough endoplasmic reticulum that was dispersed throughout the cytoplasm (37 of 38 or 97.0 %) (see **Figures 95, 96 and 97, 98 and 99**). Only one neuron possessed stacked rough endoplasmic reticulum (**Figures 100 and 101**). In this sample, no neurons were observed that possessed perinuclear rough endoplasmic reticulum.

Glial Cells

Large glial cells such as fibrous astrocytes were clearly distinguishable from nerve cells because they had irregular somatic shapes, possessed scant cytoplasm which was largely occupied by fibrils, and extended astrocytic processes as opposed to dendrites and axons (see **Figure 102 and 103**). Large glial cells such as protoplasmic astrocytes were distinguished because they had irregular somatic shapes and scant cytoplasm and extended astrocytic processes from the cell body as opposed to dendrites or axons (see **Figure 104**). Oligodendrocytes were distinguished because they had a dense, darkly staining cytoplasm which contained few organelles (**Left in Figure 100**).

Summary of Neuronal Ultrastructure

Regardless of somatic shape and nuclear morphology, most neurons in this sample possessed rough endoplasmic reticulum that was dispersed throughout the cytoplasm.

DISCUSSION

DNLL AFFERENTS

The combined retrograde and anterograde data suggest that the DNLL of the rat receives projections from contralateral DNLL and CN, the LSO bilaterally and the ipsilateral IC, INLL, VNLL, MSO, SPO, VTB, and LTB. In the discussion which follows the retrograde and anterograde findings will be compared and the results will be related to findings of other studies (mainly studies of the cat and bat). The discussion is divided into the following areas: CN, SOC, NLL, and IC. Afferents to neighbouring nuclei will be discussed in a separate section.

Cochlear Nucleus:

In the present study it was found that the contralateral CN projected to the DNLL. This finding is in accordance to previous reports in cat and a recent report in mustache bat (cat: Glendenning et al., 1981; Shneiderman et al., 1988; mustache bat: Yang et al., 1996). The projection from CN to DNLL in the bat was not reported by earlier authors (Covey and Casseday, 1994). Friauf and Ostwald (1988) stated that no collaterals were observed in DNLL of the rat following injection of anterograde tracer into the CN. Glendenning et al. (1981) specified that the contralateral VCN (the AVCN and PVCN) projected to the DNLL, which is in accordance with the present study. The direct projection from CN to DNLL may account for the monaural response patterns (17%) that are found in DNLL in addition to the more frequently observed binaural response patterns (Buckthought, 1993).

Retrograde labelling was concentrated in the dorsal (high frequency) parts of the VCN, and mainly cells with small round somata were labelled. Little labelling was found in the ventral (low frequency) tip of the VCN. The anterograde results support the finding that the contralateral AVCN projects to the DNLL and also suggest that the dorsal parts of VCN send heavier projections to the DNLL than ventral parts. Following injection of anterograde tracer into the dorsal (high frequency) part of AVCN ring-like labelling was

found in caudal DNLL that was most dense ventrally. Following Biotinylated Dextran injection into the low frequency, ventral tip of the AVCN only a few labelled terminals were found in the middle of contralateral DNLL.

The anterograde results in the present study suggest that in the rat the projection from AVCN is tonotopic with high frequency parts projecting to outer rings of DNLL and low frequency parts projecting to the middle of DNLL. This idea is supported by recent reports that DNLL projects in an onion-like tonotopic fashion to IC, with middle parts projecting to dorsal (low frequency) parts of IC and outer rings projecting to progressively higher frequency parts of IC (Merchan et al., 1994). These authors stated that the rings in DNLL were denser ventrally following an injection into a high frequency area. Hence it is consistent that the projection from the high frequency part of AVCN to DNLL was denser ventrally as well. The tonotopic labelling patterns observed in other nuclei following these injections support the notion that the projection of AVCN to DNLL could be tonotopic. Following an injection into a high frequency area of AVCN, labelling was found in the ventral high frequency part of contralateral IC and the medial high frequency limb of ipsilateral LSO. Following an injection into a low frequency area of AVCN labelling was found dorsolaterally in low frequency areas in contralateral IC, in the low frequency (lateral) limb of the ipsilateral LSO, and bilaterally in the MSO (a nucleus with a disproportionate representation of low frequencies (Irvine, 1986)).

A previous study in cat (Shneiderman et al., 1988) reported that the dorsomedial high frequency part of AVCN projected to contralateral ventral DNLL and the ventrolateral (low frequency) AVCN projected to contralateral dorsal DNLL (Shneiderman et al. 1988). In the report by Shneiderman et al. (1988), labelling in ventral DNLL following injections into high frequency areas coincides with the report of the present study that denser terminal labelling was found ventrally in contralateral DNLL following an injection into a high frequency. Yet following a low frequency injection labelling was found centrally in rat, not dorsally, as in the cat. The difference between the tonotopy in

the projection found in the present report in rat from that in cat will require further investigation. The difference may reflect a species variation or differences in interpretation between authors.

The projection from VCN to DNLL was not found by earlier authors in the rat (Friauf and Ostwald, 1988). These authors focused on the projection from VCN to VNLL. The DNLL is a smaller nucleus than the VNLL and receives a smaller projection from contralateral VCN. Since the projection from VCN to DNLL is tonotopically organized the whole nucleus will not be labelled following all VCN injections. Thus the lighter projection from VCN to DNLL might have been overlooked by Friauf and Ostwald (1988).

Preliminary results suggest that terminations in DNLL from dorsal high frequency AVCN form asymmetrical contacts with small dendrites and dendritic spines. This study was the first to investigate this projection at the ultrastructural level. More data will have to be obtained to see if there are symmetrical contacts, if there are contacts on cell bodies, and to determine the relative contribution of each type of contact. Previous studies of the bilateral projection from AVCN to IC in the cat at the ultrastructural level found asymmetric contacts on dendrites and cell bodies of IC neurons from contralateral AVCN (Oliver, 1987).

In the present study, in most cases, little retrograde labelling of the contralateral DCN was found, yet a few labelled cells were observed in dorsal parts in every case, and labelled cells were obvious in dorsal parts following lateral DNLL injections. A few labelled cells have been found in DCN in an early study in cat (Glendenning et al., 1981). Yet the DCN of the cat has been reported not to project to the DNLL by Shneiderman et al. (1988), who found no terminal labelling in DNLL following injection of anterograde tracer into DCN. They suggested that the results of Glendenning were due to uptake by fibers of passage. No injections of anterograde tracer were made into the rat's DCN in the present study, thus I cannot be sure of the direct projection from DCN to DNLL. On the

other hand I cannot rule out the possibility of a small projection that originates in dorsal DCN and terminates mainly laterally in DNLL. In support of the present result is the recent finding of sparse retrograde labelling in DCN following DNLL injections in mustache bat (Yang et al., 1996). These authors suggested that the discrepancy with reports in cat could simply represent a species-specific difference.

A very small ipsilateral projection from AVCN to DNLL was found in the present anterograde study in rat. Shneiderman et al. (1988) reported an ipsilateral projection to dorsal DNLL from the ventrolateral (low frequency) part of AVCN but not from the dorsal (high frequency) part of AVCN. A small bilateral projection was uncovered following injections into low and high frequency areas of AVCN in the present study.

SOC:

The finding that the **LSO** projects bilaterally to the DNLL of the rat is in accordance with the reports of many authors (cat: Glendenning and Masterton, 1983; Glendenning et al, 1981; Shneiderman et al, 1988; bat: Vater et al., 1995; Covey and Casseday, 1994; Yang et al., 1996). There also appears to be a nucleotopic organization of the projection from the LSO to the DNLL of the rat. Lateral DNLL injections did not label contralateral LSO yet labelled ipsilateral LSO. Middle DNLL injections labelled LSO bilaterally. Labelling was concentrated in the medial limb of the contralateral LSO while labelling in the ipsilateral LSO was evenly distributed. The results suggest that the ipsilateral LSO projects more laterally within DNLL while the contralateral LSO projects more medially. This report is in accordance with previous reports in the cat. Shneiderman et al (1988) reported that in ventral DNLL the projection from the LSO showed a mediolateral gradient with heavier projections from the contralateral LSO to medial DNLL and heavier projections from the ipsilateral LSO to the lateral DNLL. This is similar to the report of Glendenning et al. (1981) that there was a bilateral projection from LSO to DNLL via medial lateral lemniscal fibers and an ipsilateral projection via lateral lateral

lemniscal fibers. Glendenning and Masterton (1983) reported that they had no evidence to conclude that the bilateral projection from LSO to DNLL was symmetrical in density or locus of termination within the DNLL. The report of Glendenning and Masterton (1983) is in accord with the present study that suggests that the projection from contralateral LSO comes mainly from the medial limb while the entire ipsilateral LSO contributes projections. Consideration of these results helps to explain why mid DNLL injections labelled contralateral LSO while lateral injections did not (there is less chance of labelling contralateral LSO with lateral DNLL injections because there is a smaller projection from contralateral LSO to the lateral part of the DNLL).

The anterograde results support the existence of the projection from LSO to DNLL. Following an injection of anterograde tracer into the dorsal-most (top-most) part of the low frequency, lateral limb of the LSO, ring-like terminal labelling was found in ipsilateral DNLL. The ring-like pattern of labelling that was found in ipsilateral DNLL suggests that there may also be an onion-like tonotopy in the projection of LSO to DNLL in the rat (as has been reported for the DNLL to IC projection in rat (Merchan et al., 1994)), with lower frequencies projecting centrally, and progressively higher frequencies projecting to outer rings. The low frequency lateral limb LSO injection did not label the middle of ipsilateral DNLL, instead it labelled an intermediate ring. The dorsal-most (top-most) of the lateral limb was injected. This is not the lowest frequency injection site possible in this limb, the ventral-most tip of this limb is the lowest frequency area (as characterized by the tonotopic projection of VCN neurons to LSO in rat by Friauf and Ostwald, 1988). An injection into the ventralmost tip of the lateral limb would probably result in middle DNLL labelling (this will have to be confirmed in further studies). The pattern of tonotopy in the LSO to DNLL projection observed herein is not in accordance with reports in cat by Shneiderman et al. (1988). Shneiderman et al., 1988 reported that the low frequency, lateral limb of LSO projected to dorsal DNLL while the high frequency medial limb projected to ventral DNLL. Yet in their paper on the onion-like

tonotopy in the DNLL to IC projection in rat, Merchan et al (1994) stated that they had preliminary evidence that the LSO projection forms annular terminal fields in DNLL on both sides.

The finding that labelled terminals were found in ipsilateral DNLL following lateral limb anterograde tracer injection is in accordance with the retrograde results presented here. Injections of FG laterally in DNLL labelled only the ipsilateral LSO. Injections of FG into middle parts of DNLL labelled the LSO bilaterally (with labelling of the medial limb on the contralateral side). These results suggest that the ipsilateral LSO projects laterally to DNLL, while the contralateral LSO projects more medially to DNLL which is in accord with other authors as outlined above (Glendenning et al., 1981 and Shneiderman et al., 1988). Thus the data suggest that the medial limb of LSO is the bilaterally projecting limb. While the anterograde results support the existence of a projection from LSO to DNLL, the bilateral topography is not apparent because no anterograde cases with medial limb injections were acquired.

Following this injection into the lateral limb of the LSO, the ipsilateral IC showed sparse labelling while denser labelling was found concentrated laterally in contralateral IC. This result is in accordance with reports in the ferret (Henkel and Brunso-Bechtold, 1993) and in contradiction to reports in cat (Shneiderman, and Henkel, 1987). In the ferret it was found that the lateral limb of LSO sent greater sources of projections to contralateral IC while the medial limb sent greater sources of projections to ipsilateral IC (Henkel and Brunso-Bechtold, 1993). A tonotopicity of the projection was found as well, with dorsolateral IC injections labelling cells in the lateral limb and ventromedial injections labelling cells in the medial limb (Henkel and Brunso-Bechtold, 1993). In light of these results it makes sense that denser terminal labelling would be found in contralateral IC than in ipsilateral IC following lateral limb injections (there are denser projections from this limb to contralateral IC). The pattern of tonotopy found in this study also corresponds with these authors (lateral limb, low frequency area injections label

lateral lower frequency areas in IC) (Henkel and Brunso-Bechtold, 1993). The findings in the ferret differ from those in cats. Glendenning and Masterton (1983) reported that in the cat there were more contralaterally projecting cells than ipsilaterally projecting cells in the medial limb of LSO, and more ipsilateral projecting cells than contralaterally projecting cells in the lateral limb. Further studies will be required to resolve these discrepancies.

The finding that the ipsilateral **MSO** of the rat projects to the DNLL is in accordance with previous reports in the cat (Glendenning et al, 1981; Shneiderman et al., 1988; Henkel, 1989 and 1997; Vater et al, 1995). The DNLL of the cat has been reported to receive substantial projections from the MSO. The MSO did not appear to be as substantial a source of afferents to the DNLL in the rat. The MSO of the rat is much smaller than the MSO of the cat. The difference in results probably reflects a species difference. There also appears to be a nucleotopic organization of the MSO projection to the DNLL of the rat. MSO labelling was observed following middle DNLL injections but not following a very lateral DNLL injection. The results suggest that MSO projects more medially within the DNLL. This suggestion is in accordance with reports from previous authors in the cat. Shneiderman et al. (1988) reported that there was virtually no projection of the ipsilateral MSO to the lateral DNLL in the cat. Henkel (1997) reported that MSO sends axons to the IC that pass along the medial side of the DNLL, where they give rise to right-angle collaterals before entering the IC. The retrograde findings reported here remain to be confirmed with anterograde methods, as no injections were made that were confined to MSO.

The finding that the **MNTB** of the rat does not project to the DNLL is in accordance with the results of Shneiderman et al. (1988) for the cat. Only cases in which there was spread of label to INLL, resulted in labelling of ipsilateral MNTB. Glendenning et al. (1981) also had reported that there were a few labelled cells in MNTB following DNLL injections. The results of the present study suggest that labelling found in MNTB

following DNLL injections by Glendenning et al. (1981) may have been due to spread of the injection site into INLL. The anterograde results presented here support the finding that there is no projection from MNTB to DNLL. Following injection of anterograde tracer into MNTB, no labelled terminals were found in DNLL while labelling was found in other areas (ipsilateral INLL, VNLL, LSO and SPO) which is in accord with the reports of other authors (Glendenning et al., 1981; Spangler et al., 1985; Irvine, 1986; Banks and Smith, 1992; Kuwabara and Zook, 1992 a and b; Sommer et al., 1993; Schofield, 1994 and 1995).

The finding in the present study that the ipsilateral **SPO** of the rat projects to the DNLL is in accord with a recent abstract employing anterograde methods (Colombo et al., 1996). The retrograde data in the present study suggests that there is a nucleotopic organization in the projection of SPO to DNLL. Retrograde labelling was found medially and dorsally in SPO. Lateral DNLL injections resulted in the strongest labelling of the SPO. More medially positioned injections resulted in less labelling of the SPO and in one case no labelling of SPO was found following a middle injection. The retrograde data suggests that medial parts of the SPO project laterally within DNLL. The anterograde data supports the idea that there is a projection from SPO to DNLL. Following injection of anterograde tracer into the lateral part of SPO, a few terminals were observed in DNLL that were positioned laterally within the nucleus. Anterograde cases with medial SPO injections will be required to confirm the topography suggested by the retrograde data.

The finding that the ipsilateral **LTB** and **VTB** send minor projections to the DNLL is in accordance with the results of Glendenning et al. (1981). That the VTB sends a small projection to DNLL is further supported with the present anterograde findings. Following injection of anterograde tracer into VTB labelled fibers mainly passed through DNLL and terminated ventrally in ipsilateral IC (as has been previously described: Adams, 1979 and 1983; Brunso-Bechtold et al., 1981; Spangler et al., 1985). Yet a few fibers gave off collaterals that terminated in DNLL.

Nuclei of the Lateral Lemniscus:

The finding that the ipsilateral **VNLL** sends projections to the **DNLL** is in accordance with results from the cat (Glendenning et al., 1981) and the bat (Yang et al., 1996). The finding that the ipsilateral **INLL** projects to the **DNLL** is in accordance with reports from the bat (Yang et al., 1996). Yang et al. (1996) found that different divisions of the **DNLL** received differential afferent projections (**VNLL** projected to the anterior part while the **MSO** projected to the posterior part of the bat **DNLL**). The results of Yang et al. (1996) also suggest that there is a nucleotopic organization of afferent projections to the **DNLL**. Thus the results of Yang et al. (1996) correspond with the suggestion in this study that there is a nucleotopic organization of **SOC** afferents to the **DNLL** of the rat.

The finding that the contralateral **DNLL** sends a large projection to its counterpart on the opposite side is in accordance with a wealth of previous reports (see Shneiderman et al., 1988 for a list of references). Shneiderman et al. (1988) reported that injection of anterograde tracer into the **DNLL** resulted in symmetrical labelling in the contralateral **DNLL** (i.e., dorsal injections label dorsal parts and ventral injections label ventral parts of contralateral **DNLL**). Following injection of retrograde tracer into the **DNLL**, regardless of the position of the injection site, (with reference to medial to lateral orientation) labelled cells in contralateral **DNLL** were arranged rostrally as a lateral group of cells with horizontal orientation, in a ringlike manner in middle **DNLL** (where cells are tightly arranged in columns between fibers of the **LL**) and centrally in caudal **DNLL**. The symmetry in **DNLL** to **DNLL** projections as reported by Shneiderman et al., (1988) was more evident upon examination of terminal labelling following injection of the anterograde tracer Biotinylated Dextran into the **DNLL**. Cells with different soma shapes were observed in contralateral **DNLL** following filling with Fluoro-Gold. Cells with elongate somata that extended across the **DNLL** were obvious, especially in rostral **DNLL**. These cells correspond to Bajo et al.'s (1993) large bipolar cells and Wu and

Kelly's (1995) various elongate cells. Large multipolar cells were obvious in caudal DNLL. These cells correspond to Bajo et al.'s (1993) and Wu and Kelly's (1995) large multipolar cells. Bajo et al. (1993) reported that these cells were mostly found in dorsal and caudal DNLL. Elongate cells were seen in all parts of contralateral DNLL. Cells with round somata were observed in all parts of the DNLL. These cells correspond to Bajo et al.'s (1993) medium round cells and Wu and Kelly's (1995) round cells. Cells with differently shaped somata also could be distinguished at the electron microscope level.

Inferior Colliculus:

The retrograde and anterograde results reported here suggest that there is a weak descending projection from ICC to DNLL in rat that mainly terminates laterally in DNLL. Labelling of the ipsilateral ICC was observed following injections of FG into the lateral DNLL, that spread to nucleus sagulum. Following more centrally placed DNLL injections little ICC labelling was observed. Injection of anterograde tracer into ventral ICC led to terminal labelling laterally in DNLL and surrounding nucleus sagulum. Thus the anterograde result confirmed the retrograde result (ICC labelling was found with injections of FG into lateral DNLL because the ICC projects laterally to DNLL and sagulum). Caicedo and Herbert 1993 reported that the IC sends a descending projection to DNLL and neighbouring sagulum. They specified that the EC and DC of IC project to sagulum while the ICC projects to DNLL. They specified that there was a topography in the projection of ICC to DNLL. Dorsal ICC injections labelled two horizontal bands in the middle of the DNLL while ventral ICC injections led to labelling of dorsal and ventral boundaries of the DNLL. Such banding in the descending projection from IC to DNLL was not observed in this study. Only ventral ICC injections were made in this study. Following ventral injections, labelled fibers from IC passed through the DNLL medially while mainly labelled fibers, and some terminals, were found laterally in DNLL and in surrounding nucleus sagulum. Merchan et al. (1994) stated that they had

preliminary evidence to conclude that there was no significant projection from ipsilateral IC to DNLL, while there was a dense projection to nucleus sagulum, which is similar to the anterograde findings herein. A recent study in the mustache bat found that few labelled cells were found in ventral parts of ICC following injections of retrograde tracer into DNLL (Yang et al., 1996). These authors stated that the descending projection from ICC to the DNLL in mustache bat is weaker than that reported previously for the rat (Caicedo and Herbert, 1993). The discrepancies in these reports remain to be resolved.

AFFERENTS TO NEIGHBOURING NUCLEI

The projections to nuclei neighbouring DNLL (INLL, VNLL, sagulum and subcollicular area), that were revealed in retrograde and anterograde studies, will be contrasted with the pattern found for DNLL afferents. The extreme rostral DNLL, which was considered separately from the main division of DNLL, is also discussed.

INLL:

The most obvious difference between the connectivity of the INLL and DNLL is that the DNLL receives input from contralateral DNLL while INLL does not. The INLL and DNLL also receive differential projections from nuclei of the SOC. The main difference between the afferents from SOC to the DNLL and the INLL is that the INLL receives a substantial projection from ipsilateral MNTB while DNLL does not. Following injections of FG into INLL, (but not DNLL), labelling is found in MNTB and following anterograde tracer injections into MNTB, labelled terminals are found in INLL (and not DNLL). These results are in accord with the results of others (Glendenning et al., 1981; Shneiderman et al., 1988 and Spangler et al., 1985).

DNLL receives more substantial projections from bilateral LSO and ipsilateral MSO while INLL receives minor projections from these nuclei. Following FG injections into INLL, sparse labelling was found only in ipsilateral LSO in addition to MNTB. These results are in accordance with reports from cat (Glendenning et al., 1981). The

anterograde findings suggest that the INLL of the rat receives a projection from ipsilateral SPO, which is in accordance with a recent abstract employing anterograde methods (Colombo et al., 1996). The retrograde injection did not reveal the projection from ipsilateral SPO. Further cases will be required to resolve this discrepancy.

The present retrograde and anterograde data show that there is a projection from contralateral VCN to INLL which is in accordance with reports from other authors (cat: Glendenning et al., 1981; bat: Zook and Casseday, 1982 and 1985; Covey and Casseday, 1986). Following injection of anterograde tracer into high frequency areas of AVCN, labelling in contralateral INLL was located ventromedially, and following low frequency area injections labelling was found laterally in INLL. The precise tonotopy of INLL has yet to be resolved. Physiological studies suggest the the frequency progression in INLL may not lie in the dorsal to ventral direction (Covey and Casseday, 1991). This direct projection from contralateral VCN to INLL may account for the high proportion (63%) of monaural sensitivity found in this nucleus (Buckthought, 1993).

Following injections into low and high frequency areas a smaller ipsilateral than contralateral projection from AVCN to INLL was found (denser than to ipsilateral DNLL). The retrograde injection did not reveal the ipsilateral projection. Further cases will be required to resolve this discrepancy.

VNLL:

The VNLL receives a greater projection than DNLL from contralateral AVCN. The dense projection from AVCN to contralateral VNLL is in accordance with previous reports (Glendenning et al., 1981; Friauf and Ostwald, 1988; Covey and Casseday, 1994). Most VNLL units are monaural and respond only to contralateral stimulation (Aitkin et al., 1970; Buckthought, 1993). This dense direct projection from CN may explain the predominance of monaural responses seen in this nucleus. The pattern of labelling found in contralateral VNLL following an injection into a high frequency area of AVCN , was

also interesting as it suggests a possible tonotopy in the CN to VNLL connection. A vertical column of horizontally oriented patches of terminal labelling was found in VNLL. Less labelling was observed in VNLL following injection into low frequency areas of AVCN than high frequency areas of AVCN. Yet still the labelling took the form of horizontally oriented patches. These observations are interesting in light of a recent report (Merchan and Berbel, 1996) showing that the VNLL has a concentric laminar organization and that the projection from it to the IC is topographically organized with peripheral laminae projecting to low frequency parts of IC and central laminae projecting to ventral high frequency parts of IC. A spiral organization of VNLL was first proposed by Willard and Martin (1983) in opossum following IC injections. Merchan and Berbel (1996) write that other observations such as reports of horizontal banding (Roth, 1978), cross bridges or ladder rung patterns (Whitley and Henkel, 1984; Glendenning et al., 1981 and Spangler et al., 1985) support the notion of a helicoidal organization in VNLL. The horizontal banding seen in this study may also support this idea. The horizontal banding seen in this study is also consistent with previous reports in rat (Friauf and Ostwald, 1988) that showed that axons along their course send horizontal collaterals to VNLL that are repetitively arranged. Following pure tone stimulation, c-fos immunoreactive horizontal bands have also been observed in VNLL (Friauf, 1992).

A weaker projection to ipsilateral VNLL was also revealed with anterograde tracing. A bilateral projection from VCN to VNLL, with contralateral inputs outnumbering ipsilateral inputs, has recently been reported in guinea pig by Schofield and Cant (1997). They reported that multipolar and spherical bushy cells in VCN send thin fibers bilaterally to VNLL (as opposed to octopus cells which send fewer numbers of thicker axons to contralateral, not ipsilateral VNLL). A small dorsomedial region of VNLL was also reported to receive an ipsilateral projection from VCN in cat (Glendenning et al., 1981). This small ipsilateral VCN projection to VNLL may help explain the small proportion of binaural responses found in VNLL (Buckthought, 1993).

Following injection of anterograde tracer into MNTB, sparse terminal labelling was found in ipsilateral VNLL but no labelling was found in DNLL. This is in accordance with reports by Spangler et al. (1985) that terminal labelling was found in VNLL following MNTB injections. Following injection of anterograde tracer into SPO, denser terminal labelling was found in VNLL than DNLL, which is in accordance with a previous abstract in rat (Colombo et al., 1996).

Sagulum:

Sagulum can be distinguished from DNLL in that it does not share the same sources of ascending inputs from SOC and CN. Sagulum receives input from the contralateral sagulum and external and dorsal cortices of IC (Hutson et al, 1991; Henkel and Shneiderman, 1988; Caicedo and Herbert, 1993). In this study it was also observed that the ventral ICC sends input to sagulum which is in accordance with reports by Merchan et al. (1994). Physiological studies (Buckthrought, 1993) have found that sagulum showed binaural response properties with relatively long latencies to first spike (longer than expected for an ascending system). The author suggested that the long latencies found in sagulum are compatible with findings that it lacks ascending input yet receives descending input from other sources (DC and EC of IC) (Buckthrought, 1993).

Subcollicular tegmentum:

Following FG injection into subcollicular tegmentum (just dorsal to DNLL and ventral to IC), labelling in the same nuclei sending ascending projections to the DNLL was observed. The labelling is also similar to retrograde labelling following IC injections, but with an exception. Injections into ICC label DNLL bilaterally whereas subcollicular injections result in no label in ipsilateral DNLL. Only contralateral DNLL labelling was found. Hence there is some specificity in this labelling pattern. A physiological study employing extracellular recording (Buckthrought, 1993) has found binaural response properties in both subcollicular tegmentum and DNLL. Hence it is reasonable that this area receives the same set of ascending afferents as the DNLL.

Rostral DNLL:

In the one case in which an injection was located in the extreme rostral DNLL, labelling was seen in the contralateral DNLL but none appeared in the SOC and the CN. If confirmed, this pattern of labelling may distinguish the rostral DNLL from its more central core. Buckthought (1993) found a predominance of monaural responses in rostral DNLL in a small sample of neurons. Buckthought (1993) found that the core region of DNLL showed a predominance of binaural responses. Differences in ascending inputs may underlie the different response patterns found in core and rostral parts of DNLL.

DNLL EFFERENTS-ANTEROGRADE TRACT-TRACING AND ELECTRON MICROSCOPY

The observation that the DNLL sends efferents mainly to the contralateral DNLL and the IC bilaterally has been extensively documented (see Shneiderman et al., 1988 and Bajo et al., 1993 for a complete list of references). The finding that the projection from DNLL to IC is tonotopically organized and denser contralaterally has also been extensively documented (see Shneiderman et al., 1988). The finding that there is a symmetry in the DNLL to DNLL connection has also been reported (Shneiderman et. al., 1988 and Bajo et al., 1993).

The observation that DNLL sends a less dense descending efferent projection to ipsilateral SOC (mainly the MNTB and SPO) is also in accordance with the literature. Kudo et al. (1981) reported a projection from DNLL to nuclei of the trapezoid body and marginal regions of the SOC in the cat. Bajo et al. (1993) reported a projection to

ipsilateral periolivary regions (SPO and the lateral part of the rostral periolivary region), MNTB and ventral, medial and dorsal periolivary regions. The finding that the DNLL sends weaker efferent projections to deep and intermediate layers of the superior colliculus bilaterally is in accordance with the literature for the rat (Bajo et al., 1993; Druga and Syka, 1984; Tanaka et al., 1985) and is in agreement with some studies in the cat (Kudo, 1981). However these observations have been challenged by Hutson et al. (1991) who reported that there was no projection from the DNLL beyond the IC to SC in the cat and that this projection is from its immediate neighbours, the INLL and nucleus sagulum.

In the present study it cannot be stated unequivocally that all of the terminations observed in SC following DNLL injections arise solely from DNLL neurons. They may also arise from its close neighbour, nucleus sagulum, since injections confined to nucleus sagulum alone resulted in terminal labelling in SC and most DNLL injections spread to nucleus sagulum. However DNLL injections that were small and confined to DNLL in rostral parts showed sparse bilateral labelling in SC (and sparse labelling of ICC). Very small injections confined to DNLL in caudal parts did not label SC but labelled ICC bilaterally. The results lend support to reports by Tanaka et al. (1985) that rostral parts of DNLL project to SC while caudal parts of DNLL project to IC in rat. Hence the discrepancy between different authors may be due to differences in efferent projections from rostral and caudal DNLL. If different authors inject into different regions of DNLL they will observe different results. The discrepancy between different authors may also be due to differences in identifying the sites of injection that produce the given results (the distinction between DNLL and its surrounding nuclei, sagulum and INLL). If authors do not document spread of injection to neighbouring nuclei or confinement of injection to DNLL, the specificity of the projection cannot be determined. It is also possible that a species difference exists between the rat and the cat.

The observation that the DNLL of the rat makes mainly symmetrical contacts with dendrites in contralateral DNLL is in accordance with the literature for the cat (Oliver and Shneiderman, 1989). Following injection of tritiated leucine into the DNLL, Oliver and Shneiderman (1989) observed mainly labelled terminals which contained pleomorphic vesicles and made symmetrical contacts with dendrites. In addition they observed a small but significant proportion of labelled terminals that made contacts with cell bodies. They took these data as evidence that the DNLL has an inhibitory influence on the contralateral DNLL (Oliver and Shneiderman, 1989). The observation that the DNLL contains some labelled terminals that form asymmetric contacts following DNLL injection is also in accordance with the literature. Oliver et al. (1987) reported that there were a few endings with round synaptic vesicles in one of their samples.

The observation that the bilateral IC in rat receives labelled terminals that make mainly symmetrical contacts with dendrites is in accord with reports in the cat (Shneiderman and Oliver, 1989). Following injection of tritiated leucine into the DNLL they observed mainly labelled terminals with pleomorphic vesicles that made symmetrical contacts with dendrites in bilateral IC (Shneiderman and Oliver, 1989). In the ipsilateral IC, they also observed a significant proportion of labelled endings (30%) that contained round vesicles and made asymmetric contacts with dendrites. Overall they took this data as evidence to indicate that the DNLL may have an inhibitory influence on the IC (Shneiderman and Oliver, 1989). In the present study although unlabelled terminals forming asymmetrical contacts were observed, labelled endings with large round vesicles forming clear asymmetric contacts were not documented. In ipsilateral IC some very large labelled endings were observed at the EM level (which is in accord to observations at the light level (Bajo et al., 1993). The projection from ipsilateral DNLL to IC appears quite complex and will require further examination.

This study is the first to describe the projection from DNLL to SC at the ultrastructural level. The observation that bilateral deep and intermediate layers of the SC contain

labelled terminals that make symmetrical contacts with dendrites is reasonable in light of the fact that the DNLL has been shown previously in the cat to provide an input whose morphology is associated with inhibition (symmetrical contacts and pleomorphic vesicles) to the IC bilaterally and to DNLL contralaterally. The possibility that some of these labelled endings in SC reflect input from sources other than DNLL (i.e. sagulum) must be kept in mind.

This study is the first to describe the descending projection from DNLL to periolivary nuclei of the SOC at the ultrastructural level. Labelled terminals arising from DNLL make symmetric and asymmetric contacts with dendrites and cell bodies in periolivary nuclei of the SOC. At the light level Bajo (1993) observed that terminals in MNTB from DNLL formed single boutons terminaux in close apposition to the somata of MNTB neurons, in accord with the results of the present study.

DNLL ULTRASTRUCTURE

DNLL neurons with different somatic shapes (round, elongate and triangular) were observed at the ultrastructural level. DNLL neurons have also been distinguished at the light level on the basis of somata shape following Nissl stain (Bajo et al., 1993). In the sample in the present study it was found that regardless of soma shape or nuclear morphology most neurons (97.0 %, N=38) possessed rough endoplasmic reticulum that was dispersed throughout the cytoplasm.

Oliver and Shneiderman (1989) suggested that there may be two main cell types in the DNLL of the cat. One type was identified that had fusiform somata, irregularly shaped nuclei and perinuclear rough endoplasmic reticulum. The other type had a rounded nucleus and lacked the perinuclear reticulum. In the present study most neurons, regardless of somata shape or nuclear morphology, possessed rough endoplasmic reticulum that was dispersed throughout the cytoplasm. Thus it was found that the

morphological classification proposed by Oliver and Shneiderman (1989) for DNLL neurons in the cat could not be applied to DNLL neurons in the rat.

Oliver and Shneiderman (1989) stated that nuclear morphology and the distribution of rough endoplasmic reticulum can be used to distinguish different types of cells. These features have been used to distinguish cell types in the cochlear nucleus and the inferior colliculus (Cant and Morest, 1979; Cant, 1981; Oliver, 1984). Oliver and Shneiderman (1989) suggested that cell types in DNLL may be related to their connections. Cells in DNLL project to either the ipsilateral or contralateral IC (few cells project bilaterally) (Tanaka et al., 1985). Some fibers from DNLL terminate directly in contralateral IC while others send collaterals to contralateral DNLL before entering ICC (Bajo et al., 1993). Oliver and Shneiderman (1989) suggest that functionally distinct cell types may have different transmitters and exert different effects on different areas. Anatomical and physiological studies may one day determine if DNLL neurons can be classified into functionally distinct cell types.

Summary and Future Research

The present study has found that the DNLL of the rat receives afferent projections from the CN, SOC and NLL. The projections from these nuclei to the DNLL appear to have a nucleotopic and tonotopic organization that merits further investigation with a combined physiological and anterograde tract -tracing approach.

The present study has found that the DNLL of the rat sends major efferent projections to bilateral IC and contralateral DNLL and smaller projections to SC and SOC, as has been described previously at the light level in cat and rat. Observations at the electron microscopic level have revealed similar findings as those in cat, in that DNLL mainly makes symmetrical contacts with the dendrites of target nuclei in bilateral IC and contralateral DNLL (and in addition in this study with bilateral SC). The descending projection from DNLL to SOC forms symmetric and asymmetric contacts with dendrites

and cell bodies. Future studies employing a combined immunohistochemical and ultrastructural approach may delineate the transmitters subserving these connections.

Investigation of DNLL neurons in the rat at the ultrastructural level suggests that neurons in the DNLL of the rat cannot be classified with the same morphological scheme as proposed for the cat. Future studies employing combined pharmacological, anatomical and physiological methods may allow for classification of DNLL neurons into functionally distinct groups.

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FIGURES

Figures 4 - 28. A series of photomicrographs and schematic drawings of coronal sections through the brain of a rat to show the pattern of cell body labelling in auditory nuclei following injection of the fluorescent retrograde tract tracer Fluoro-Gold into the right dorsal nucleus of the lateral lemniscus (DNLL) or neighbouring nuclei.

Figures 29-52. A series of photomicrographs of coronal sections through the brain of a rat to show the pattern of terminal labelling in auditory nuclei following injection of the anterograde tract tracer Biotinylated Dextran into auditory nuclei seen to project to the DNLL following retrograde studies (the CN, the SOC, and the IC) .

Figures 53-94. A set of light and electron micrographs of terminal labelling following injection of Biotinylated Dextran into the right DNLL of the rat. Light micrographs show the pattern of terminal labelling in bilateral IC, contralateral DNLL, bilateral SC and ipsilateral SOC. Electron micrographs show the site of contact and the nature of the contact formed by labelled terminals in target nuclei.

Figures 95-104. A series of electron micrographs of nervous tissue from the dorsal nucleus of the lateral lemniscus (DNLL) of the rat.

Figure 4. A schematic representation of the location of Fluoro-Gold (FG) injection sites within the DNLL. Most DNLL injections were in middle parts of DNLL with reference to rostral to caudal position. Injections located in DNLL varied with respect to their medial to lateral position. Some injections were located centrally (star) (cases RL 17, 37 and 75) others were located intermediately (filled circle) (cases RL 19 and 20) while still others were positioned more laterally and spread into nucleus sagulum (open circle) (cases RL16 and 83).

DNLL INJECTION SITE COMPOSITE

LEGEND

- ★ = CENTRAL DNLL INJECTION SITE
- = INTERMEDIATE DNLL INJECTION SITE
- = LATERAL INJECTION SITE

Figure 5. Photomicrographs of labelling within the contralateral DNLL from rostral (5A) to caudal (5C) following an injection of FG into the center of the DNLL (Figure 5D). Also shown is light labelling of the ipsilateral INLL and more pronounced labelling of the VNLL (Figure 5D). Scale bars = 100 μm .

A

B

C

DNLL

INLL

VNLL

D

Figure 6. Photomicrographs of Fluoro-Gold labelled cells in contralateral DNLL following injection of FG into the center of DNLL (as shown in Figure 5D). **Figure A** shows an example of a large multipolar shaped cell which was observed in the caudal part of DNLL. Scale bar = 10 μ m. **Figure B** shows an elongate shaped cell. These cells were seen throughout the DNLL. In some instances in rostral to mid DNLL, their processes were seen to cross the DNLL from medial to lateral. Scale bar = 10 μ m.

Figure 7. Photomicrographs of bilateral labelling of the SOC following injection of FG into the center of the DNLL (as shown in Figure 5D). The contralateral LSO is labelled (mainly the medial limb) (Figure 7A). Labelling of the ipsilateral LSO, MSO and the medial part of the SPO is shown in Figure 7B. Scale bars = 100 μ m.

Figure 8. Photomicrographs of labelling of the contralateral CN following injection of FG into the center of the DNLL (as shown in Figure 5). No labelling of ipsilateral CN was observed. The AVCN (Figure 8A) and the PVCN (Figure 8B) were labelled in dorsal parts. Little labelling in the ventral tip was found in both divisions. The DCN showed very sparse labelling in dorsal parts (Figure 8C). The arrows indicate the areas shown at higher power in the insets in each figure. Scale bars = 100 μm for the main figures; scale bars for the insets = 10 μm .

Figure 9. Photomicrographs of labelling of the contralateral DNLL from rostral (Figure 9A) to caudal (Figure 9C) following an intermediately placed (with reference to medial to lateral) DNLL FG injection (Figure 9D). Also shown is sparse labelling of the ipsilateral INLL and VNLL (Figure 9D). Scale bars = 100 μ m.

A

B

C

DNLL

INLL

VNLL

D

Figure 10. Photomicrographs of bilateral labelling of SOC following an intermediately placed DNLL FG injection (as shown in Figure 9). The contralateral LSO is labelled (mainly the medial limb) (Figure 10A). Labelling of the ipsilateral LSO, MSO and medial part of the ipsilateral SPO is shown in Figure 10B. Scale bars = 100 μ m.

Figure 11. Photomicrographs of labelling of the contralateral CN following an intermediate DNLL FG injection (as shown in Figure 9). No labelling of the ipsilateral CN was observed. The AVCN (Figure 11A) and the PVCN (Figure 11B) showed strong labelling dorsally while the DCN (Figure 11C) showed sparse labelling in dorsal parts. Little labelling of the ventral tip was found in all divisions. The arrows indicate the areas of each nucleus that are shown in the insets for each figure. Scale bars = 100 μm for the main figures; scale bars for the insets = 10 μm .

Figure 12. Photomicrographs of labelling in the contralateral DNLL from rostral (Figure 12A) to caudal (Figure 12C) following a lateral DNLL injection which spread into nucleus sagulum (Figure 12D). Also shown is weak labelling of the ipsilateral INLL and VNLL (Figure 12D). Scale bars = 100 μm .

A

B

C

DNLL

INLL

VNLL

D

Figure 13. Photomicrographs of Fluoro-Gold labelled neurons in contralateral DNLL following a laterally placed injection of FG into the DNLL (as shown in Figure 12). Figure A shows a multipolar shaped cell which was observed in caudal DNLL (also shown at lower power in Figure 12C). Scale bar = 10 μm . Figure B shows an elongate shaped cell. These cells were observed throughout the DNLL. In some instances in rostral to mid DNLL, their processes crossed the DNLL from medial to lateral. Scale bar= 10 μm .

Figure 14. Photomicrographs of the SOC following an FG injection situated laterally within the DNLL (as shown in Figure 12). The contralateral LSO is not labelled (Figure 14A). Labelling was found in the ipsilateral LSO and the medial part of the SPO (Figure 14B). Note the absence of MSO labelling. Scale bars = 100 μ m.

LSO

— A

SPO

LSO

↖
MSO

— B

Figure 15. Photomicrographs of labelling in the ipsilateral SPO following a lateral DNLL injection (as shown in Figure 12). Strong labelling in the medial part of the SPO can be seen in Figure 15A. Figure 15B is a higher power photomicrograph of the area shown in Figure 15A. Scale bar= 100 μ m in Figure 15A. Scale bar = 20 μ m in Figure 15B.

Figure 16. **A.** A photomicrograph of labelling in ipsilateral IC following an injection of Fluoro-Gold laterally into the DNLL that spread to nucleus sagulum (as shown in Figure 12). There are many labelled cells in the ICC ipsilaterally. The white arrow indicates the area that is shown in close-up Figure 16B. Contrast this pattern of labelling to that seen in Fig 16C following an injection placed in mid DNLL. In this case there is little labelling of the ICC. Scale bar = 100 μm . **B.** A close-up of the area indicated by the white arrow in Figure 16A. Scale bar = 20 μm . **C.** A photomicrograph of labelling in ipsilateral ICC following an injection of Fluoro-Gold in mid DNLL (as shown in Figure 5). In this case there is little labelling of ICC. There is some labelling of the EC (white arrow indicates a cell shown in higher magnification in Figure 16D) and the area just ventral to IC (the subcollicular area). Scale bar = 500 μm . **D.** A close-up of a cell indicated in Figure 16C in EC by the white arrow. Scale bar = 20 μm .

Figure 17. **A** A photomicrograph of a small injection of Fluoro-Gold in mid DNLL (case RL75). Scale bar= 100 μ m. **B.** A photomicrograph of labelling in mid contralateral DNLL following injection into DNLL as shown in Figure 17A. Note the ring-like pattern of labelling. Scale bar= 100 μ m.

Figure 18. **A** A photomicrograph of labelling in ipsilateral SOC following injection of FG into mid DNLL as shown in Figure 17A. Labelling is diffuse throughout all limbs of the LSO. Some labelling is seen laterally in MSO. No labelling is found in the SPO in this case. Scale bar=100 μ m. **B** Labelling of the contralateral SOC. Scale bar= 100 μ m.

Figure 19. **A** A photomicrograph of labelling in contralateral DNLL following a large laterally placed DNLL injection that spread to INLL (Figure 19B). Labelled cells are arranged in vertical columns between fibers of the lateral lemniscus. The white arrows indicate the cells seen with higher magnification in Figures 19C and D. Scale bar=100 μm . **B** A photomicrograph of a large injection site in DNLL that is laterally placed and spread to the INLL (case RL83). Scale bar= 200 μm . **C** To the right is a magnified view of a Fluoro-Gold filled neuron seen in contralateral DNLL (arrow to the right in A) following the injection seen in Figure 19B. This elongate neuron, which was oriented medio- laterally, could be seen to contact a cell in a neighbouring column of labelled cells. Scale bar = 10 μm . **D** To the left is a close up of a Fluoro-Gold filled indicated in 19A (arrow on the left). This neuron had a round soma. Neurons with round somas were seen throughout DNLL. Scale bar= 10 μm .

—A

—B

—C

—D

Figure 20. A Labelling in ipsilateral SOC following a large laterally concentrated FG injection that spread to INLL (as shown in Figure 19B). Labelling is distributed throughout the ipsilateral LSO. There is little labelling of the MSO. The LTB is labelled. The medial part of the SPO is labelled. The MNTB in this case is labelled which is in contrast to most cases in which injections were contained within DNLL and did not spread to INLL (these cases showed no MNTB labelling). Scale bar = 100 μ m. **B** Labelling of the contralateral SOC in this case. The medial limb of the LSO shows the most labelling while the rest of the LSO shows little labelling. Scale bar = 100 μ m.

MNTB

SPO

MSO

LSO

LTB

_A

LSO

_B

Figure 21. Labelling of the contralateral CN for the case shown in Figure 19 that had a large laterally concentrated DNLL injection that spread to INLL. The ipsilateral CN showed virtually no labelling. **A** The dorsal part of the contralateral AVCN showed dense labelling. Scale bar = 50 μm . **B**. The dorsal part of the contralateral PVCN was labelled. Scale bar = 100 μm . **C** The dorsal part of contralateral DCN was labelled in this case. In most DNLL injection cases the contralateral DCN showed few labelled cells (Figures 8C, 11C). The ventral tip of all divisions showed little labelling. Scale bar = 100 μm .

Figure 22. Photomicrographs of labelling patterns in SOC following injection of FG into the INLL. **A** This figure shows an injection site localized within the INLL at rostral levels. There is no labelling of ipsilateral DNLL or contralateral DNLL (not shown). There is sparse labelling of ipsilateral VNLL. Scale bar = 100 μ m. **B** This figure shows labelling of ipsilateral MNTB following an INLL injection (labelling was found mainly in rostral MNTB and was concentrated more laterally within MNTB). This is in contrast to DNLL injection cases that did not spread to INLL, in these cases the MNTB was not labelled. This case is similar to a DNLL injection case that spread to INLL in that it also showed ipsilateral MNTB labelling (Figures 19 and 20). Scale bar = 50 μ m. **C** This figure shows labelling of ipsilateral SOC following INLL injection. There is sparse labelling of ipsilateral LSO. No labelling of contralateral SOC was observed in this case (not shown). Scale bar = 100 μ m.

Figure 23. Photomicrographs of labelling in contralateral CN following an INLL injection as shown in Figure 22. No labelling of ipsilateral CN was observed. **A** This figure shows some labelling in the dorsal part of the contralateral AVCN. Scale bar = 100 μm . **B** This figure shows some labelling in the dorsal part of the contralateral PVCN. Scale bar = 50 μm . No labelling of the contralateral DCN was observed in this case (not shown).

Figure 24. This set of figures shows an injection of FG just dorsal to the DNLL and just ventral to the IC (**in subcollicular tegmentum**). **A** Labelling of contralateral DNLL following an injection in subcollicular tegmentum. The contralateral DNLL was labelled from rostral to caudal following this injection. Scale bar = 100 μm . **B** A photomicrograph of an injection site in subcollicular tegmentum. The ipsilateral DNLL shows little labelling. The ipsilateral INLL and VNLL were labelled (not shown). Scale bar = 200 μm .

Figure 25. This set of figures shows labelling of the SOC following the subcollicular injection shown in Figure 24. **A** Labelling in the ipsilateral SOC. Labelling is dispersed throughout the ipsilateral LSO. The ipsilateral MSO is heavily labelled. The SPO is labelled. Scale bar = 100 μm . **B** Labelling in the contralateral SOC. Labelling is dispersed throughout the contralateral LSO. Scale bar = 100 μm .

Figure 26. This set of figures shows labelling of the contralateral CN following the subcollicular injection shown in Figure 24. The ipsilateral CN was not labelled (not shown). **A** Labelling was found in the dorsal part of the contralateral DCN. Scale bar = 100 μm . **B** Labelling was found in the dorsal part and medial and lateral edges of the PVCN. Scale bar = 100 μm . **C** Labelling was found in the dorsal part of the contralateral AVCN. Little labelling of the ventral tip was found in all cases. Scale bar = 100 μm .

Figure 27. This set of figures shows an injection into the **nucleus sagulum** (case RL 14) which borders the DNLL laterally. **A** Labelling in contralateral sagulum following injection into nucleus sagulum. Very few labelled cells were seen in contralateral DNLL. Scale bar = 100 μm . **B** This figure shows the injection site of FG into the sagulum (at rostral levels) which surrounds the DNLL laterally. The injection did not spread to DNLL. Scale bar = 100 μm . Injection into nucleus sagulum did not result in labelling of ascending auditory nuclei (NLL, SOC and CN) (not shown) unlike DNLL injections that resulted in labelling of ascending auditory nuclei.

Figure 28. A schematic representation of the general pattern of labelling found in auditory nuclei following injections of Fluoro-Gold into the DNLL of the rat. Labelling was found in the contralateral DNLL and CN; bilaterally in the LSO; and ipsilaterally in the MSO, SPO. Labelling was also found in the IC following lateral injections (not shown). Labelling in ipsilateral VTB and bilateral LTB was found sporadically. Adapted from Paxinos and Watson, (1986).

**A SCHEMATIC REPRESENTATION TO SHOW A SUMMARY OF
RETROGRADE LABELLING IN ASCENDING AUDITORY NUCLEI
FOLLOWING INJECTION OF FLUORO-GOLD INTO THE DNLL**

Figure 29. Light micrographs of terminal labelling in contralateral DNLL following injection of Biotinylated Dextran into the dorsal (**high frequency**) part of the anterior ventral cochlear nucleus (AVCN). **A.** Shows a high frequency (22,000 Hz) injection site which is situated dorsally in AVCN. **B.** Shows terminal labelling in contralateral DNLL following this injection. The labelling is most obvious ventrally in DNLL (arrow) and can be seen at higher magnification in **C.** **C.** Shows a magnified view of labelled terminals which were indicated by the arrow in **B.** See also Figure 30 for contralateral DNLL labelling. Scale bars: A and B= 100 μm , C= 20 μm .

Figure 30. Light micrographs of terminal labelling of contralateral lateral lemniscal nuclei following injection of Biotinylated Dextran into the high frequency dorsal part of AVCN as shown in Figure 29. **A** Shows labelling of contralateral DNLL, INLL and VNLL (arrows). Terminal labelling in DNLL is ring like but is most dense ventrally (arrow) and was seen above in Figure 29 and is also shown in close-up in **B** of this figure. Terminal labelling in INLL (arrow) is concentrated ventromedially and is seen at higher magnification in **C** of this figure. Terminal labelling in VNLL (arrow) is arranged in a column of horizontally organized patches, one of which is shown in close in **D** of this figure. The densest terminal labelling in lemniscal nuclei was found in VNLL. Scale bar = 100 μm . **B** A magnified view of terminal labelling in ventral contralateral DNLL which was indicated with the arrow in **A** (also shown in Figure 29). Scale bar = 20 μm . **C** A magnified view of terminal labelling in INLL which was indicated with the arrow in **A**. Thick and thin terminal fibers are labelled. Scale bar = 20 μm . **D** A close up of a patch of terminal labelling in VNLL which was indicated with the arrow in **A**. Scale bar = 20 μm .

Figure 31. A set of figures of dense terminal labelling in contralateral ICC following injection of Biotinylated Dextran into a high frequency area of the AVCN (as shown in Figure 29). **A.** A schematic drawing indicating that labelled terminals were located ventrally in ICC (shaded area) following high frequency AVCN injections. **B.** A magnified view of labelled terminals in ICC. Scale bars: A=1mm, B=20 μ m. Ipsilateral ICC was also labelled ventrally but to a lesser extent (not shown).

Figure 32. A set of micrographs of terminal labelling in ipsilateral SOC following injection of Biotinylated Dextran into the dorsal high frequency part of the AVCN as shown in Figure 29. **A** Labelling was found in the medial (high frequency) limb of the ipsilateral LSO. See the magnified view shown in Figure 32B and C. Scale bar = 100 μm **B** A more magnified view of labelling of the medial limb of the ipsilateral LSO. Arrow indicates the area shown in magnified view in Figure 32C. Scale bar = 50 μm **C** A high power micrograph of labelled terminals in ipsilateral LSO. The labelled terminals and fibers are arranged in a vertical column. Scale bar = 20 μm .

Figure 33. A set of micrographs of terminal labelling in contralateral SOC following injection of Biotinylated Dextran into the dorsal high frequency part of the AVCN as shown in Figure 29. **A** Labelling was found in periolivary nuclei on the contralateral side. Labelling was found in SPO, VTB and just lateral to MNTB. The arrow indicates the area shown in magnified view in **B**. Scale bar = 100 μm . **B** A high power micrograph of the labelling of contralateral periolivary nuclei that was shown in Figure 33A. Scale bar = 50 μm .

Figure 34. Electron micrographs of terminal labelling in contralateral DNLL following injection of Biotinylated Dextran into the dorsal high frequency part of the AVCN as shown in Figure 29. Preliminary results have revealed that labelled terminals from dorsal AVCN form asymmetric contacts on small dendrites or dendritic spines of DNLL neurons. **A** A labelled terminal (At) forms an asymmetric contact (arrow) with a dendritic spine (ds). Also seen is an unlabelled terminal (star) that forms an asymmetric contact with a neighbouring dendrite (D). Scale bar = 0.5 μm . **B** A labelled terminal (At) forms an asymmetric contact (arrow) with a small dendrite (D). Scale bar = 0.5 μm .

Figure 35. Light micrographs of terminal labelling in contralateral DNLL following injection of Biotinylated Dextran into the **ventral low frequency part of the AVCN**. **A.** Shows a low frequency (1,300 Hz) injection site which is situated ventrally in AVCN. **B.** Shows terminal labelling in contralateral DNLL. The scant labelling is located in the middle of the DNLL. The arrow indicates labelled terminals which can be seen more clearly in C. **C.** Shows a magnified view of the labelled terminals that were indicated by an arrow in B. See also Figure 36 for contralateral DNLL labelling. Scale bars: A and B= 100 μm , C=20 μm .

Figure 36. A set of light micrographs of terminal labelling in contralateral nuclei of the lateral lemniscus following injection of Biotinylated Dextran into the ventral low frequency part of the AVCN as shown in Figure 35. **A** Terminal labelling in DNLL, INLL and VNLL is indicated by arrows. There is little labelling in DNLL and the labelling is situated in the middle of the DNLL as was seen in Figure 35B and C and can be seen again in close up in Figure 36B. Sparse labelling in INLL is situated laterally (see close up in Figure 36C). The VNLL shows the densest labelling, yet the labelling is confined to a small horizontally oriented patch (arrow) (see magnified view in Figures 36D and E). Scale bar = 100 μm . **B** A high power micrograph of terminal labelling in the middle of DNLL as indicated in Figures 36A and 35B and C. Scale bar = 20 μm . **C** A high power micrograph of the sparse terminal labelling found in INLL (Figure 36 A). The terminal labelling is situated laterally within the INLL and can be seen to consist of boutons en passant coming off of labelled fibers. Scale bars = 20 μm . **D** A closer view of labelled terminals in VNLL indicated with the arrow in 36A. The labelled terminals are arranged in horizontal row. See Figure 36E as well. Scale bar = 50 μm . **E** A magnified view of labelled terminals in VNLL as shown in 36A and D. The small arrow indicates larger boutons terminaux and the larger arrow indicates some very small boutons terminaux. Scale bar = 20 μm .

Figure 37. A set of micrographs to show sparse terminal labelling in ipsilateral DNLL following high frequency (dorsal) and low frequency (ventral) AVCN Biotinylated Dextran injections (as shown in Figure 29 and 35). **A** The arrow indicates a few labelled terminal fibers in ipsilateral DNLL (as indicated in B) following a high frequency (dorsal) AVCN injection as shown in Figure 29. Scale bar = 20 μm . **B** The arrow indicates a very few labelled terminal fibers in ipsilateral DNLL following a high frequency AVCN injection. See the close up in A. Scale bar = 100 μm . **C** This figure shows a labelled fiber in ipsilateral DNLL, following a low frequency (ventral) AVCN injection, giving off a collateral which gives rise to a few terminals. See Figure D for a lower magnification view. Scale bar = 20 μm . **D** The arrow indicates a labelled fiber in ipsilateral DNLL following a low frequency AVCN injection. The fiber gives rise to only a few terminals (see C for a close up). Scale bar = 100 μm .

-A

-B

-C

-D

Figure 38. Light micrographs of terminal labelling in contralateral ICC following injection of Biotinylated Dextran into a ventral low frequency area of AVCN (as shown in Figure 35). **A.** A schematic drawing indicating that labelled terminals were located dorsolaterally in ICC (shaded area) following high frequency AVCN injection. **B.** A photograph showing the dorsolateral location of labelled terminals in contralateral ICC following this low frequency AVCN injection. **C.** A magnified view of labelled terminals in ICC. Scale bars: A= 1mm, B= 100 μ m, C=20 μ m. There was very little labelling of the ipsilateral ICC in this case (which was also located dorsolaterally) (not shown).

Figure 39. A set of micrographs of labelling in the ipsilateral SOC following a ventral low frequency AVCN injection as seen in Figure 35. **A** Terminal labelling can be seen in the low frequency (lateral) limb of the ipsilateral LSO. The ipsilateral MSO is also labelled. The arrows indicate the areas that are shown at higher magnification in Figure 39B to D. Scale bar = 100 μm . **B** A closer view of labelling in the lateral limb of the LSO. The arrow indicates the area of the lateral limb shown in close up in 39C. Scale bar = 50 μm . **C** A magnified view of labelled terminals in ipsilateral LSO. The fine terminal fibers are arranged horizontally. Scale bar = 20 μm . **D** A close up of terminal labelling in ipsilateral MSO. Fine fibers are diffusely distributed throughout the MSO. Scale bar = 20 μm .

Figure 40. A set of micrographs of labelling in the contralateral SOC following a ventral low frequency AVCN injection as seen in Figure 35. **A** Labelling was observed in contralateral MSO (arrow). Scale bar = 100 μm . **B** A close up of terminal labelling in contralateral MSO. Labelled terminals and fine fibers are diffusely distributed throughout the MSO. Scale bar = 20 μm .

Figure 41 A set of micrographs of terminal labelling in ipsilateral DNLL and nucleus sagulum following injection of Biotinylated Dextran into the ICC. **A** The injection site is located ventromedially within the ICC. Scale bar = 1 mm. **B** Labelling in the ipsilateral DNLL and sagulum is indicated with the arrow and can be seen at higher magnification in Figure 41C. Labelled terminals and fibers are found laterally within the DNLL and surrounding nucleus sagulum. Scale bar = 100 μ m. **C** A magnified view of labelling in the ipsilateral DNLL. Labelled fibers and terminals are found laterally within the DNLL and nucleus sagulum. Scale bar = 20 μ m.

Figure 42. A set of micrographs of terminal labelling in ipsilateral DNLL following an injection of Biotinylated Dextran into the top of the lateral (low frequency) limb of the LSO. **A** This figure shows the injection site of Biotinylated Dextran into the top of the lateral limb of the LSO. Scale bar = 100 μm . **B** Terminal labelling in ipsilateral DNLL forms a central (as opposed to peripheral) ring which is not that clear in this photomicrograph. The ring is incomplete medially and laterally. The arrows indicate the ventral part of the ring which can be better seen in a closer view in Figure 42C. Scale bar = 100 μm . **C** A high power micrograph of terminal labelling in the ventral part of the ring in ipsilateral DNLL. The band consists of patches of terminal labelling that appeared between the fibers of the lateral lemniscus (where cell bodies are located). Ventrolaterally the labelled terminals and fibers are oriented horizontally (open arrow indicates this area which is shown in close up in Figure 42E). More medially labelled fibers and terminals are oriented in a vertical column (open arrow indicates this area which is shown in close up in Figure 42D). Scale bar = 50 μm . **D** A high power micrograph of the vertically oriented column of labelled fibers found medially within the band of terminal labelling in ipsilateral DNLL. Scale bar = 20 μm . **E** A close up of the horizontally oriented patches of labelled terminals and fibers ventrolaterally located in the band of terminal labelling in DNLL. Scale bar = 20 μm .

Figure 43. A set of micrographs of contralateral DNLL following a lateral limb LSO injection (the top of the lateral limb) as shown in Figure 42A. **A** There was little labelling of the contralateral DNLL following this lateral limb LSO injection. Contrast this to the dense ring like pattern of labelling in ipsilateral DNLL found following lateral limb injection (Figure 42). The arrow indicates a few labelled terminals which are shown at higher magnification in **B**. Scale bar = 100 μm . **B** A close up of the few labelled terminals found in contralateral DNLL that were indicated with the arrow in **A**. Scale bar = 20 μm .

Figure 44. A set of figures illustrating labelling of contralateral ICC following a lateral limb LSO injection (the top of the lateral limb) as shown in Figure 42A. **A** A schematic representation to show that the terminal labelling was located laterally in ICC (shaded area) following this lateral limb LSO injection (as opposed to more ventrally where higher frequencies are represented in IC). Scale bar = 1mm. **B** A micrograph of the band of terminal labelling found laterally in ICC as indicated in the schematic (see A). The arrow indicates the area shown in magnified view in C. Scale bar = 50 μ m. **C** A high power micrograph of terminal labelling found laterally in ICC following a lateral limb LSO injection which was indicated by the arrow in B. The labelled terminals are arranged in a column oriented dorso-laterally. Scale bar = 20 μ m. Little labelling of the ipsilateral ICC was observed following this lateral limb LSO injection (not shown).

Figure 45. A set of micrographs of labelling in ipsilateral SOC following an injection into the MNTB. **A** A magnified view of labelling of the medial limb of ipsilateral SPO as indicated with the arrow in C. Scale bar = 20 μm . **B** A magnified view of labelling of the medial part of the ipsilateral LSO as indicated with the arrow in C. Scale bar = 20 μm . **C** This figure shows an injection of Biotinylated Dextran into the MNTB (laterally) and the arrows indicate terminal labelling found in medial parts of ipsilateral SPO and LSO (see close ups in A and B). Scale bar = 100 μm .

Figure 46. A set of micrographs to show terminal labelling in ipsilateral nuclei of the lateral lemniscus following an MNTB injection as shown in Figure 45C. **A** Labelling was not observed in the DNLL. Sparse labelling was observed in both the INLL and VNLL. The arrows indicate labelled terminals which are shown at higher magnification in B and C. Scale bar = 100 μm . **B** A close up of labelled terminals in INLL as indicated by the arrow in A. Scale bar = 30 μm . **C** A magnified view of labelled terminals in VNLL as indicated by the arrow in A. Scale bar = 30 μm . No terminal labelling was found in the contralateral lemniscal nuclei or the IC (not shown).

Figure 47. A set of micrographs of labelling of ipsilateral DNLL following an injection of Biotinylated Dextran into the VTB. **A** This figure shows the injection site in the VTB. Scale bar = 100 μm . **B** This figure shows the ipsilateral DNLL following VTB injection. Labelled fibers clearly pass right through the DNLL en route to the ipsilateral ICC (see Figure 48). The arrow indicates collaterals coming off a labelled fiber that can be seen at higher magnification in C. Scale bar = 100 μm . **C** This is a close up of the area indicated with the arrow in B. In this figure (to the right) it can be seen that labelled fibers pass through the DNLL. A thick labelled fiber can be seen in the left of this figure. This fiber sends off a fine collateral that branches, to terminate in DNLL. There were few labelled terminals seen in ipsilateral DNLL following VTB injection but many labelled fibers. No labelling in contralateral DNLL was observed (not shown). Scale bar = 20 μm .

Figure 48. A set of figures to illustrate labelling in ipsilateral ICC following a VTB injection as shown in Figure 47A. **A** A schematic representation indicating that terminals from VTB were found ventrally in ICC (shaded area). Figures 48 B and C show close ups of this labelling. Scale bar = 1mm. **B** A micrograph to show the fine terminal fiber labelling in ICC following VTB injection. The small arrow indicates the area that is shown in close up in C. Scale bar = 50 μ m. **C** A magnified view of the area indicated with the arrow in B. Fine labelled fibers give off tiny terminals in ICC. Scale bar = 20 μ m. Note that most labelled fibers passed though ipsilateral DNLL (Figure 47) to terminate in ICC. No labelling was observed in contralateral ICC (not shown).

Figure 49. This figure shows labelling in ipsilateral SOC following an injection of Biotinylated Dextran into the lateral part of the SPO. **A** The injection site is situated laterally in SPO. Some terminal labelling in ipsilateral LSO can also be seen. Scale bar = 100 μm . **B** Labelling of the ipsilateral LSO (middle part) and MSO (arrows) following injection into the SPO. Scale bar = 100 μm . **C** A high power micrograph of labelling of ipsilateral MSO as shown in B. Scale bar = 20 μm . **D** A magnified view of labelling of the middle part of the ipsilateral LSO as shown in B. Scale bar = 50 μm .

Figure 50. This figure shows labelling in ipsilateral nuclei of the lateral lemniscus following a lateral SPO injection as seen in Figure 49. **A and B** Show a few small labelled terminals from lateral DNLL (as indicated by the arrows in F). Scale bars = 20 μm . **C** A labelled terminal fiber found in DNLL (as indicated by the arrow in F). Scale bars = 20 μm . **D** A magnified view of a few very small labelled terminals and fibers in INLL (as indicated by the arrow in F). **E** A high power micrograph of terminal labelling in VNLL as indicated in F. **F** Terminal labelling was densest in VNLL and least dense in DNLL. The few labelled terminals found in DNLL were found laterally. Terminal labelling was also sparse in INLL and was concentrated ventrally. The arrows indicate the areas shown at higher magnification in A to E. No labelling of contralateral nuclei of the lateral lemniscus was observed although some labelled fibers were seen in contralateral VNLL (not shown). Scale bar = 100 μm .

Figure 51. This figure shows labelling in ipsilateral ICC following a lateral SPO injection as seen in Figure 49. **A** Labelling in ipsilateral ICC was dense and concentrated in the middle of ICC. Scale bar = 100 μm . **B** A closer view of labelling in the ipsilateral ICC. The arrow indicates terminals that are shown in close up in C. Scale bar = 50 μm . **C** A magnified view of labelling in ipsilateral ICC that was indicated with the arrow in B. Scale bar = 20 μm .

Figure 52. This figure shows labelling in the contralateral CN following an injection of Biotinylated Dextran laterally into the SPO. Ipsilateral labelling was also observed but was not as dense (not shown). **A** The arrow indicates labelled terminals and fibers in caudal VCN. No labelling was observed in rostral AVCN. See the magnified view in **B**. Scale bar = 100 μm . **B** A high power micrograph of labelled terminals and fibers in caudal VCN as shown in **A**. Scale bar = 20 μm . **C** The arrow indicates labelled terminals and fibers in PVCN. See the close up in **D**. Scale bar = 100 μm . **D** A magnified view of labelled terminals and fibers in PVCN as shown in **C**. Scale bar = 50 μm . **E** The arrow indicates labelled terminals and fibers in DCN. See the magnified view in **F**. Scale bar = 100 μm . **F** A magnified view of labelled terminals and fibers in DCN as shown in **E**. Scale bar = 20 μm .

A

B

C

D

E

F

Figure 53. A set of micrographs to show fiber paths through the inferior colliculus bilaterally following injection of Biotinylated Dextran into the right DNLL. **A** Fiber paths in the contralateral IC take a dorsolateral or dorsomedial route through the IC (curved arrows). Scale bar = 500 μm . **B** Fiber paths exit from DNLL dorsally taking a dorsolateral or dorsomedial route through ipsilateral IC as indicated by the curved arrows. Scale bar = 500 μm .

Figure 54. A set of micrographs to show labelling of the commissure of Probst (CP) from caudal to rostral in representative coronal sections following injection of Biotinylated Dextran into the DNLL. **A** At caudal levels the labelled fibers in the CP exit the DNLL medially and curve back dorsomedially (as indicated by the arrow). Scale bar = 500 μm . **B** In the middle part of the DNLL (with reference to rostral to caudal) the CP exits the DNLL medially and runs nearly straight across to the contralateral DNLL. Scale bar = 500 μm . **C** At rostral levels the CP exits the DNLL medially and runs farther down ventrally than it does at middle levels (with reference to rostral to caudal position). Scale bar = 500 μm .

Figure 55. A photomicrograph of an injection site of Biotinylated Dextran into the right DNLL. This injection site is located ventrally within the DNLL. Scale bar = 100 μ m. B. A schematic representation of the general pattern of labelling observed following injections into the DNLL. The contralateral DNLL and bilateral IC showed heavy labelling (fine stippling) while bilateral SC and ipsilateral periolivary nuclei of the SOC showed less heavy labelling (shading). The trapezoids indicate the areas that were extracted for EM analysis. [Adapted from the atlas of Paxinos and Watson, (1986); Bajo (1993) and Oliver and Shneiderman, (1989)].

FIG. . **A.** A photomicrograph of an injection site of Biotinylated Dextran into the right DNLL. The injection site is located ventrally within the DNLL. Scale bar = 100 μ m. **B.** A schematic diagram of the pattern of labeling observed following injections into the DNLL. The contralateral DNLL and bilateral IC showed heavy labeling while bilateral SC and ipsilateral periventricular nuclei of the SOC showed less heavy labeling. The trapezoids indicate the areas that were extracted for EM analysis.

LEGEND

- = injection site
- ▨ = heavy labeling
- ▤ = moderate labeling
- ▥ = weak labeling

Figure 56 .Photomicrographs of labelling of the contralateral DNLL following injection of Biotinylated Dextran ventrally into the DNLL as shown in **Figure 55A**. **A and C**. In contralateral DNLL labelled terminals and fibers were dense ventrally in symmetry to the injection site. **C** Shows a magnified view of the terminal labelling as shown in **A**. **B** A high power micrograph of labelled terminals in the contralateral DNLL. **D and E**. High power micrographs of the labelling shown in **C**. In **D** the terminal labelling found medially in this ventral band is arranged in a column. In **E** it can be seen that terminal labelling found ventrally in this ventral band is arranged in horizontally oriented patches. Scale bars: **A**= 100 μm ; **C**= 50 μm ; **B, D and E** = 10 μm .

Figure 57. Light micrographs to show different injection sites in the DNLL and patterns of terminal labelling seen in contralateral DNLL. **A** Terminal labelling in mid contralateral DNLL following an injection in mid DNLL as in **B** (with reference to caudal to rostral position). The labelled terminals are arranged in vertical columns between fibers of the lateral lemniscus (where cell bodies are located). Scale bar = 50 μm . **B** An injection in mid DNLL (with reference to rostral to caudal position) that spreads laterally into nucleus sagulum. This injection leads to the columnar like pattern of terminal labelling in mid DNLL as seen in **A**. Scale bar = 100 μm . **C** A diffuse terminal labelling pattern (not columnar as in **A**) seen in rostral DNLL following a rostral DNLL injection shown in **D**. More caudally the labelling in contralateral DNLL was concentrated laterally in symmetry to the injection site (see **Figure 58**), while rostrally as shown, the labelling was diffusely distributed throughout rostral DNLL. Scale bar = 50 μm . **D** An injection into very lateral rostral DNLL that spreads to nucleus sagulum. See **C** for contralateral rostral DNLL labelling and **Figure 55** for caudal DNLL labelling. Scale bar = 100 μm .

A

C

B

D

Figure 58. A set of micrographs to show labelling in contralateral DNLL at more caudal levels following injection of Biotinylated Dextran into the DNLL laterally as shown in **Figure 57 D**. **A** Terminal labelling is concentrated laterally (arrow) at this level, in symmetry to the lateral injection site. At more rostral levels the labelling is spread diffusely throughout the DNLL (**Figure 57 C**). See **B** for a magnified view. Scale bar = 100 μm . **B** A high power micrograph of terminal labelling found laterally in the DNLL as shown in **A**. Scale bar = 20 μm .

Figure 59. A set of micrographs of labelling in contralateral DNLL following small injections into caudal (A and B) and rostral (C and D) DNLL that do not spread to nucleus sagulum. **A** A few labelled terminals are found in caudal contralateral DNLL following a minute injection into caudal DNLL that does not spread to nucleus sagulum (see B). Labelled terminals were also found bilaterally in ICC following this injection while bilateral SC was not labelled following this caudal DNLL injection (not shown). Scale bar = 50 μm . **B** A very small injection of Biotinylated Dextran into the caudal DNLL that does not spread to sagulum. See A for contralateral DNLL labelling pattern. Scale bar = 100 μm . **C** A few labelled terminal fibers are found in rostral contralateral DNLL following a small injection into rostral DNLL that does not spread to nucleus sagulum (see D). Few labelled terminals were found in ICC bilaterally yet sparse terminal labelling was found bilaterally in SC following this rostral DNLL injection (not shown). Scale bar = 50 μm . **D** A small injection of Biotinylated Dextran into the rostral DNLL that does not spread to sagulum. See C for contralateral DNLL labelling pattern. Scale bar = 100 μm .

Figure 60. Micrographs to show laterally situated injection sites in surrounding nucleus sagulum. **A** Only a very few labelled terminals are found in contralateral DNLL following the injection shown in **B**. Scale bar = 20 μm . **B** An injection site of Biotinylated Dextran into surrounding nucleus sagulum. This injection leads to few labelled terminals in contralateral DNLL. (as shown in **A**) but leads to extensive labelling of ipsilateral SC and EC of the IC. Scale bar = 100 μm . **C** An injection site of Biotinylated Dextran into surrounding nucleus sagulum at rostral levels. This injection did not lead to labelling of contralateral DNLL (not shown) few terminals were also found bilaterally in IC but the SC was labelled bilaterally (not shown). Scale bar = 100 μm .

Figure 61. Electron micrographs of labelled terminals in contralateral DNLL. **A and B** show labelled terminals (At) contacting cell bodies (CB) of DNLL neurons. Contacts on cell bodies were not as common as symmetrical contacts formed on dendrites (see **Figures 62 and 63**). Scale bars: C= 0.5 μm , D= 1 μm .

Figure 62. Electron micrographs of labelled terminals in contralateral DNLL following injection of Biotinylated Dextran into the DNLL. **A** and **B** show labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D). These types of contacts were common in DNLL. Scale bars: A and B = 0.5 μm .

Figure 63 Electron micrographs of labelled terminals in contralateral DNLL following DNLL injection. **A** and **B** show labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D). These types of contacts were common in DNLL. Scale bars: 0.5 μm .

Figure 64. An electron micrograph of a labelled terminal (At) in contralateral DNLL following injection of Biotinylated Dextran into the DNLL. The terminal forms an asymmetric contact (arrow) with a dendrite (D). These types of contacts were observed in addition to the more frequently occurring symmetrical contacts shown in **Figures 62 and 63**. Scale bar = 0.5 μm .

Figure 65. An electron micrograph of a labelled terminal (At) in contralateral DNLL following injection of Biotinylated Dextran into the DNLL. The terminal forms an asymmetric contact (arrows) with a small dendrite (D). These types of contacts were observed in addition to the more frequently occurring symmetrical contacts shown in Figures 62 and 63. Scale bar = 0.5 μm .

Figure 66. Photomicrographs of labelling of the contralateral IC (left IC=LIC) following injection of Biotinylated Dextran into the right DNLL. **A** and **B** show labelled terminals and fibers in the contralateral ICC. **C** Magnified view of labelled terminals in contralateral ICC. Scale bars: **A**= 50 μm ; **B** and **C** = 10 μm .

Figure 67. Electron micrographs of labelled terminals in contralateral ICC following injection of Biotinylated Dextran into the DNLL. **A and B** show labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D). These types of contacts were common in contralateral ICC. Scale bars = 0.5 μ m.

Figure 68. An electron micrograph of a labelled terminal in contralateral ICC following injection of Biotinylated Dextran into the DNLL. The labelled terminal (At) forms a symmetrical contact (arrow) with a dendrite (D). Scale bar = 0.5 μ m.

Figure 69. An electron micrograph of a labelled terminal in contralateral ICC following injection of Biotinylated Dextran into the DNLL. The labelled terminal (At) forms a symmetrical contact (arrow) with a dendrite (D). Scale bar = 0.5 μm .

Figure 70. An electron micrograph of a labelled terminal in contralateral ICC following injection of Biotinylated Dextran into the DNLL. The labelled terminal (At) forms a symmetrical contact (arrow) with a dendrite (D). Scale bar = 0.5 μ m.

Figure 71. An electron micrograph of a labelled terminal in contralateral ICC following injection of Biotinylated Dextran into the DNLL. The labelled terminal (At) forms a symmetrical contact (arrow) with a small dendrite (D). Scale bar = 0.5 μm .

Figure 72 .Photomicrographs of labelling of the ipsilateral IC (right IC=RIC) following injection of Biotinylated Dextran into the DNLL. **A and B** show labelling of the ipsilateral ICC. **C** A magnified view of labelled terminals in ipsilateral ICC. Scale bars: **A**= 50 μm ; **B and C**= 10 μm .

Figure 73 . Electron micrographs of labelled terminals in ipsilateral ICC following injection of Biotinylated Dextran into the DNLL. **A and B** show labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D). These types of contacts were common in ipsilateral ICC. Scale bars = 0.5 μ m.

Figure 74. An electron micrograph showing a small labelled axon terminal (At) forming a symmetrical contact (arrow) with a dendrite (D) in ipsilateral IC. Scale bar = 0.5 μm .

At

Figure 75. An electron micrograph showing a labelled terminal (At) contacting a large dendrite (D) in ipsilateral IC. Scale bar = 0.5 μm .

Figure 76. An electron micrograph of **unlabelled** terminals (At) in ipsilateral ICC forming asymmetric contacts with a dendrite. Scale bar = 0.5 μm .

Figure 77. Photomicrographs of labelling of the contralateral SC following injection of Biotinylated Dextran into the right DNLL. **A and B** show labelled fibers and terminals in deep and intermediate layers of contralateral SC. **C** A magnified view of labelled terminals in contralateral SC. Scale bars: **A** = 50 μm ; **B and C** = 10 μm .

Figure 78. Electron micrographs of labelled terminals in contralateral SC. **A and B** show labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D). These types of contacts were common in contralateral SC. Scale bars = 0.5 μm .

Figure 79. An electron micrograph of a labelled ending (E) in contralateral SC forming a symmetrical contact (arrow) with a dendrite (D). Scale bar =0.5 μm .

FIG 80. An electron micrograph of a dendrite (D) in contralateral SC receiveing contacts (arrows) from two labelled terminals (At). Scale bar = 0.5 μ m.

AT

D

AT

Figure 81. Photomicrographs of labelling of the ipsilateral SC following injection of Biotinylated Dextran into the right DNLL. **A and B** show labelled fibers and terminals in ipsilateral deep and intermediate layers of SC. **INSETS:** Magnified views of labelled terminals in ipsilateral SC. Scale bars: **A** = 50 μm , **B and Insets** = 10 μm .

Figure 82. Electron micrographs of labelled terminals in ipsilateral SC. **A and B** show labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D). These types of contacts were common in ipsilateral SC. Scale bars = 0.5 μm .

Figure 83. An electron micrograph of a small labelled terminal (At) forming a symmetrical contact (arrow) with a dendrite (D) in ipsilateral SC. Scale bar = 0.5 μm .

Figure 84. An electron micrograph of a labelled terminal (At) forming a symmetrical contacts (arrows) with a dendrite (D) in ipsilateral SC. Scale bar = 0.5 μm .

Figure 85. An electron micrograph of a labelled terminal (At) in ipsilateral SC seen coming off a labelled fiber to form symmetrical contacts at several sites (arrows) on neighbouring processes. Scale bar = 0.5 μm .

Figure 86. A set of micrographs to show labelling in contralateral intercollicular nuclei following injection of Biotinylated Dextran into the DNLL of the rat. **A** A schematic representation to show the position of labelled terminals (shading). Small areas with dense labelling were observed contralaterally. Scale bar = 500 μm . **B** This figure shows fiber labelling and patches of terminal labelling in this area which is seen at higher magnification in C. Scale bar = 100 μm . **C** A high power micrograph of a dense patch of terminal labelling in this intercollicular area. Scale bar = 10 μm .

Figure 87. A set of micrographs to show labelling in ipsilateral intercollicular nuclei following injection of Biotinylated Dextran into the DNLL of the rat. **A** A schematic representation to show the position of labelled terminals (shading). More diffuse labelling is observed ipsilaterally in this area (contralaterally labelling is more dense). See magnified view in B. Scale bar = 500 μm . **B** A magnified view of diffuse terminal labelling in this area following DNLL injection. Scale bar = 10 μm .

Figure 88. Labelling of the ipsilateral superior olivary complex (SOC) following injection of Biotinylated Dextran into the right DNLL. **A** A schematic diagram of sparsely distributed labelling (shading) in the ipsilateral medial nucleus of the trapezoid body (MNTB) and the superior paraolivary nucleus (SPO). **B** A photomicrograph of sparse labelling of the ipsilateral MNTB and SPO. **C** A magnified view of labelling of the SPO. The inset shows a magnified view of the area indicated with an arrow. **D** A high power micrograph of labelling of the MNTB. The inset shows a magnified view of the area indicated with an arrow. Scale bars: **B**= 50 μm ; **C, D and INSETS**= 10 μm .

Figure 89 . A and B Electron micrographs of labelled terminals (At) forming symmetrical contacts (arrows) with dendrites (D) in ipsilateral periolivary nuclei of the superior olivary complex. Scale bars = 0.5 μ m.

Figure 90. An electron micrograph of a labelled terminal (At) forming a symmetrical contact with a dendrite (D) in ipsilateral periolivary nuclei. Scale bar = 0.5 μm .

Figure 91 . Electron micrographs of labelled terminals in ipsilateral periolivary nuclei. **A and B** show labelled terminals (At) forming asymmetrical contacts (arrows) with dendrites (D). Scale bars = 0.5 μ m.

Figure 92. An electron micrograph of a labelled terminal (At) forming an asymmetric contact (arrow) with a dendrite (D) in ipsilateral periolivary nuclei. Scale bar = Scale bar = 0.5 μm .

At

Figure 93. An electron micrograph of a labelled terminal (At) (shown in the inset in the top left and indicated with the thick black arrow in the main figure) forming a contact with a cell body in periolivary nuclei of the SOC. Scale bar for the main figure and the inset is 0.5 μm .

Figure 94. An electron micrograph of a labelled terminal (At) (shown in the inset on the top right and indicated with the arrow in the main figure) forming a contact with a cell body in periolivary nuclei of the SOC. Scale bar for the main figure and the inset is 0.5 μm .

Figure 95. An electron micrograph of a **round neuron** in DNLL. It has an eccentrically placed nucleus (N). The nucleus of this cell has a regular contour. The cytoplasm is abundant. Long strands of rough endoplasmic reticulum (black arrows) are evident. Long stacked cisternae forming a circle constitute the Golgi apparatus (G), lysosome (L). Scale bar = 1 μm .

Figure 96. In the center of this figure is a neuron from DNLL with a large **elongated soma** (as indicated with the black arrow in the white circle). The nucleus (N) is also elongated and eccentrically placed. The nucleus has a very irregular shape. Visible in the nucleus is a large nucleolus (Nu). In the cytoplasm (the part which is indicated by the open white arrow, is shown at higher magnification in Figure 97) long strands of rough endoplasmic reticulum are dispersed (small black arrows). Stacks of long cisternae form the Golgi complex (G) (large black arrows). A large dendrite (D) extends from the soma contains long strands of rough endoplasmic reticulum (small black arrows). Scale bar = 1 μm .

Figure 97. A magnified view of part of the nucleus (N) and cytoplasm of the neuron in DNLL shown in the middle of Figure 96 (the area indicated with the open white arrow in Figure 96) along with the surrounding neuropil. It can be seen that the nucleus had a very irregular contour. Strands of rough endoplasmic reticulum are dispersed throughout the cytoplasm (black arrows). Scale bar = 100 nm.

Figure 98. In the middle of this figure is a cell with a **triangular shaped soma** in DNLL. The nucleus (N) which is centrally located and triangular shaped, has a smooth contour. The cytoplasm contains mitochondria and short strands of rough endoplasmic reticulum which are dispersed throughout the cytoplasm. See the magnified view of the area indicated with the open black arrow in Figure 99. Two dendrites (D) can be seen extending from the cell.

Figure 99. This is a magnified view of the area shown in the cell in Figures 98. Shown is the Golgi apparatus (G) near the nucleus. The cisternae of the rough endoplasmic reticulum are short and are dispersed throughout the cytoplasm (small black arrows). Scale bar = 100 nm.

Figure 100. To the right of the electron micrograph is a neuron with a round soma. It has a very irregular nucleus (N) which is centrally placed. The cytoplasm contains both stacked (RER) and dispersed rough endoplasmic reticulum (black arrows). The Golgi apparatus is indicated (G). The part of the cytoplasm which is shown at higher magnification in Figure 101 is indicated by the large black arrow. To the left of the figure is part of a darkly stained glial cell (an oligodendrocyte (O)). Scale bar = 1 μm .

Figure 101. A magnified view of the cytoplasm of the neuron shown in Figure 100. The nucleus (N) of the neuron is seen on the right. In the cytoplasm of the neuron a **stack of long strands of rough endoplasmic reticulum** can be seen (RER). At the top of the figure the Golgi apparatus is indicated (G), lysosome (L). Scale bar = 100 nm.

Figure 102. This figure shows the cell body of a glial cell (a fibrous astrocyte). The nucleus (N) occupies a large portion of the cell body so that there is scant cytoplasm. The two opposing black arrows indicate the part of the cytoplasm of the cell that is shown at higher power in Fig 103. Scale bar = $1\mu\text{m}$.

Figure 103. This figure shows a magnified view of the part of the cytoplasm (indicated by the arrows) of the astrocyte that was shown in Figure 102. The thin rim of cytoplasm contains bundles of fibrils which are shown in longitudinal section (fl) and in cross section (fx). Also seen in the cytoplasm are small mitochondria (M). Scale bar = 100 nm.

Figure 104. **A** This figure shows the cell body of a glial cell (a protoplasmic astrocyte). This glial cell has an elongate soma containing an irregularly shaped nucleus (N) which occupies most of the cell body. The scant cytoplasm contains a sparse population of organelles (as indicated by the long black arrow and shown in close up in B) which is a feature characteristic of glial cells (Peters et al., 1976). Yet the cytoplasm of this cell is devoid of fibrils which are characteristic of fibrous astrocytes (see Figure 102 and 103). The cell also sends out short stubby protrusions (astrocytic processes) (see B and C). The black arrows indicate the areas of the cell that are shown in close up in B and C. Scale bar = 1 μm . **B** This figure shows a closer view of the area indicated by the long black arrow in A. The nucleus (N) is shown on the bottom right. Long stacks of cisternae form the Golgi complex (black star). Mitochondria (M) and strands of rough endoplasmic reticulum are dispersed throughout the cytoplasm (black arrows). A short process characteristic of glial cells is seen at the top of the figure (black asterisk). Scale bar = 100 nm and applies to C as well. **C** This figure shows a magnified view of the area of the cell in A that was indicated by the short black arrow to the left of the figure. The nucleus (N) is seen on the right. A mitochondrion (M) is visible in the thin rim of cytoplasm. A short protrusion from the cell (black asterisk) extends from the cell body. Scale bar in B applies here = 100 nm.

APPENDIX A

List of Abbreviations

At	axon terminal
AVCN	anteroventral cochlear nucleus
BD	Biotinylated Dextran
CB	cell body
CN	cochlear nucleus
CP	commissure of Probst
D	dendrite
DC	dorsal cortex of the inferior colliculus
DCN	dorsal cochlear nucleus
DNLL	dorsal nucleus of the lateral lemniscus
EC	external cortex of the inferior colliculus
EI	binaural response type- cell excited by contralateral stimulation and inhibited by ipsilateral stimulation
EM	electron microscopy
EO	binaural response type-cell excited by contralateral stimulation, ipsilateral stimulation has no effect
FG	Fluoro-Gold
G	Golgi complex
IC	inferior colliculus
ICC	central nucleus of the inferior colliculus
IE	binaural response type-cell inhibited by contralateral stimulation and excited by ipsilateral stimulation
IID	interaural intensity difference
INLL	intermediate nucleus of the lateral lemniscus
ITD	interaural time difference
L	lysosome
LIC	left inferior colliculus

LSO	lateral superior olivary nucleus
LTB	lateral nucleus of the trapezoid body
MGB	medial geniculate body
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olivary nucleus
N	nucleus
Nu	nucleolus
O	oligodendrocyte
PVCN	posteroventral cochlear nucleus
RER	rough endoplasmic reticulum
RIC	right inferior colliculus
SAG	sagulum
SC	superior colliculus
SOC	superior olivary complex
SPO	superior paraolivary nucleus
VCN	ventral cochlear nucleus
VNLL	ventral nucleus of the lateral lemniscus
VTB	ventral nucleus of the trapezoid body

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