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City University of New York, 1989

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GLYCINERGIC PATHWAYS OF THE CENTRAL AUDITORY SYSTEM AND ADJACENT RETICULAR FORMATION OF THE RAT

by

CHYREN HUNTER

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1989

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ABSTRACT

GLYCINERGIC PATHWAYS OF THE CENTRAL AUDITORY SYSTEM AND ADJACENT RETICULAR FORMATION OF THE RAT

by

CHYREN HUNTER

Advisor: Melvin H. Van Woert, M.D.

The development of techniques to visualize and identify specific transmitters of neuronal circuits has stimulated work on the characterization of pathways in the rat central nervous system that utilize the inhibitory amino acid glycine as its neurotransmitter. Glycine is a major inhibitory transmitter in the spinal cord and brainstem of vertebrates where it satisfies the major criteria for neurotransmitter action. Some of these characeristics are: uneven distribution in brain, high affinity reuptake mechanisms, inhibitory neurophysiological actions on certain neuronal populations, uneven receptor distribution and the specific antagonism of its actions by the convulsant alkaloid strychnine.

Behaviorally, antagonism of glycinergic neurotransmission in the medullary reticular formation is linked to the development of myoclonus and seizures which

may be initiated by auditory as well as other stimuli. In the present study, decreases in the concentration of glycine as well as the density of glycine receptors in the medulla with aging were found and may be responsible for the lowered threshold for strychnine seizures observed in older rats.

Neuroanatomical pathways in the central auditory system and medullary and pontine reticular formation (RF) were investigated using retrograde transport of tritiated glycine to identify glycinergic pathways; immunohistochemical techniques were used to corroborate the location of glycine neurons.

Within the central auditory system, retrograde transport studies using tritiated glycine demonstrated an ipsilateral glycinergic pathway linking nuclei of the ascending auditory system. This pathway has its cell bodies in the medial nucleus of the trapezoid body (MNTB) and projects to the ventrocaudal division of the ventral nucleus of the lateral lemniscus (VLL). Collaterals of this glycinergic projection terminate in the ipsilateral lateral superior olive (LSO). Other glycinergic pathways found were afferent to the VLL and have their origin in the ventral and lateral nuclei of the trapezoid body (MVPO and LVPO). Bilateral projections from the nucleus reticularis pontis oralis (RPOo), to the VLL were also identified as glycinergic. This projection may link motor

output systems to ascending auditory input, generating the auditory behavioral responses seen with glycine antagonism in animal models of myoclonus and seizure.

Autoradiographic studies also revealed that glycinergic pathways with terminals in the medullary and pontine reticular formation project from cell bodies in sensory nuclei associated with the descending control of nociception. These are short axoned projections and interneurons.

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I would like to thank my advisor Dr. Melvin Van Woert in whose lab I recieved my training. Under his direction and with the combined guidance and support of himself and Dr. Eunyong Chung, I was provided with a creative scientific environment for my intellectual growth and expression. Their efforts on my behalf have made this thesis possible. They will remain excellent role models and friends.

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ABBREVIATIONS USED IN ILLUSTRATIONS AND TEXT

AVCN	Anteroventral Cochlear Nucleus (see VCOA)
AR	Acoustic Middle Ear Reflex
ARAS	Ascending Reticular Activating System
DCN	Dorsal Cochlear nucleus
DLL	Dorsal nucleus of the Lateral Lemniscus
DMPO	Dorsomedial periolivary nucleus
GABA	gamma-aminobutyric acid
GEPR	Genetically Epilepsy Prone Rat
GCS	Glycine Cleavage System
HAc	acetic acid
HRP	horseradish peroxidase
IC	Inferior Colliculus
ipsi	ipsilateral
kg	kilogram
KF	Kolliker Fuxe nucleus
LNTB	Lateral nucelus of the Trapezoid Body
LL	Lateral Lemniscus
LSO	Lateral Superior Olive
LSO_d	dorsal aspect of LSO
LSO_l	lateral aspect of LSO
LVPO	Lateroventral periolivary nucleus
M	midline

ABREVIATIONS (cont'd)

mg	milligram
MONV	motor trigeminal nucleus
MNTB	Medial nucleus of the Trapezoid Body
MSO	Medial Superior Olive
MVPO	Medioventral periolivary nucleus
nA	nanoamperes
NLL	Nuclei of the Lateral Lemniscus (VLL & DLL)
NV	nucleus of the trigeminal nerve
NVII	nucleus of the facial nerve
PGl	Nucleus Paragigantocellularis Lateralis
PL	Paralemniscal zone
PL$_m$	medial aspect of PL
Pon N.	nucleus of the pons
PRNVL	ventrolateral principal sensory trigeminal nucleus
RAM	Nucleus Raphe Magnus
RAP	Nucleus Raphe Pallidus
RGc	Nucleus Reticularis Gigantocellularis
RF	Reticular Formation
RMc	Nucleus Reticularis Magnocellularis
RPc	Nucleus Reticularis Parvocellularis
RPO	Rostral periolivary nucleus
RPOc	Nucleus Reticularis Pontis Caudalis pars alpha
RPOc	Nucleus Reticularis Pontis Caudalis pars beta

ABBREVIATIONS (cont'd)

RPOo	Nucleus Reticularis Pontis Oralis
RtTg	Nucleus Reticularis Tegmenti Pontis
S5	sensory root of the trigeminal nerve
SHMT	serine hydroxymethyl transferase
SOC	Superior olivary complex
SPO	Superior periolivary nucleus
SP5O	oral aspect of spinal Trigeminal nucleus
St	Stapedius muscle
TT	Tensor Tympani muscle
Tz	Trapezoid body (auditory fiber pathway)
VCOA	Anteroventral Cochlear nucleus
VCN	Ventral Cochlear nucleus (includes AVCN & PVCN)
VLL	Ventral nucleus of the Lateral Lemniscus
VNTB	Ventral nucelus of the Trapezoid Body

I. LITERATURE REVIEW

1.1. Introduction

The central auditory system of the lower brainstem is essential to both the central processing of afferent acoustic information and the feedback regulation of auditory input to the cochlea. Within the central auditory system, the complex properties of a sound stimulus are integrated and transmitted to cortical and subcortical areas in order to direct somatomotor responses to sound. These responses to acoustic stimuli are in part mediated by connections between the auditory system and the reticular formation.

Behavioral evidence linking the auditory system with motor output via the reticular formation (RF) was first described by Forbes and Sherrington (1914). They noted widespread muscle reflex responses when acoustic stimuli were presented to cats with midcollicular transections. This muscle reflex response to acoustic stimuli, termed 'acoustic startle' can be elicited most effectively by stimulation of lower brainstem auditory or RF nuclei caudal to the level of the inferior colliculus. The functional connections between the auditory nuclei and the brainstem RF are thus important elements in the genesis of normal and paroxysmal motor output in response to sound.

The 'acoustic middle ear reflex' is a less complex circuit than that for acoustic startle; it is localized to the auditory brainstem and RF and governs the transduction of sound from the middle ear (Borg, 1972, 1973).

Sound sensitive tonic-clonic seizures have been reported in rats (Morgan 1940), mice (Vicari 1947), rabbits (Antonitis 1954), and humans (Forster 1970). This disorder, termed 'audiogenic' seizures, occurs in the absence of cortical epileptiform electroencephalographic discharges, suggesting that these motor seizures may originate in the brainstem.

Electrophysiological studies in humans, cats and rabbits have attempted to identify the functional components of the central auditory system regulating motor responses to sound. Correspondence between single unit recordings and field potentials of the brainstem auditory nuclei and the RF have established that these two nuclear groups are part of a brainstem circuit modulating motor responses to sound. Inhibitory interactions within this circuit may be fundamental to its functional integrity. Identification of the inhibitory pathways within this circuit could provide a model by which to analyze the role of inhibition in sensorimotor integration.

Glycine is a major inhibitory neurotransmitter in the brainstem. Recent evidence suggests that glycine acts as a neurotransmitter in some pathways of both the auditory

system and the RF (Faingold et al 1983). The direct visualization of possible glycinergic pathways however, have not yet been demonstrated. The major goal of my research has been to identify glycinergic neuronal projections in the brainstem auditory system and adjacent RF of the rat.

1.2 Glycine as a Central Nervous System Neurotransmitter

Glycine neurotransmission within the vertebrate central nervous system (CNS) has been well documented since the late 1960's. Aprison et al (1969) found that the spinal cord contained the highest levels of this amino acid along the entire neuroaxis in seven vertebrates (3.81 umoles/g wet weight in the rat), closely followed by the medulla (2.72 umoles/g wet weight in the rat). These values were approximately three times the amount found in more rostral regions of the CNS. The uneven distribution of glycine led to the hypothesis that glycine may function as a neurotransmitter in the CNS. A large body of evidence now confirms this hypothesis on physiological, pharmacological and biochemical grounds.

1.1a. Physiology

The neurophysiological actions of glycine on CNS neurons are inhibitory when tested on most adult preparations. Curtis (1968,1968a,) found that iontophoretic application of glycine onto the soma and proximal dendrites of lumbosacral motoneurons led to a decrease in postsynaptic potentials (PSPs) as recorded from intracellular electrodes. The hyperpolarizing effects of glycine were rapid in onset, dose dependent (from 0 to 100 nanoamps electrophoretic current) and

completely reversible within 4 seconds after termination of the ejection current. In addition, hyperpolarization was accompanied by an increase in membrane conductance of chloride ion. After glycine application, higher currents were necessary to depolarize alpha motoneurons by ventral root stimulation or iontophoretic application of DL-homocysteic acid (this amino acid is generally excitatory when applied to CNS neurons). Curtis and collegues (see Krnjevic 1974), found that glycine increased the chloride ion (Cl-) flux. Serine, b-alanine and taurine also have depressant effects on CNS neurons similar to that of glycine but all are less potent than glycine (Curtis 1968,1968a, Curtis and Watkins, 1960). These glycine-like amino acids however, are not viable neurotransmitter candidates based on other neurotransmitter criteria (McGeer and McGeer 1981).

The inhibitory postsynaptic potential (IPSP) resulting from iontophoretic application of glycine is physiologically relevant, since other studies show that the reversal potential for glycine closely mimics that of the endogenous substance released presynaptically (Curtis 1968). In the mouse spinal cord brainstem-cerebellar preparations (Barker and Ransom, 1978), the somatic regions appear to be more sensitive to the effects of glycine than the distal dendritic region in cat motoneurons (Zieglgansberger and Champagnat, 1979).

1.2b. Receptor Binding

Pharmacologic tools to identify the neurotransmitter actions of glycine are limited in number. The most potent antagonist of glycine-induced hyperpolarization is the convulsant alkaloid strychnine. It has been known since the early 1920's that intravenous administration of strychnine is capable of reducing the inhibition of motoneurons induced by stimulation of peripheral nerves (Eccles 1954). Werman et al (1968) and Curtis et al (1968) were among the first to demonstrate that strychnine's action on spinal cord neurons was to block glycine induced hyperpolarization (Curtis et al 1968a). The effects of strychnine were rapid and reversible, and strychnine did not antagonize GABA induced hyperpolarization. In addition, picrotoxin and bicuculline, antagonists of the postsynaptic hyperpolarization induced by GABA, are ineffective at glycine sensitive synapses in the spinal cord (Biscoe 1972, Johnston et al 1972).

Receptor binding studies of glycine and strychnine, based largely on the work of Young and colleagues, have established the nature of their interaction with membrane receptors. Young and Snyder (1973, 1974) found that 3H strychnine binds to rat spinal cord synaptic membranes in a saturable fashion. Scatchard analysis showed a dissociation constant (K_D) for strychnine in the range of 2.6 - 4.0 nM with a maximum number of binding sites

(B_{max}) of 1.8 picomoles/mg protein. This data has been confirmed by others in cow and human spinal cord membrane preparations (Lloyd 1983, DeMontis 1982). 3H strychnine binding is displaceable by both strychnine and glycine. Double reciprocal plots of specific strychnine binding showed a nonlinear displacement of 3H strychnine by glycine indicating positive cooperativity. In contrast, the displacement of 3H strychnine by strychnine is linear. The specificity of 3H strychnine binding to the glycine receptor in tissue slices has proven useful in locating receptor dense areas in the CNS.

1.2c. Glycine Uptake Systems

The variety of metabolic roles served by glycine have made the identification of glycinergic neurons a difficult task. Analysis of the high affinity uptake mechanism for glycine has become the cornerstone by which glycine's neurotransmitter and metabolic roles are distinguished. The uptake of glycine into glycinergic nerve endings may terminate its transmitter actions similar to monoamine neurotransmitter systems. Glycine is primarily synthesized de novo in the CNS from serine by the enzyme Serine Hydroxymethyltransferase (SHMT). The activity of this mitochondrial enzyme closely parallels glycine content in the rat CNS; spinal cord levels of glycine are fourfold higher than in the telencephalon; spinal cord

SHMT is approximately twofold higher as compared to the cortex (Daly 1974). The Glycine Cleavage System (GCS), a complex of three enzymes (Motokawa and Kikuchi 1974), is primarily responsible for the catabolism of glycine in brain. GCS activity varies inversely with glycine content and SHMT activity; GCS activity is twofold higher in the telencephalon than in the spinal cord (Daly 1976). Aprison (1976) has hypothesized that the high glycine levels in the spinal cord and medulla reflect both the neurotransmitter and metabolic pools for glycine, while the low levels in the cortex represent metabolic glycine pools only. This hypothesis is corroborated by neurophysiological studies showing that glycine does not hyperpolarize neurons in the cerebral cortex of vertebrates (Kelly and Krnjevic 1969, Crawford 1963). Extending this hypothesis further, it could be suggested that to maintain levels of glycine for neurotransmission, high affinity specific uptake mechanisms may exist to remove and recycle glycine after it has been released presynaptically. Evidence supporting this hypothesis is provided by Johnston and Iverson (1971). High affinity glycine uptake rates in rat tissue slices and homogenates varies along the neuroaxis directly proportional to glycine levels and SHMT activity. The Km for glycine uptake in the spinal cord is 36 uM compared to 250 uM in the cortex, representing rates for high and low affinity uptake systems respectively. Glycine's high affinity

uptake system is sodium and temperature dependent and specific for glycine and the glycine-like amino acids b-alanine and taurine. GABA and 2-aminoisobutyric acid do not compete with glycine for this uptake system. The low affinity uptake system, also present in the spinal cord and medulla, is relatively nonspecific, thereby allowing a number of small neutral amino acids (i.e. L-a-alanine and 2-aminobutyric acid) to compete with glycine for uptake.

The specific high affinity uptake system for glycine has been utilized to identify glycinergic pathways in the CNS. Hunt et al (1977) demonstrated that when 3H glycine was injected into the nucleus isthmus parvocellularis of the pigeon visual system, 3H glycine was taken up by terminals via a specific high affinity uptake system and then retrogradely transported to a subpopulation of neurons in the pigeon optic tectum. Previous neurophysiological (Barth and Felix 1974), uptake and release (Henke 1976) and receptor binding (Zukin 1975) data suggested that glycine might be the transmitter released in the nucleus isthmus parvocellularis from cell bodies in the optic tectum of the pigeon. The specificity of the uptake and retrograde transport was substantiated by the fact that L-serine and b-alanine but not GABA, were capable of labelling this subpopulation of neurons. In addition, studies with the nonspecific tracers horseradish peroxidase (HRP) or leucine (retrograde and anterograde tracers respectively) indicated that 3H glycine only

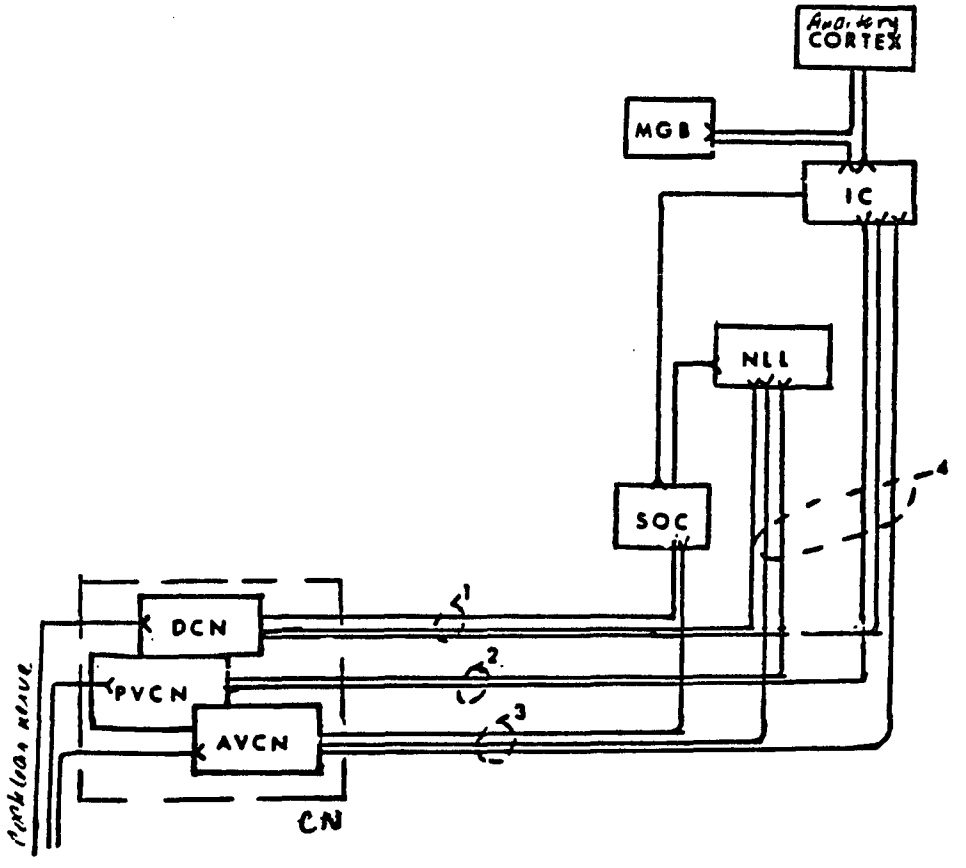
labelled a subset of neurons projecting to the optic tectum. These initial studies laid the foundation for the utilization of the high affinity uptake system in identifying glycine specific neuronal pathways. Carlson (1984), using this technique, has confirmed glycine as the transmitter of inhibitory neurons R#-R14 in the parietovisceral ganglion of Aplysia. In addition, iontophoretic injection of 3H glycine into the abducens nucleus in the cat has been shown to selectively label cell bodies in the medial vestibular nucleus, prepositus hypoglossi and the pontine medullary RF (Spencer and Baker 1985).

1.3. Glycinergic Neurotransmission in the Auditory System of the Lower Brainstem.

1.3a. General Anatomy of the Central Auditory System

The diagram below shows the major pathways of the ascending auditory system of the brainstem. Afferent acoustic information enters the central auditory system via the cochlear nerve, terminating in three major divisions of the cochlear nucleus (CN), located in the pons, the Anteroventral Cochlear Nucleus (AVCN), Posteroventral Cochlear Nucleus (PVCN) and Dorsal Cochlear Nucleus (DCN). The principal cell types in the 3 CN divisions project to the auditory nuclei in the brain stem via three major pathways: 1) the DCN projects to the contralateral Superior Olivary Complex (SOC) and, Nuclei of the Lateral Lemniscus (NLL) located in the pons, and Inferior Colliculus (IC) in the midbrain via the dorsal acoustic stria or Stria of Monakow 2) the PVCN projects to the contralateral NLL and IC via the intermediate acoustic stria or stria of Held, and 3) the AVCN projects to the SOC, NLL and IC via the ventral acoustic stria.
Major descending pathways to the brainstem auditory nuclei originate in the auditory cortex to terminate bilaterally in the IC and ipsilaterally into the medial geniculate body (MGB). The IC in turn projects to the SOC. The

olivocochlear bundle, emanating from the SOC terminates in the contralateral cochlea. All three divisions of the cochlear nuclei also project ipsilaterally to nonauditory nuclei, e.g. DCN projections to the cerebellum (Whitfield 1967).



Pathways:
1 = dorsal acoustic stria (Stria of Monakow)
2 = intermediate acoustic stria (Stria of Held
3 = ventral acoustic stria
4 = Lateral Lemniscus

Efferent fibers of the SOC ascend to rostral auditory centers in the inferior colliculus and MGB, primarily as part of the lateral lemniscus (LL) and adjacent lateral tegmentum, called the "paralemniscal zone" (PL) (Glendenning 1981, Davis et al 1982). Short axons from the MNTB (part of the SOC) also project to the adjacent RF (Warr 1966). The SOC also projects to the nuclei of the lateral lemniscus, which is located within the ascending and descending auditory fibers comprising the LL.

Nuclei of the ascending and descending pathways of the lower brainstem auditory system have been shown to have levels of glycine similar to that found in the spinal cord (Godrey et al 1978). Physiological and pharmacological critera also suggest that glycine is a neurotransmitter within the brainstem auditory system.

1.3b. The Cochlear Nuclei (CN)

There are three major divisions of the CN: the PVCN, AVCN and the DCN. Glycine is unevenly distributed in the rat and cat CN, with levels in the DCN the highest found in the CNS. The PVCN has levels intermediate between the DCN and AVCN, while glycine content in the auditory nerve is minimal (Godfrey et al 1977, 1978). Immunocytochemical analysis with anti-glycine antibodies confirms the uneven distribution of glycine in the CN. In the guinea pig (Wenthold 1987b, 1985) and cat (Adams and Wenthold 1987)

intense glycine immunoreactivity is seen in small and medium cells of the superficial and deep region of the DCN. In the guinea pig, large immunoreactive cells are also present in the AVCN adjacent to the auditory nerve while small immunoreactive cells are localized to the granule cell cap of the CN (Wenthold et al 1987b). An interaction between glycine and GABA, another inhibitory amino acid neurotransmitter, is suggested by the colocalization of glycine and GABA immunoreactivity in the small cells of the DCN (Oberdorfer and Wenthold 1987).

In addition to innervation from the cochlear nerve, all divisions of the CN receive the following centrifugal inputs: the nuclei of the lateral lemniscus, the IC, and the brainstem RF. Fibers from the SOC and the contralateral medial superior olive (MSO), which together comprise the olivocochlear bundle, also innervate the CN. The subdivisions of each CN are also interconnected.

Immunohistochemical identification of glycine in the rat and guinea pig (Wenthold 1987) indicate that glycinergic terminals and postsynaptic receptors also are present in the CN. High affinity uptake of 3H glycine has been demonstrated in the DCN (Schwarz 1981). In the rat (Zarbin et al 1981) and mouse (Frostholm and Rotter 1985), 3H strychnine receptor autoradiography shows high grain densities in the deep (granule) layer of the DCN with moderate densities over the entire nucleus. Few

strychnine receptors were localized to the VCN (AVCN and PVCN) with the exception of the dorsal surface. Immunostaining with antibodies to glycine and glutamic acid decarboxylase (GAD) however has localized intense punctate labelling to fusiform and other cell types throughout the VCN (Wenthold 1987, Adams and Mugnaini 1987). 3H strychnine autoradiography in the gerbil VCN shows similar labelling patterns, including a gradient in receptor density from the dorsal to the ventral surface (Sanes 1987). At the EM level, glycine immunoreactivity in VCN was observed postsynaptic to terminals containing flattened (inhibitory type) vesicles (Altschuler et al 1986). Symmetrical synaptic contacts (indicative of inhibitory synapses) containing flattened vesicles were also seen in the VCN (Cant 1984). Calcium dependent release of C14 glycine in the guinea pig PVCN and DCN after axotomy of the cochlear nerve suggests that the cell bodies of origin of these glycinergic terminals are not located in the cochlea (Staatz-Benson and Potashner 1986). Retrograde transport of HRP combined with glycine immunohistochemistry suggests the existence of a glycinergic pathway connecting the CN of both sides in the guinea pig (Wenthold 1987). In the cat AVCN, this combined technique was used to trace glycinergic inputs from the contralateral CN to medium sized cells of the deep layer of the DCN (Adams and Wenthold 1987).

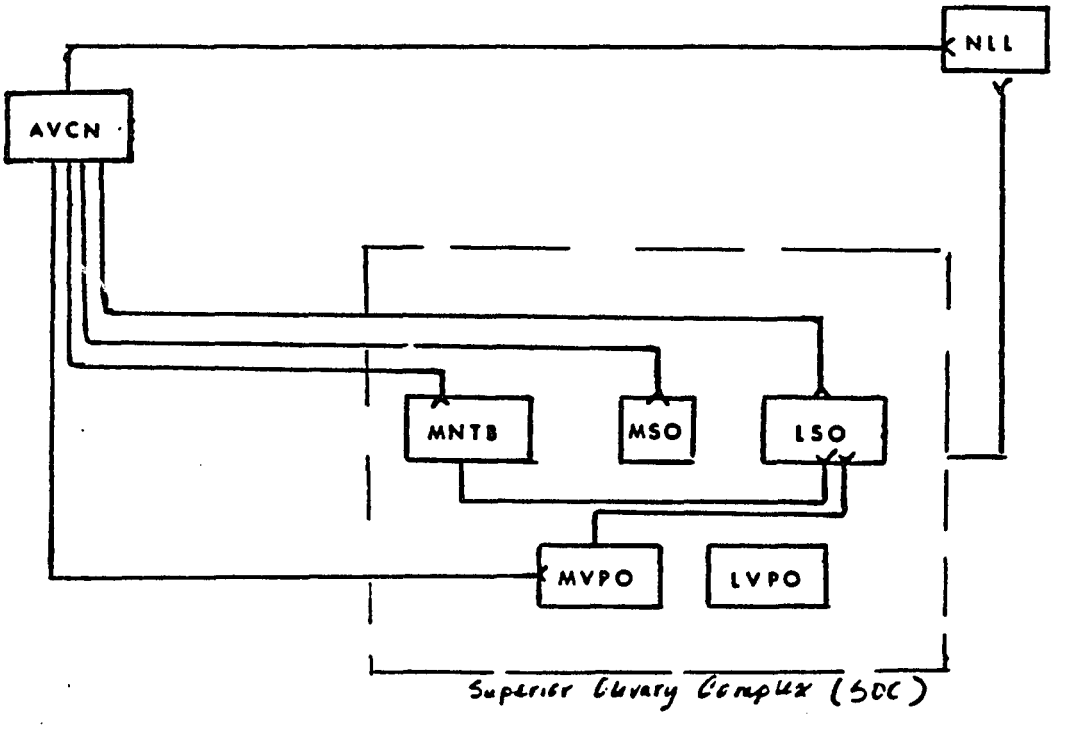
Neurons in the CN are responsive to microionto-

phoretically applied glycine and strychnine (Caspary et al 1979, 1985, Martin et al 1982). Glycine, or the related mino acids, b-alanine and taurine, lead to reversible depression of neuronal firing in the AVCN which is specifically antagonized by strychnine. GABA has similar actions on AVCN neurons but its actions are unaffected by strychnine. The functional importance of glycine-induced depression of AVCN responses however, is controversial. Caspary (1979) found that strychnine did not counteract the normal suppression of the AVCN in response to two consecutive tones of increasing intensity (two tone suppression). On the other hand, Prisig (1968) found intravenous strychnine effective in blocking two tone suppression. In the chinchilla DCN, the fusiform cell, (representing the principal output neuron of the DCN) changes its response characteristics with the iontophoretic application of glycine and strychnine; bicuculline is ineffective (Caspary et al 1979,1985). The source of glycine inhibition to these neurons is unknown but may arise from interneurons within the DCN (Caspary 1986).

1.3c. The Superior Olivary Complex (SOC)

The SOC consists of 5 distinct nuclear groups diagrammed below: the lateral superior olive (LSO), medial superior olive (MSO), medial nucleus of the

trapezoid body (MNTB), and the medioventral (MVPO) and lateroventral (LVPO) periolivary (or preolivary) nuclei (see Figure I for brainstem location). Studies with anti-glycine antibodies have localized densely immunoreactive neurons in the principal and elongate cell populations of the MNTB in the guinea pig and rat (Wenthold 1987b, Campistron et al 1986). Fusiform and oval cells in the LSO and fusiform cells of the superior periolivary nucleus,(an auditory nuclear group lying dorsal to the LSO) are also immunostained. Small (15-20 um) cells of the MVPO and the LVPO were also immunoreactive for glycine in the rat and cat (Adams and Wenthold 1987). In addition to glycine immunoreactivity, all of the above nuclei are immunoreactive for GABA. (Helfert 1987). This suggests, that similar to the CN, GABA and glycine may be co-localized in these nuclei.



Anterograde (3H leucine) and retrograde (HRP) transport studies, summarized in the diagram above, demonstrate afferents to the LSO originating from the ipsilateral AVCN and the MNTB and ipsilateral and contralateral MVPO (Spangler et al 1985, Warr 1966). Affinity purified antibodies to glycine identify many of the axons within the neuropil of LSO as glycinergic; immunoreactive puncta representative of terminals are also seen surrounding LSO somata. The origin of these glycinergic afferents to LSO however have not been identified. Corroborating the existence of glycinergic synapses in LSO is the fact that there is a nonuniform distribution of grains overlying the LSO with 3H strychnine autoradiography (Sanes 1987). As a corrollary to this, antibodies to the 93 kdal subunit of the glycine receptor immunostain the soma and dendrites of LSO neurons (Wenthold 1987b). At the EM level, this immunoreactivity was visualized postsynaptic to terminals containing flattened vesicles, suggestive of inhibitory transmitter terminals.

The LSO and the closely associated MSO are the only two nuclei of the SOC to receive auditory information from both the ipsilateral and contralateral CN. Spherical bushy cells in AVCN project bilaterally to principal cells of the MSO; the MSO firing patterns mimic those projecting to it from the AVCN (Brawer et al 1974, Cant 1986). The LSO receives exclusively ipsilateral input from the AVCN;

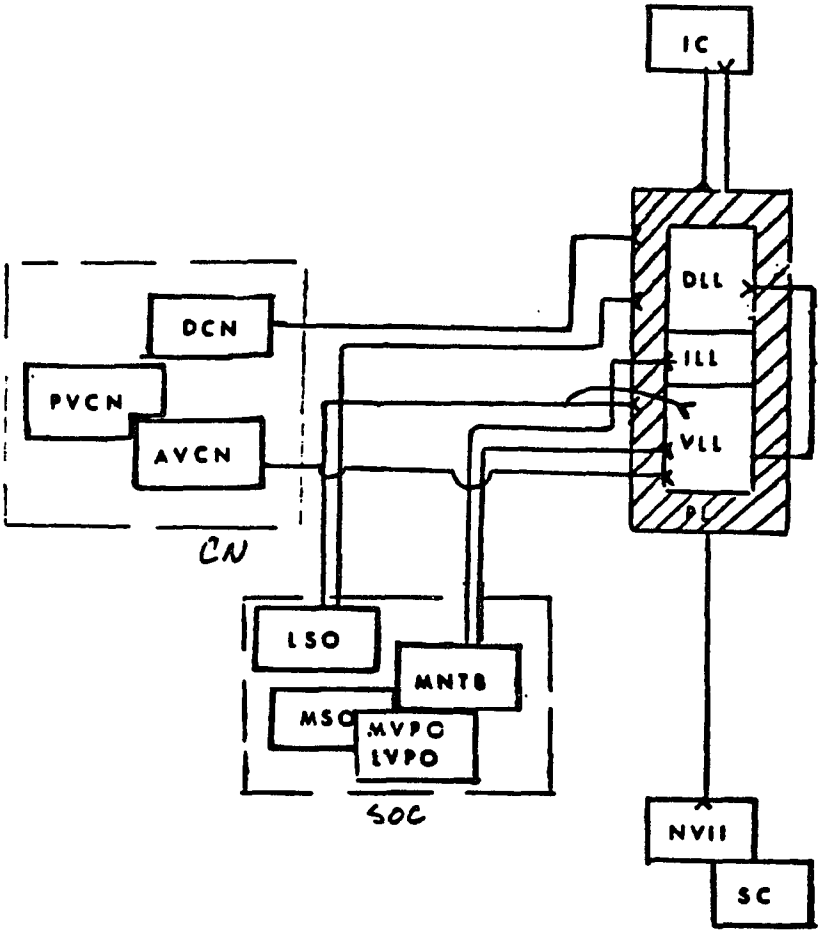
contralateral input is relayed through principal cell axons of the ipsilateral MNTB (Cant 1984). The connections of these two nuclei make them candidates for the integration of binaural temporal and spatial acoustic information entering the brainstem (Tsuchitani 1978). This integration presumably involves the interaction of both excitatory and inhibitory synapses in the MSO and LSO, as both of these nuclei contain type I and type II synaptic endings (Schwartz 1978, Cant 1979). Extracellular recordings from LSO and MSO have established that these neurons are excited by ipsilateral monaural stimulation; by contrast, contralateral tone pips or simultaneous stimulation of both ears leads to a decrease in the responsiveness of these neurons (Tsuchitani 1977, 1978). Moore and Caspary (1983) have shown that binaural inhibition in the LSO may be mediated by glycine. Iontophoretic application of glycine to monaurally activated units in the LSO lead to reduced firing, mimicking natural binaural inhibition in response to sound. Strychnine was effective in counteracting the reduced firing resulting from binaural inhibition and was also capabable of blocking the inhibition of monaural firing induced by iontophoresed glycine. Bicuculline, the GABA-A receptor antagonist was ineffective. These investigators suggested that terminals in the LSO which mediate binaural inhibition may originate from the MNTB. Thus it appears that glycinergic neurotransmission in the

LSO and MSO has functional significance in information processing within the auditory system.

Terminals within SOC nuclei are the large calyses of Held (Stotler 1953) originating from globular and spherical bushy cells of the AVCN (Harrison 1960). Ultrastructural studies in the cat and chinchilla indicate that type I and type II endings are present in these calyses suggesting both inhibitory and excitatory synapses (Schwartz 1980, Perkins 1973). In addition to these endings, two other types of axon terminals, possibly arising from the nuclei of the lateral lemniscus have been distinguished morphologically, (Kudo 1981); somatostatin immuno- reactivity is present in some of these noncalyceal endings in the MNTB (Morely 1985). 3H strychnine autoradiography and antibodies to the glycine receptor (Frostholm and Rotter 1985, Wenthold 1987b) and glycine immunocytochemistry (Wenthold 1985) have ruled out glycinergic terminals or receptors in MNTB, LVPO, and MVPO. GABA immunoreactive terminals however have been demonstrated in the MVPO and LVPO with minor staining in the MNTB. A study using 3H GABA high affinity uptake and subsequent retrograde transport from the CN combined with GABA immunocytochemistry suggests that GABAergic terminals in the CN are MVPO and LVPO efferents (Saint-Marie et al 1986). There are no electrophysiological studies to date to assess the transmitter role of either glycine or GABA in these nuclei. Investigation of the physiological

actions of these two transmitters on MNTB somata would be of particular interest since it has been reported that 3H strychnine receptors have been found in the MNTB (Sanes 1987); inhibitory interactions at the MNTB may influence on binaural inhibition generated in the LSO.

1.2d. The Nuclei of the Lateral Lemniscus (NLL)



The ventral (VLL) and dorsal (DLL) nuclei of the lateral lemniscus shown in the diagram above, are located

within the ascending and descending auditory fibers comprising the lateral lemniscus (LL). An interstitial nucleus (ILL) has been identified in the cat, bat, and most recently in the rat (Glendenning et al 1981, Zook and Casseday 1979, Paxinos and Watson 1986 - see Figure I for location). The VLL in the rat has two subdivisions: a ventrocaudal nucleus lying immediately dorsal to the rostral periolivary nucleus and a larger division rostral and dorsal to the first and separated by fibers of the LL.

Glendenning and colleagues (1981) have conducted an exhaustive study of afferents to the nuclei of the lateral lemniscus (NLL) in the cat using anterograde degeneration, 3H leucine anterograde transport and HRP retrograde transport. These authors suggest that afferents to the nuclei of the lateral lemniscus from the SOC arise primarily from the ipsilateral MNTB, LVPO, MSO and LSO and project virtually exclusively to the ILL and DLL. The contralateral LSO also projected to these nuclei, sending collaterals to the paralemniscal zone (PL) in the lateral tegmentum. Studies of SOC efferents however suggest that the VLL receives a large projection from the MNTB. Spangler and collegues (1985) have examined the projections of the principal cells of the MNTB using the technique of 3H leucine anterograde transport; they found that a major projection of MNTB is to the ipsilateral VLL with minor projections to the ILL. Corroborating this observation, injections of HRP into VLL labelled a

majority of MNTB principal cells. Kudo (1981) also found many labelled fibers in the VLL after 3H leucine injections in the MNTB.

Afferents to the nuclei of the lateral lemniscus from the CN arise primarily from the contralateral AVCN and project exclusively to the VLL (Glendenning et al 1981), although Kudo (1981) has found that the contralateral VCN (including the AVCN), projects to the PL only. The contralateral DCN sends collaterals to the PL only. A small ipsilateral projection from the AVCN to the ventrocaudal VLL has been demonstrated in the macaque (Strominger (1978). In addition to ascending input, centrifugal pathways of the rostral brainstem also send afferents to all divisions of the nuclei of the lateral lemniscus, primarily from the IC, which in turn receives a majority of efferents from the nuclei of the lateral lemniscus (Noback 1985).

Efferents of the nuclei of the lateral lemniscus project primarily to the inferior colliculus and the contralateral nuclei of the lateral lemniscus. The commissure of Probst crossing the midbrain tegmentum links the DLL bilaterallly; in addition the ipsilateral DLL and VLL are connected (Brunso-Bechtold and Thompson 1978). The dorsal and dorsolateral MNTB are also innervated by the DLL and VLL respectively (Spangler 1985). In contrast to the VLL and DLL, the PL projects primarily to nonauditory structures. Martin (1979) has demonstrated

projections from the interstitial nucleus of the lateral lemniscus to the spinal cord, where it may mediate reflex responses to acoustic stimuli. A projection to the trigeminal nucleus (Henkel 1978) from the VLL and /or DLL is proposed to mediate the facial components of the acoustic startle reflex (Davis 1982).

Glycine receptors in the nuclei of the lateral lemniscus has been localized with 3H strychnine autoradiography (Frostholm 1986, Zarbin et al 1981). Dense grain distributions were found in the VLL in amounts greater than that observed in the LSO. By contrast, label was absent in DLL. The source of glycine terminals in VLL are at present unknown. Antisera to GAD, the synthetic enzyme for GABA have localized GAD positive cell bodies and terminals to the DLL in the cat (Adams and Mugnaini 1987). These cell bodies are thought to project to the IC, which also contains many GAD immunoreactive terminals and cell bodies (Roberts et al 1985). Only scattered small cells and terminals in VLL are GAD immunoreactive.

The segregation of glycinergic and GABAergic neurotransmission in the two main divisions of nuclei of the lateral lemniscus may be functionally significant to inhibitory interactions in auditory pathways. The physiological roles for glycine and GABA in the nuclei of the lateral lemniscus however may be inferrred from studies on monaural and binaural stimulation. Aitken and collegues (1969) have provided the only study to date on

the reponse characteristics of single units in the nuclei of the lateral lemniscus of the cat. The response characteristics of DLL neurons are influenced by binaural presentation of tones while neurons in VLL are primarily modulated by contralateral monaural stimulation. At sound intensities greater than 45 dB, unit activities of VLL neurons display fewer spikes during the latter part of the tone pip. Increasing the intensity above 45 dB results in a reduction in the number of spikes at earlier stages of the response and finally the response itself is delayed. This may suggest that an inhibitory process with a longer onset latency modulates unit responsiveness. At higher frequencies, the latency of this inhibition may progressively decrease affecting earlier components of the VLL response. By contrast, for DLL units, total suppression of spontaneous activity occurs beyond the duration of the tone above 45 dB; suggestive of more complex interactions. The nature of inhibition in VLL and DLL units may reflect glycinergic and/or GABAergic modulation.

1.4. The Reticular Formation (RF)

Historically, the term " reticular formation " arose from observations of classical neuroanatomists before the turn of the century. They noted areas in the vertebrate brain with a netlike structure composed of cells of various sizes traversed by fibers travelling in many directions. These areas were termed reticular, and they were found in areas throughout the brain. Emphasis on the RF of the brainstem arose from Nissl staining and Golgi impregnation studies in vertebrates which revealed particularly large areas of the brainstem devoted to this reticular structure. These areas comprised by and large the medial two-thirds of the mesencephalon, pons and medulla and were thought to serve as an anatomic matrix for the morphologically distinct cranial nerve nuclei.

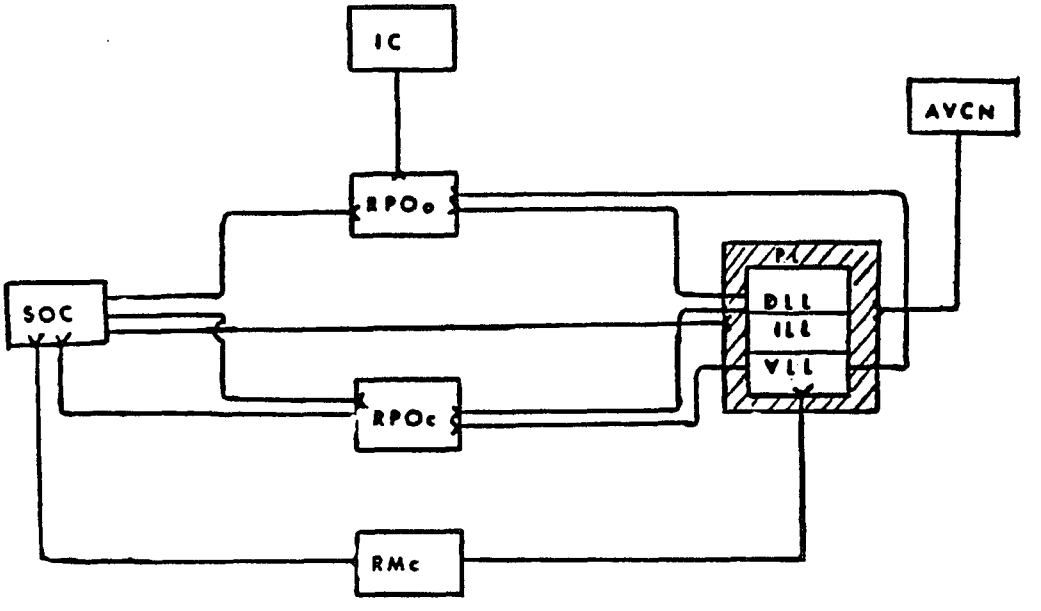
The diffuse morphology of the so called reticular core of the brainstem can in fact, be subdivided into discrete nuclear groups based upon morphological and cytoarchitectonic features. Newman (1985 a,b) reexamined the somatodendroarchitectonics of the brainstem reticular formation using a modified Golgi technique, horseradish peroxidase, and Nissl staining in the rat. These complementary techniques revealed unique perikaryal shapes and dendritic arrangments characteristic of individual nuclear groups. The degree of variation in dendritic orientation within some nuclei necessitated further

subdivision of these areas.

According to Newman's studies, there are 5 major reticular nuclei that are in close proximity to the nuclei of the central auditory system of the lower brainstem. The most caudal group is the nucleus reticularis gigantocellularis (RGc) in the medial medulla. The rostral extension of RGc can be found at the caudalmost region of the SOC which lies ventral to it. Lateral to the RGc at medullary and pontine levels is the nucleus reticularis parvocellularis (RPc). The nucleus reticularis pontis caudalis (RPOc) and the more rostral nucleus reticularis pontis oralis (RPOo) replace RGc at the level of the pons and mesencephalon (see Figure I p. 113). Each of these nuclei are subdivided into a medial and lateral area both of which lie dorsal to the SOC (referred to pars alpha and pars beta respectively for RPOc). The RPOo is found dorsomedial to the rostral periolivary nucleus (RPO) and medial to the nuclei of the lateral lemniscus. The nucleus reticularis magnocellularis (RMc) pars alpha and pars beta are located dorsomedial to the MNTB at medullary and pontine levels (see Figure I p. 113 for location of reticular nuclei). Rostral to these 5 RF nuclei, 2 nuclear groups, the nucleus reticularis pedunculopontinus pars compacta and pars dissipatus border the VLL and DLL at midbrain levels. These 2 reticular nuclei and the RPOo above are functionally identical with the diffuse "ascending

reticular activating system", (ARAS) defined in the lesion studies of Morruzi and Magoun (1949). The relationship of these ARAS structures to the auditory system is discussed below.

1.3a. RF/Auditory Pathways



Sensorimotor integration of acoustic impulses to motor output pathways probably has its neuroanatomical basis in the connections between the RF and the brainstem auditory system (see diagram above). Early studies reported preterminal degeneration in the region of the cat RPOc and RMc following lesions of the periolivary nuclei of the SOC

(Stotler 1953). However, HRP iontophoresed into the RPOc leads to cell body labelling in the contralateral nuclei of the lateral lemniscus with no labelling of SOC or any other rat auditory nuclei (Davis et al 1982). However, lesion studies of the nuclei of the lateral lemniscus suggest that the nuclei of the lateral lemniscus sends fibers to the more rostral RPOo (Stotler 1953). The PL, medial to the nuclei of the lateral lemniscus, is of interest in this discussion of RF-auditory system connections because it has been defined as a secondary ascending auditory pathway (Morest 1969) and as part of the lateral tegmentum, which is coextensive with the lateral division of the RPOo. Heavy fiber and terminal degeneration is found in the PL after lesions of the ipsilateral SOC. Furthermore, HRP injections into the VLL, which usually involve the PL, produce cell body labelling in the contralateral LSO of the SOC (Spangler et al 1985). Anterograde degeneration studies also suggest that other afferents to the PL zone are from the contralateral CN (Glendenning et al 1981). The identification of the PL as reticular or auditory however, is subject to the discretion of the anatomist or physiologist.

The lateral RPOo also receives afferent input from the SOC as well as the nucleus sagulum (a midbrain auditory nucleus) (Irvine and Jackson 1983). Fibers from cell bodies in the inferior colliculus projecting to lateral

pontine RF have been demonstrated in the guinea pig (Woolard 1940) monkey (Mortimer 1975) and the cat (Powell 1969, Kawamura 1975). This pathway is thought to mediate acoustic input to the parafloculus and floculus of the cerebellum.

Very few studies have evaluated connections between RF efferent systems and brainstem auditory nuclei. The existence of extensive collaterals of most reticular nuclei presuppose terminations in most all cranial nerve nuclei (Brodal 1957, Scheibel and Scheibel 1958). Specific terminations from RF nuclei onto auditory nuclei have only been adequately described for the inferior colliculus (Noback 1985). In addition, the RMc is retrogradely labelled after HRP injections into the ipsilateral VLL (Spangler et al 1985) and the neurons of the DCN have been shown to respond to ARAS stimulation (see section 1.4a). The ARAS, primarily the rostral pontine nuclei, receive afferents from the inferior colliculus (Brodal 1981). This connection may be relevant to audiogenic seizure models as discussed in section 1.4c. Finally, some fibers of the MNTB project to the RPOc (Warr 1966).

What then, is the evidence for glycinergic pathways linking the RF and the auditory system? Moderate densitites of 3H strychnine binding sites are found in the pontine and medullary RF in the mouse (Frosholm and Rotter 1985) and rat (Zarbin et al 1981). Glycinergic cell

bodies have recently been demonstrated in the area rostral and medial to the MNTB corresponding to the RMc (Pourcho and Goebel 1985).

Electrophysiological studies demonstrating the role of glycine in linking the auditory system and the RF are limited in number. Stimulation of brainstem auditory nuclei (with the exception of the IC) has been correlated with enhanced responses in RF neurons in cats (Faingold et al 1983a). The systemic administration of strychnine and bicuculline, the respective antagonists of glycine and GABA, had their greatest effects (inhibition) on RF neurons when RF units were activated by sensory stimuli (including sound) (Faingold et al 1983). To localize these effects Faingold et al (1984) recorded extracellular potentials in the rat RPOo (or cat ARAS) in response to sensory stimuli including auditory 'clicks'. Glycine and GABA were both effective in decreasing spontaneous firing of RPOo neurons; reversal of these effects was greatest with strychnine application. Sequential iontophoresis of strychnine and bicuculline showed that their effects were additive. In addition, the effects of strychnine application were reversed by both glycine and GABA. Glycine receptors have been demonstrated within the RF, and GABA and glycine have been shown to coexist in certain neuronal populations (Oberdorfer and Wenthold 1987); thus it is possible that the seemingly interchangeable roles of these transmitters may be mediated by functional postsynaptic

receptors on RPOo neurons.

In conclusion, glycine is a neurotransmitter in the auditory system and RF where it mediates neuronal responsiveness to acoustic stimulation. It remains to be considered how glycinergic neurotransmission is involved in sensorimotor integration jointly mediated by these two systems.

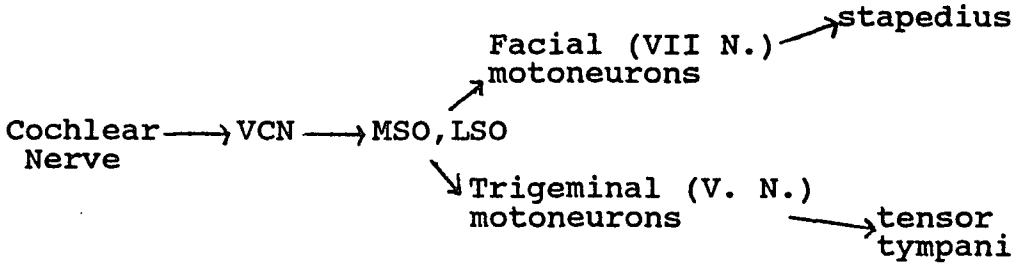
1.5. Behavioral correlates of glycinergic neurotranmission in the RF and Central Auditory System

The acoustic middle ear reflex may be viewed as a model for studying glycinergic effects on the brainstem auditory system and RF. Acoustic startle and sound sensitive seizures represent a continuum of this simple reflexive behavior and exemplify the importance of the chemical neuroanatomy of glycine in the coordination of auditory input with motor output.

1.5a. The Acoustic Middle Ear Reflex (AR)

The AR is a brainstem feedback loop which serves to attenuate afferent acoustic input to the central auditory system when it is above a certain threshold. The activity of this reflex is measured by monitoring the stimulus induced, time-locked contraction (defined as a change in immitance) of the stapedius (St) and tensor tympani (TT) muscles which indirectly control tension of the tympanic membrane of the middle ear. In animals and human subjects, the presentation of monaural tones results in immitance changes in both the ipsilateral and contra-lateral St and TT muscles (in humans,the TT only activated stimulus intensities sufficient to induce a startle

reaction - Brodal 1981, Djupesland 1980). The anatomic organization of the uncrossed (ipsilateral) AR as originally defined by Borg (1972, 1973) consists of a 3 or 4 neuron chain :



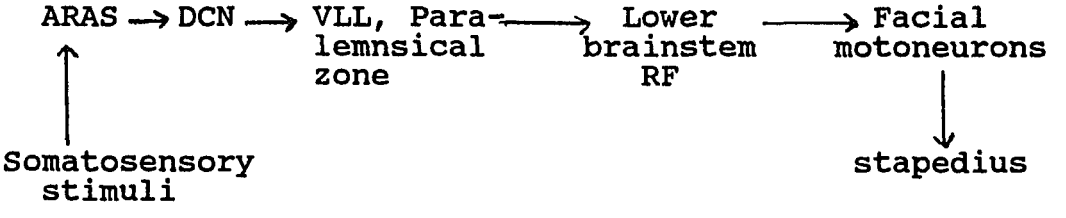
Of interest to the present discussion is the polysynaptic AR (pAR), which has been anatomically and physiologically distinguished from the AR by its connections to the RF. The pAR is slowly conducting because of connections with the RF; RF connections most likely lead to the increased latency of reflex activation of pAR (Borg 1972). In addition, this reflex is very sensitive to the effects of barbiturate anaesthesia (Borg 1972, Borg and Moller 1973) which is also suggestive of RF involvement. Single and multiple unit recordings of auditory evoked activity in the anesthetized cat demonstrate that RF units are more depressed than that of the cochlea (Mori 1968) and the lower brainstem auditory nuclei (Holstein et al 1969) in the cat. Anatomically, the RF units with the most influence on the pAR were localized to the ARAS. The level of activity in the ARAS

is directly related to the strength of the AR in the cat (Holstein 1969) and the rabbit (Borg and Moller 1975). Corollary to this, tonic activity in the ARAS is profoundly influenced by acoustic stimuli (see review by Rossi and Zanchetti 1957) and barbiturate anesthetics (Borg and Moller 1975). Gonzalez-Lima and Scheich (1984) have expanded on this concept of reciprocal interactions between the auditory system and ARAS in a 2-deoxyglucose study in rats either electrically stimulated in the ARAS or given 4-5 kHz tones. ARAS stimulation lead to an increase in 2-deoxyglucose uptake above control levels in the DCN and the VLL. 4-5 kHz tones by contrast lead to an increase in label in the LSO and MSO only. The pAR, although not measured in this study, might be expected to increase its response amplitude in parallel with the heightened activity of the ARAS.

Other evidence favoring ARAS involvement in the pAR is suggested by the the fact that initiation of the AR occurs in response to tactile or electrical stimulation or air puffs delivered to specific areas of the face; this results in bilateral activation of TT and St muscles in humans (Djupesland 1980). In the unrestrained cat, auditory as well as skeletal and facial stimulation evoke responses in RF neuronal populations throughout the rostrocaudal extent of the reticular core. Neuroanatomical evidence has corroborated multisensory afferents to RF neurons (Siegel 1983, Schulz 1983).

Single unit recordings in the mesencephalic and rostral pontine extensions of the ARAS have demonstrated that a majority of RF neurons in these areas are responsive to acoustic stimulation over a broad range of frequencies. In addition, 60% of sound responsive units were also activated by somatosensory or visual stimuli (Irvine 1983).

The longer latency pAR then, is modulated at the level of the pontomesencephalic RF (site of the ARAS) which receives impulses derived from numerous sensory modalities. With respect to acoustic input, the ARAS relays information taken from a broad spectrum of tones, which suggests that the ARAS is concerned primarily with the fluctuation in acoustic input and not its frequency or tonal qualities. Based upon this data and the results of the 2-deoxyglucose study above, the longer latency pathway (pAR) outlined by Borg may be redefined as follows:



The role of glycinergic neurotransmission within this circuit is implied from observations in humans suffering

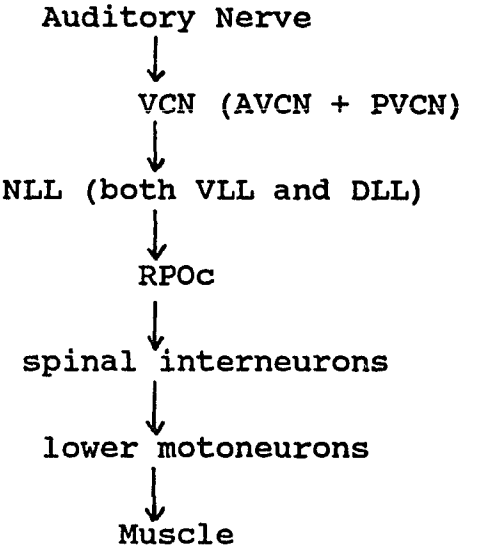
from strychnine toxicity. Ingestion of subconvulsant doses of strychnine result in increased auditory awareness and responsiveness to sound (Gosselin 1976, Clark 1938). Rats receiving subconvulsant and convulsant doses of strychnine also exhibit heightened responses to sound stimuli; at convulsant doses, such stimuli can initiate and aggravate seizure activity (personal observations). Strychnine administered systemically or iontophoresed into the mesencephalic RF (coextensive with the ARAS) leads to localized sensory response enhancement of ARAS units in the cat; glycine reverses this effect (Faingold 1984). As reviewed above, 3H strychnine autoradiography has localized glycine receptors to the DCN, VLL and PL, and the brainstem RF, and glycine antisera has localized glycine positive cell bodies and terminals (presumably interneurons) in the DCN. Glycine positive cell bodies have also been demonstrated in the dorsolateral aspect of the LSO (Adams and Wenthold 1987) cited as one area of origin of stapedial motoneurons (Lyon 1978). Single unit responses of DCN fusiform cells have inhibitory characteristics which are strychnine sensitive and thus may be mediated by glycine (Caspary 1987); these responses are also diminshed by barbiturate anaesthesia (Evans and Nelson 1973) to a greater extent than responses in the VCN.

Glycinergic neurotransmission in the pAR is thus involved in the arousal components of the circuit via the

ARAS; polysynaptic connections of pAR within the lower brainstem RF may also use some of the same neural circuits as involved in the acoustic startle reflex and sound sensitive seizures.

1.5b. The Acoustic Startle Reflex (ASR)

The ASR, as described by Forbes and Sherrington (1914), is a generalized muscle reflex response to acoustic stimuli. Since ASR could be elicited in cats decerebrated at midcollicular levels, it was suggested that ASR had its origin within the lower brainstem. ASR, as measured by electromyographic (EMG) recordings from the gastrocnemius muscle (Prosser and Hunter 1936, Szabo and Hazafi 1965) or the quadriceps femoris muscle (Davis et al 1982), appears to be dependent upon transmission via the caudal pontine and medullary RF. Davis et al (1982) found that lesions confined to the ventral RPOc/rostral RGc were effective in diminishing EMG amplitude, while lesions of more rostral RF nuclei were ineffective. Furthermore direct stimulation of the ventral RPOc was effective in producing the ASR. Injections of the retrograde tracer HRP into the RPOc result in labelling of cell bodies in the contralateral DLL and VLL. The VLL in these studies was defined as the second synapse in a 5 synapse, 7 millisecond circuit:



The startle circuit briefly outlined above is of interest in light of its similarity to the pAR. Of note are the fact that 1) both reflexes are highly sensitive to auditory stimuli, but are also activated by somatosensory stimuli; 2) both reflexes are mediated by synapses in the VLL; and finally, 3) the RF is important in the activation of both reflexes. The type of the reflex response (pAR, ASR or audiogenic seizure) outcome may rest solely on the RF area activated and the intensity of the afferent auditory stimulus. The ARAS may act as a sensory filter for afferent acoustic input, while the more ventral RPOc subserves the motor commands of the sensory filter. When the sensory stimulus is of sufficient intensity, both the ARAS and the pontomedullary RF may be activated to affect motor output. The pAR and ASR may be

activated simultaneously by integration at the level of the VLL. (Auditory input sufficient to drive the ASR transmits information to the VLL/PL area where it drives both the motor component of the ASR and the facial motoneurons of the pAR). Davis (1982) points out that the ASR can also be activated by high electrical stimulation of the RPOo. The authors suggest that activation of the startle reflex from RPOo stimulation may result from current spread to the RPOc. An alternative explanation for this data is that electrical stimulation of the RPOo has a higher threshold for activation of the startle reflex. Generation of the startle reflex from the RPOo at such a current intensity may also activate the pAR.

The simultaneous activation of these reflexes is of interest in light of observed facial movements that occur with the ASR and pAR. Cutaneous stimulation of the side of the face up to and including the pinna of the ear can activate the pAR (Djupesland 1980)(mediated by the RF) or the ASR (Davis 1980). Thus the facial components of the ASR suggest that pAR activation is also occurring. Of interest to this discussion is the finding by Siegel (1983b) that a subclass of facial movement responsive RF cells increase their discharge rate in response to auditory stimulation. These cells are located in the area of the RPOc where electrical stimulation elicits acoustic startle. The cells in this area were found to project to the facial motoneuron pool (Peterson 1979).

The above data suggests that the RPOc nucleus of the ASR circuit is also a relay nucleus for the pAR.

The effect of the glycine antagonist strychnine on ASR has been investigated. Systemic, intrathecal or intracisternal administration of subconvulsant doses of strychnine to rats leads to an increase in whole body ASR as measured by the velocity of displacement of animals in a suspended cage (Kehne et al 1981). The presence of glycine receptors and cell bodies in the brainstem auditory system and RF, as well as the effect of glycine antagonism on whole body startle in the above study suggests that glycine may provide tonic inhibition of ASR at certain points along the startle pathway. Given the similarities between ASR and pAR, it would be of interest to determine the contribution of the pAR to the increase in startle amplitude in these strychnine treated animals.

To summarize, the ASR may be regarded as an extension of the AR, transmitting acoustic and other sensory information via similar circuits in the medullary RF and lower brainstem auditory system to affect motor output. The normal activity of both reflexes appear to be dependent upon intact glycinergic neurotransmission in auditory / RF pathways.

1.5c. Sound Sensitive Seizures

The disinhibitory effect of subconvulsant doses of strychnine on the pAR and ASR is of interest because of their possible involvement in the genesis of reticular reflex myoclonus and sound sensitive seizures induced by strychnine. In rats, systemic administration of subconvulsant doses of strychnine (1.00 to 2.5 mg/kg) leads to reticular reflex myoclonus which can be triggered by acoustic or somatosensory stimulation. At convulsant doses of strychnine, auditory stimulation can initiate myoclonus and tonic-clonic seizures (Hunter et al 1987). Reticular reflex myoclonus has been defined as " a fragment of a type of generalized epilepsy (Hallet 1977) and its site of origin in cats has been localized to the medullary RF (MRF), especially the RGc (Zuckerman and Glaser 1972). In the rat, direct injection of strychnine (Chung and Van Woert 1984) or the less potent antagonist brucine (Chung, personal communication) into the MRF including RGc and RPOc elicits stimulus sensitive reticular reflex myoclonus. GABA antagonists injected into the MRF do not produce myoclonus. Reticular reflex myoclonus is blocked by oral administration of milacemide (Hunter et al 1986), a glycine precursor (Van Dorsser et al 1983, Christophe et al 1983).

Inasmuch as strychnine may elicit a fragment of sound sensitive seizures or the seizure itself depending on the

dosage given, strychnine induced enhancement of the AR and/or ASR may represent a component of strychnine induced audiosensitive seizures; again dependent upon the extent of glycine antagonism.

Sound induced seizures have been reported in cats, rats, rabbits, dogs and man for over 6 decades (see Swinyard 1972 for review). Progress in determining the etiology of this phenomenon has been facilitated by the colonization of rats and mice with a genetic predisposition to acoustically induced seizures. The genetically epilepsy prone rat (GEPR) and DBA/2J mouse are two strains that have been extensively studied with regard to the anatomy and pharmacology underlying audiogenic seizures (AGS). Centrally, paroxysmal EEG activity seen in AGS animals before, during and after AGS episodes has been localized to the brainstem (Kesner 1966, Browning 1985, Jobe 1981). Peripherally, these animals have hearing impairments most likely due to the degeneration of inner and outer hair cells of the cochlea (Portmann 1971, Glenn et al 1979). Behaviorally, electric stimulation delivered to central auditory and RF nuclei in AGS rodents induce seizures at lower thresholds than for non-susceptible animals; thus in the AGS animal reduced auditory input to the CNS may cause a compensatory response enhancement of RF and auditory neurons to acoustic stimuli (Kesner 1966, Maxon and Cowan 1976).

Lesion and stimulation studies have begun to define

the neuroanatomical pathways involved in AGS genesis. Lesions of the VCN (see Browning 1986) and inferior colliculus (Kesner 1966, Willot et al 1980) attenuate or abolish AGS in rodents. No studies on the SOC or the nuclei of the lateral lemniscus have been reported. A number of studies have implicated the pontomedullary RF in the genesis of AGS (Kesner 1966, Burnham 1985). The most marked attenuation of AGS has been produced by lesions of the RPOo. All phases of the AGS episode from initial running to maximal seizure (tonic extension of body with no refractory or clonic period) can be abolished by electrolytic or mechanical lesions of RPOo (Browning et al 1985).

Neurotransmitter studies in AGS animals suggest that there is a deficit in inhibitory neurotransmission that results in RF neuronal hyperexcitability and enhanced motor output via the reticulospinal pathways. the following evidence suggests that desensitization of GABA receptors on inferior colliculus efferents to the RF leads to an excess of excitatory output (Faingold et al 1986): 1) There is an increase in GABA immunoreactive terminals in the inferior colliculus of the GEPR (Roberts et al 1985), probably causing the down-regulation of GABA receptors,and 2) the inferior colliculus projects to RF neurons (Scheibel and Scheibel 1958).

Only one report to date has examined the effects of glycine in AGS. Systemic administration of 11.25g/kg body

weight of glycine to DBA/2J mice significantly reduced the incidence and severity of audiogenic seizures (Toth and Lathja 1984).

1.5d. Summary

Glycinergic synapses within the central auditory system and RF are most likely relevant to audiomotor reflexes and seizure. Disinhibition of glycinergic neurotransmission, e.g. by strychnine administration, leads to enhanced pAR, ASR and an increased incidence of seizures. Knowledge of the glycinergic pathways common to these three systems would provide a basis for understanding sound induced audiomotor reflexes and behaviors in the normal animal and may provide new therapeutic approaches to auditory deficits of genetic origin or occurring as a result of the aging process.

II. METHODS

2.1. AUTORADIOGRAPHIC STUDIES

2.1a. Animals/Stereotaxic injections

Twenty six male Sprague Dawley rats (Ace Animals, Boyerton, PA) weighing 250-390 grams were used (see Table 1 p. 104 for details on experimental subjects). Animals were housed in groups of 5 and were maintained on a 12 hour light/dark cycle receiving food and water ad libitum.

Stereotaxic surgery was performed during dark cycle hours. For pressure injections, animals were given an intraperitoneal injection of Chloropent (Fort Dodge) containing 14mg/kg chloral hydrate and 3 mg/kg pentobarbital. For iontophoretic injections, rats were given an intraperitoneal injection of 30 mg/kg Ketamine (Parke Davis) followed by an intramuscular injection of 30 mg/kg Rompun (Haver-Lockhart). Booster injections of 15mg/kg Rompun were given when necessary. Stereotaxic injections into the nuclei of the central auditory system and the medullary and pontine RF were made at an angle of 90 degrees to the horizontal plane of the skull (see Appendix I p. 132 for injection sites and coordinates). All stereotaxic coordinates are based on the atlas of Paxinos and Watson (1982, 1986). Sham injections of 1 - 1.5 ul of 0.1% methylene blue were performed for each injection site prior to iontophoretic or pressure

injection of tracer.(See Appendix I p. 132 for injection coordinates)

2.1b. Tracer Preparation/Deposition

For pressure injections, 3H glycine with a specific activity of 44.3 Ci/mmole in 1 uCi/ul (New England Nuclear) was dried under a gentle stream of nitrogen and reconstituted in 0.01 N HCl to a concentration of 50 uCi/ul for injection, representing 1 mM glycine. Reconstituted 3H glycine was injected using glass micropipettes pulled to achieve an inner diameter of 110 um, outer diameter of 200 um and a length greater than 3 mm. Micropipettes were calibrated to deliver 50 nl over an 8 minute period. Five additional minutes were allowed for diffusion of the tracer before micropipette was withdrawn.

For iontophoretic injections, the parameters governing deposition of glycine were first tested on tissue blocks. Fresh slabs of rat brainstem was placed in a shallow dish containing 1 ml of isotonic saline. 3H glycine concentrated to 25 uCi/ul was iontophoresed into tissue slabs at a constant current of 1 uA at time periods of 5 to 30 minutes. Slabs were homogenized in 0.1N HCl and the supernatant and solubilized pellet were subjected to liquid scintillation spectroscopy. For actual injections, a similar protocol was used. 3H glycine dried under a

gentle stream of nitrogen was reconstituted in 0.01 N HAc to a concentration of 50 uCi/ul, corresponding to a concentration of 1 mM. Micropipettes were prepared from microfilament capillary tubing (A-M Systems). They were pulled to achieve an inner tip diameter of 6 - 8 um and a length greater than 8 mm. 3H glycine was iontophoresed into anesthetized animals using a constant current generator (Micro-Iontophoresis Programmer Model 161 WPI Instruments). A retaining current of 1 nA was used while the micropipette was being lowered to the site of injection. A current of 1 uA was used over 30 minutes to deposit 3H glycine. The micropipette was left in place for an additional 5 minutes with 1 nA retaining current to allow for diffusion of the deposited glycine.

2.1c. Perfusion/Fixation

6 - 18 hours after stereotaxic injections, animals were deeply anesthetized with Chloropent and transcardially perfused with 500 mls of 0.1 M phosphate buffer containing 5% glutaraldehyde and 0.05% MgSO4 (pH 7.4) at 25 degrees C. A vascular rinse with Ringer solution at 25 degrees C. preceeded perfusion with fixative. Rats were decapitated and the in situ CNS and spinal cord preparations were placed in fresh fixative for 2 hours at room temperature or at 4 degrees overnight. Brain and spinal cord were then removed. Tissue was

prepared for cryostat or freezing stage sliding microtome sectioning by immersion in 30% sucrose/fixative and sotrage at 4 degrees C. until tissue was completely infiltrated such that it sank.

2.1d. Autoradiographic Processing

Sucrose/fixative infiltrated brain and spinal cord were sectioned at 16 or 20 microns with a cryostat or freezing stage microtome. Every fourth or tenth section was thaw mounted onto slides subbed in gelatin chrome alum and allowed to dry. Prepared slides for autoradiography were thoroughly rinsed in distilled water and dried in a 37 degree oven. Dried slides were coated with photographic emulsion (equal parts of 15% glycerol in distilled water and Kodak NTB-2 photographic emulsion) at 50 degrees in a darkroom. After drying, slides were transferred to light tight slide boxes and kept in a dessicator for 6 - 8 weeks under vacuum at 4 degrees. Slides were developed in Kodak D-19 photographic developer diluted 1:1 with distilled water. Sections were counterstained with cresylecht violet and coverslipped prior to viewing under dark and bright field optics.

2.2. HORSERADISH PEROXIDASE HISTOCHEMISTRY AND AUTORADIOGRAPHY - DUAL INJECTION

2.2a. Stereotaxic Injection

A pressure injection of Horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA) was injected into the ventral nucleus of the lateral lemniscus (VLL) of one rat. Iontophoretic injection of 3H glycine into the ipsilateral lateral superior olive (LSO) preceeded HRP-WGA injection.

2.2b. Tracer Preparation/Deposition

A pressure injection of HRP-WGA (lectin from Triticum vulgaris, Peroxidase labelled - HRP Wheat germ agglutinin (Sigma Type VI) was used. 600 nl of a 2% solution of HRP-WGA (weight/volume) in 0.1 M phosphate buffer (pH 7.4) was injected into VLL over 5 minutes using a 10 ul Hamilton syringe. The needle was left in place for an extra 5 minutes to allow for diffusion of HRP-WGA.

3H glycine was injected into the LSO by iontophoresis as described above.

2.2c. Perfusion/Fixation

Eighteen hours following the completion of stereotaxic injection, the rat was deeply anesthetized with an intraperitoneal injection of Chloropent and transcardially perfused with 0.1M phoshate buffered saline at 25 degrees followed by fixation with a mixture of 0.5% pure formaldehyde and 3% glutaraldehyde (buffered to pH 7.4 with sodium phosphate and containing 2.5% beta-D-glucose and 0.1% MgSO4). Fixation was immediately followed by perfusion with 10, 20 and 30% phosphate buffered sucrose at 4 degrees. Brain was removed and stored in 30% sucrose at 4 degrees prior to sectioning on a freezing stage microtome.

2.2d. Processing

20 or 30 micron frozen sections were collected into 0.1M phosphate buffer and alternate sections were processed for either HRP-WGA detection or processed for autoradiography. Sections for HRP-WGA were incubated in 5% acetate buffer (pH 3.3) and reaction product visualized with the chromogen 3,3'5,5' tetramethylbenzidine (TMB). The chromogen was stabilized with sodium nitroferricyanide. Mounted sections were dehydrated or stained with neutral red and coverslipped. Sections were viewed using bright and darkfield optics.

Sections designated for autoradiography were of two groups: unreacted tissue and those already processed for HRP-WGA. Sections were processed for autoradiography as described above (2.1d). Sections were viewed under bright and darkfield optics and compared with sections processed only for HRP-WGA.

2.3. GLYCINE IMMUNOCYTOCHEMISTRY

2.3a. Animals/Surgery

450 - 500 gram male Sprague Dawley rats were used. Animals were anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital.

2.3b. Perfusion/Fixation

Rats were given a bolus arterial injection of heparin/sodium nitrite and then perfused via the abdominal aorta with isotonic saline at room temperature. Fixative composed of 1% paraformaldehyde and 1% glutaraldehyde at room temperature was injected following the saline wash. Following fixation, the brain was removed and postfixed by cutting tissue into small blocks and immersing them in fresh fixative at 4 degrees C. for 60 minutes. Blocks were prepared for sectioning by infiltration successively with 7%, 10% and 20% sucrose in phosphate buffer. Tissue was then freeze-thawed twice in isopentane cooled in a dry ice acetone bath to enhance penetration of the primary antibody. Blocks were sectioned on a vibratome into 50 um thick sections.

2.3c. Processing

Immunocytochemical visualization of tissue glycine was accomplished using the peroxidase-antiperoxidase (PAP) technique. Tissue sections treated with normal sheep serum were incubated with the primary antibody - a rabbit antibody to glycine conjugated to bovine serum albumin via a glutaraldehyde linkage (a gift of Dr. Pedro Pasik). A final antibody dilution of 1/1750 was used for the studies. Incubation proceeded for 18 hours. A secondary antibody raised in sheep against rabbit sera (SHANTI Cappel Labs.) (dilution of 1:40) was incubated with tissue sections to enhance visualization of reaction product. Rabbit PAP complex (Cappel Labs) at a dilution of 1:100 was complexed with the secondary antibody and then treated tissue sections were reacted with 3,3 diaminobenzidine (DAB, Sigma)/H2O2 . Tissue sections were dry mounted on clean slides and counterstained with methyl green.

Controls for nonspecific staining of the antibody were assessed by applying antibody that had been deactivated as follows: primary antibody was incubated with glycine conjugated to bovine serum albumin and coprecipitated with an antibody to bovine serum albumin.

III. RESULTS

3.1. 3H Glycine Iontophoretic Injections - General

The relationship between duration of iontophoretic ejection and the amount of 3H glycine deposited is presented in Figure III p 114. The amount of radioactivity delivered with a constant current source over 5 - 30 minutes into slabs of rat brain tissue is linearly related to the duration of ejection (r=0.97). Using 1 uA and a 25 uCi/ul solution, a 30 minute ejection deposited approx- imately 0.3 uCi of 3H glycine. Since the specific activity of the solution was 44.3 Ci/mmole, this represents 6 picomoles of glycine. Shubert and Hollander (1972) have hypothesized that iontophoresed amino acids, including glycine, diffuse throughout of interstitial fluid in the section to approximately 500 microns in diameter. Based on this data and the present observations the injection site was hypothesized to diffuse throughout a cylindrical volume measuring 500 um in diameter and 1 mm rostro-caudally. Six picomoles of glycine in this volume represents a concentration of 31 uM. This concentration is within the range of 40 uM for the high affinity uptake for glycine in spinal cord slices and homogenates (Johnston and Iverson 1971). Based on these results, the iontophoretic deposition of 3H glycine was carried out using 3H glycine concentrated to 50 uCi/ul and deposited using 1uA ejection current for 30 minutes.

3.2. 3H GLYCINE INJECTIONS INTO NUCLEI OF THE CENTRAL AUDITORY SYSTEM

3.2a. General

Analysis of the autoradiographic material from experiments of 3H glycine injections into areas of the auditory system and adjacent RF focussed first on the evaluation of the injection site. The injection site is characterized by dense deposits of reduced silver halide grains in the photographic film over the surface of the tissue, reflecting the presence of beta particles in the top three microns of the section. Under darkfield illumination, individual grains appear white against a dark background. Under brightfield illumination these grains can be localized to structures using histological stains such as cresyl echt violet and neutral red. The injection site is defined as the section containing the ventralmost evidence of the injection tract made by the micropipette used for injection. Surrounding this site section is the dense accumulation of silver grains, representing the deposit of 3H glycine. A short distance from this area, the density of the label falls off precipitously, thus defining the limits of the injection site within the section. The rostral and caudal confines of the injection site (the injection site section defined

as 0.00 mm) are determined in a similar manner after examination of all sections: the rostral or caudalmost section from the injection site which exhibits silver grain deposits consistent with background (the scant, nonspecific accumulation of grains seen on the slide adjacent to the tissue section in question) is defined as the caudal or rostral limit of the injection. Appendix II, page 133 lists the rostro-caudal dimensions of all stereotaxic injections performed.

Specific cell body label after 3H glycine injections is determined as the dense accumulation of grains over a cell body seen at 160X magnification. Specific terminal labelling is defined as a uniform distribution of grain density above background levels at a sufficient distance (rostral, caudal, medial or lateral) from the injection site to distinguish it from the confines of the injection site.

The specific labelling patterns seen after 3H glycine injections were found to be of two general types. Specific retrograde labeling was recognized as the dense accumulation of silver grains over neuronal cell bodies. Specific terminal labelling was also found after 3H glycine inejections. This terminal labelling in most cases represented collaterals of labelled cell bodies. Other labelled terminals however likely represent the specific anterograde transport of 3H glycine (glycinergic neurons). This conclusion is based on the fact that very

few efferent projections of the injection site showed terminal label, as would be expected with a nonspecific anterograde marker. In fact, Beaudet et al (1981) have shown selective bidirectional transport of the the amino acid neurotransmitter aspartate in the pigeon retino-tectal pathway. In addition, within the present study, these areas with terminal labelling, e.g. the trigeminal (NV) and facial nuclei (NVII) possess moderate to high densities of glycine receptors (Frostholm and Rotter 1985).

Thus, 3H glycine injected into the rat CNS was transported mostly retrogradely with minor anterograde transport in a few instances. Based on the present study and the available literature, both labelling patterns (cell bodies and terminals), were found to reflect a selective, high affinity uptake and transport system for glycine.

3.2b. 3H Glycine Injections into VLL

Figure IV p. 115 is a an illustration of iontophoretic injections of 3H glycine made into the VLL of 4 rats (VLL/1, VLL/2, VLL/3 and VLL/6). Corresponding cell counts of retrogradely labelled somata detected by the autoradiographic method for these 4 cases are presented in Appendices III- VI p. 134 to 137. Comparison of the injection site with the location of labelled somata within

MNTB illustrates the correspondence between injections involving the different rostrocaudal divisions of VLL and labelling of MNTB somata. The summary table, Table III, p. 106 illustrates this point. Case VLL/6 has 485 labelled MNTB cell bodies; this injection site is centered in the main nuclear group of VLL. VLL/2 with 463 labelled MNTB cells has its injection site slightly ventral and caudal to that of VLL/6 (i.e. the ventrocaudal division of VLL). Deposition of tracer centered in more rostral (VLL/3) or caudal (VLL/1) extensions of the nucleus lead to fewer labelled MNTB somata; 157 and 62 cells respectively. Figure IV also demonstrates that all VLL injections include some deposition of label in and adjacent to the pontine nuclei and the middle cerebellar peduncle. Injection of 3H glycine into these areas alone (and the adjacent RPO nucleus - see case # 5 in section 3.2c) does not result in MNTB cell body labelling; thus label in MNTB is from 3H glycine retrogradely transported from terminals in VLL.

There are 3 types of MNTB neurons: principal, multipolar and elongated cells (Morest 1968). Labelled principal cells were found throughout the rostrocaudal extent of the nucleus, irrespective of the site of 3H glycine injection into VLL. Less numerous but nonetheless consistent labelling of multipolar and small elongated cells within MNTB also were found after all VLL injections (see photo p. 122).

The MVPO/LVPO were also labelled after all VLL injections. The number of labelled cells in LVPO/MVPO were greater when 3H glycine was deposited in the more rostral VLL sites (VLL/6, 93 cells, and VLL/3 39 cells) compared with more caudal VLL injection sites (VLL/2, 12 cells and VLL/1, 4 cells). Since the PL borders the VLL, the 3H glycine injection site most likely includes the PL, especially at rostral levels. Cell body label observed in LVPO/MVPO could therefore reflect 3H glycine deposits in the PL zone adjacent to VLL at these rostral injection sites.

Injections, VLL/2, VLL/3, and VLL/5 resulted in retrograde labelling of LSO somata in addition to that of the MNTB and LVPO/MVPO. In these three injections, clusters of grains over cells were generally confined to the dorsal and lateral margins of LSO (Photo p. 121). In case VLL/6, cell body label is also found in the A5 region. These labelled A5 neurons may represent additional labelled LSO somata; the border between the lateral LSO and the A5 region are ill defined neuroanatomically. Similar to label in LVPO/MVPO, LSO label may in fact be due to 3H glycine deposits in the PL.

Cases VLL/1 and VLL/2 represent the only instances of VLL injections resulting in accumulation of silver grains over RF somata (RPOo) as shown in Table III and Appendices III-IV. In case VLL/2, multipolar, medium sized neurons

were present bilaterally with a contralateral predominance in the RPOo immediately dorsal to the reticulotegmental nucleus of the pons. In case VLL/1, RPOo label was detected only ipsilateral to the injection site.

Terminal label found after VLL injections was largely confined to sites ipsilateral to the tracer deposit as seen in Table IV p. 107. Terminal label was especially prominent over the LSO where reduced silver grains were uniformly distributed over the entire nucleus. Silver grains suggestive of terminal label were also found over the DMPO, located immediately dorsal to MNTB. (Photo p. 121).

A high density of silver grains were consistently found overlying the ipsilateral AVCN and granule cell layer above AVCN in all VLL injections. Label in AVCN was difficult to define as strictly terminal or somatic because of the generally high density of grains present (Photo p. 124).

Accumulation of label was found in the pontine nuclei, primarily ipsilateral, in all VLL cases. Similar to labelling in AVCN, the density of grains overlying the pontine nucleus generally made determination of cell body versus terminal labelling difficult. In cases VLL/2 and VLL/3 however, a few distinctly labelled cell bodies were detected bilaterally as noted in Table III p.106 and Apendices IV and V p. 135 and 136.

There were two cases (#4 and #5 of this series) in

which the injection site was not in VLL. Case # 4 had its injection site in NV at the level of the principal sensory nucleus of NV. No cell body labelling was apparent outside of the confines of the injection site in this case. In case # 5, 3H glycine was deposited in the middle cerebellar peduncle lateral to VLL. This resulted in some cell body labelling in VLL and RPO with no other labelling of neurons in the medulla. Caudally, terminal labelling was detected in the NV.

Terminal labelling overlying RPO was greatest for cases VLL/2 and case # 5. Case # 5 in particular resulted in terminal and cell body labelling in the RPO adjacent to the injection site. Given the high density of label in the RPO, this nucleus was thus regarded as continuous with the confines of the injection site. In some sections of Case VLL/2, RPO cell body labelling could be distinguished from the high general backround close to the injection site. These labelled neurons may represent glycinergic interneurons.

3.2c. 3H Glycine Injections into LSO

A total of 3 unilateral iontophoretic injections of 3H glycine were made into the LSO nucleus as shown in Figure V. p. 116. Case LSO/4 is the most rostral of these injection sites, followed by LSO/3 and LSO/5.

Cases LSO/3, LSO/4 and LSO/5 lead to retrograde labelling of brainstem auditory nuclei as listed in Appendices VII - IX (p.138 to p. 140). All three injections resulted in cell body labelling in the MNTB, as summarized in Table V p.108. Following all 3 LSO injections, greater than 84% of all retrogradely labelled cells were found in MNTB. The numbers of labelled somata found in various rostro-caudal subdivisions of MNTB however differ in the three cases. In cases LSO/3 and LSO/4,the majority of labelled MNTB somata are located 0.64 - 1.120 mm caudal and 0.4 - 0.720 mm caudal respectively to the injection site. LSO/5 has the majority of its MNTB labelled cells 0.12 mm rostral - 0.360 mm caudal to the site of injection. Most cell body labelling in MNTB then, occurs caudal to the injection site, despite the fact that the MNTB extends rostral to the injection site. Thus the area of the MNTB labelled is likely related to the rostro-caudal location of the LSO injection site.

All three cases, LSO/3, LSO/4 and LSO/5 also result in labelling of the LVPO/MVPO located ventral and lateral to the MNTB. Label here most likely reflects short glycinergic projections to the LSO from LVPO/MVPO. The number of labelled cell bodies in these periolivary nuclei are generally similar to that seen following VLL injections.

Terminal labelling patterns following 3H glycine injections into the LSO is summarized in Table VI p 109.

Similar to VLL injections, all 3 injections resulted in terminal labelling in the pontine nucleus ipsilateral to the injection site. Given the proximity of this label to the injection site in cases LSO/3 and LSO/4, this label was regarded as part of the injection site. Label here does not contribute to retrograde labelling of brainstem auditory nuclei; this is based on the results of an injection of 3H glycine into the pontine nuclei (see case # 2 below). 3H glycine deposited here may in fact account for terminal label seen in the lateral lemniscus and the more rostral Kolliker Fuxe and DLL nuclei in case LSO/4. It will be recalled that the lateral lemniscus projects to the DLL and Kolliker Fuxe nuclei via the RPO (as well as other brainstem auditory nuclei). Thus labelled terminals in the DLL and Kolliker Fuxe nuclei may represent specific 3H glycine terminals labelled anterogradely from the pontine nuclei and the RPO.

Cases # 1 and # 2 in this series had their injection sites outside of LSO. These injections were medial or rostral to the LSO, and did not label brainstem auditory nuclei. In case # 2, the injection site is inclusive of the pontine nuclei and RPO; LSO is located slightly more caudally. Retrograde cell body labelling was absent following this injection. Terminal label was observed over the contralateral sensory root of the trigeminal nerve (S5). An increase in silver grains was also seen in the ipsilateral LL and the extreme external cortex of the

IC. Case # 1 has its injection site in the lateral RPOc (RPOc alpha); this injection also resulted in terminal label in the contralateral s5. Faint cell body labelling was seen in the ipsilateral NV and NVII; however, due to the generally high density of silver grains present, this labelling could not be defined unequivocally as being labelled cell bodies or terminals.

3.2d. 3H Glycine Injection into LSO and HRP Injection into VLL

Given that labelled somata are found in the MNTB after 3H glycine injections in either VLL or LSO, the possibility that some glycinergic neurons in MNTB project to both nuclear groups was investigated. For this purpose, 3H glycine was injected into LSO and HRP - WGA was injected into VLL in the same rat. Case LSO/4 and VLL/4 represent alternate sections from one rat treated in this manner. 3H glycine labelled somata found in case LSO/4 are shown in Appendix VIII p.139. Auditory brainstem nuclei labelled after the retrograde transport of HRP - WGA in the same animal - case VLL/4 - are detailed in Appendix X p. 141. A comparison of the two labels in the summary Table VII p.110 shows that five auditory brainstem nuclear groups labelled after 3H glycine deposits in LSO are similarly labelled after HRP deposits in VLL. In general, HRP-labelled cells were found bilaterally in contrast to tritium labelled cells which were located only ipsilateral to the site of injection. In addition, more brainstem nuclear groups, both auditory and nonauditory, are labelled with HRP. The reasons for these differences in labelling patterns with tritium and HRP are twofold. HRP is a nonspecific retrograde marker and is assumed to label all afferents to VLL; in addition, VLL and LSO afferents arise in many nuclei, some of which are not

overlapping; thus the cells labelled after retrograde transport from these sites would be different. There is however, some overlap in the distribution of labelled cells with the two markers which suggest that a number of the same nuclear groups send afferents to both VLL and LSO. The MNTB and the LVPO/MVPO are especially significant in this regard. It will be recalled that both of these auditory nuclear groups are labelled after 3H glycine injections in either the LSO or VLL. The number of HRP labelled cells found in the LVPO/MVPO and MNTB are higher than the number of 3H glycine labelled cells in these nuclei (Table VII). Due to the large injection of HRP-WGA made into the VLL (shown in Figure VI), it is likely that almost all MNTB or LVPO/MVPO afferents to the VLL are labelled (Photo p. 125); 3H glycine then labels a subpopulation of these labelled somata.

The number of HRP-WGA labelled cells in Table VII p. 110 are greater in number than those labelled with 3H glycine. This is true for virtually all brainstem nuclei labelled by both tracers as seen in Table XII. The uptake of HRP-WGA has been suggested to occur via binding to a carbohydrate moiety at the axon terminus; this binding site is ubiquitous in the CNS. 3H glycine uptake however, is selective as discussed in section 3.1b. The pattern of 3H glycine uptake in the present study confirms the selectivity of 3H glycine as a retrograde tracer.

The highest number of HRP label of all brainstem nuclei is in the contralateral CN, inclusive of the AVCN and DCN as seen in Table VII. This is of interest since following 3H glycine injections into the VLL (section 3.2b), the AVCN and granule cell cap was labelled only ipsilaterally and not contralaterally. The designation of the tritium label as representing somatic or terminal labelling however, could not be determined conclusively due to the high density of the reduced grains (Photo p. 124). 3H glycine was also injected into the AVCN of 2 rats. In both cases, cell body labelling was confined to the injection site. Close to the injection site, the VIII nerve and the DCN were very densely labelled. However, no VLL neurons were retrogradely labelled. This is consistent with the lack of evidence for projections from the VLL to the ipsilateral AVCN (Kudo 1981). Thus label in the ipsilateral AVCN most likely represents somatic labelling which was technically difficult to differentiate from terminal labelling.

3.2e. 3H Glycine Injections into Nuclei of the Reticular Formation

Stereotaxic injections of 3H glycine directed into the nucleus reticularis gigantocellularis (RGc) were performed in six rats to investigate possible glycinergic projections linking the auditory system and reticular formation (RF); Figure VII, (p. 118 and photo (p. 126). Table XIII (p. 111) shows the location and number of cells labelled by injections into RGc. In cases RGc/1 and RGc/2, injections of tracer was limited to RGc. In these rats, the greatest number of labelled cells were found in the ipsilateral paragigantocellularis lateralis (Pgl) and nucleus reticularis magnocellularis (RMc). Although some cell body labelling was within the rostro-caudal dimensions of the injection site,labelling could still be differentiated from background. Labelled cell bodies were also seen in the nucleus raphe magnus (RAM).

In two other cases, injections of 3H glycine that were primarily located in the ventral or rostral portion of RGc (RGc/3 and RGc/4) also partially labelled the nucleus reticularis magnocellularis (RMc) or the RPOc. In case RGc/4, the 3H glycine injection bordered the RMc. This was associated with an increase in the number of labelled cell bodies in the raphe pallidus (RAP) as compared to injections that were restricted to RGc. Specific cell body labelling was also observed in the PGl and RMc (Photo

p. 127) in case RGc/4, similar to cases RGc/1 and RGc/2, where the injection sites were restricted to RGc. In case RGc/3, the 3H glycine injection bordered both RMc and RPOc; hence this injection was the most rostrally placed of all injections in RGc. This injection did not result in cell body label in RAM (unlike cases RGc/1, RGc/2 and RGc/4); this may be due to the involvement of RPOc in this injection site.

When injections of 3H glycine were localized to the lateral portion of RGc (Case RGc/5) or outside of RGc (Case # 6), no labelled cell bodies were found in RAM, RMc, or PGl. However, specific cell body labelling in RGc was seen in case RGc/5.

Taken together, the data suggest that injections of 3H glycine into the RGc result in labelling of a subpopulation of RGc afferents. Short axoned glycinergic projections to RGc have their cell bodies in RAM, PGl, and RMc of the RF; these nuclei have been implicated in the descending control of nociception. Thus glycine neurotransmission stemming from these nuclei may play a role in mediating nociception. Glycinergic cells in the RAP, which is the caudal extension of RAM, may also participate in descending nociceptive pathways.

3.2f. 3H Glycine Injections into the Cerebellum, DLL and the Spinal Cord

3H glycine was injected into the cerebellum, DLL and the spinal cord. The cerebellum and DLL were chosen because of negative evidence in the literature for glycinergic neurotransmission in these areas. The lack of evidence for glycinergic descending pathway(s) to the spinal cord made spinal cord injections useful in evaluating the selectivity of 3H glycine uptake and retrograde transport.

Pressure or iontophoretic injections of 3H glycine into the cerebellar cortex (2 rats) or the DLL (3 rats) led to deposition of label in the neuropil and overlying cell bodies at the site of injection. In the cerebellum, nonspecific labelling of granule and Purkinje cells near the injection site were observed. DLL injections resulted in dense nonspecific accumulation of silver grains over small and medium sized cells within the DLL. Infrequent labelling of glial elements was also observed in the DLL and cerebellar cortex. No cell body labelling however, was observed outside the confines of the injection site in either case. Glycinergic receptors in DLL and the cerebellum are low as assessed with 3H strychnine autoradiography (Zarbin et al 1981, Frostholm and Rotter 1985), and glycine uptake rates (Johnston and Iverson

1971) and glycine levels (Aprison et al 1971) are low in the cerebellum. This suggests that glycine uptake and retrograde transport is a selective process, dependent upon the presence of glycinergic terminals with a high affinity glycine uptake process. The labelling of cells and terminals at the site of injection most likely reflects the low affinity transport system for small amino acids, including glycine. In some cases, it is possible that glycinergic interneurons at the site of injection were labelled. The high density of label in the neuropil surrounding these cells however obviated an accurate determination of specific labelling of these somata.

Two rats received unilateral pressure injections of 3H glycine into the thoracic cord (at T7-T9). Silver grains were found bilaterally overlying cell bodies in Lamina I - III (Photo p. 128) of the dorsal horn at cervical and thoracic levels of the cord. A high density of grains were also found in the ventrolateral white matter of the cord at these segments. No cell body or terminal labelling was found rostral to the first cervical segment of the cord, suggesting that there are no glycinergic descending pathways projecting to the cord. The spinal cord of vertebrates is known to contain high levels of glycine that are markedly diminished after aortic occlusion (Davidoff et al 1967) probably due to the loss of glycinergic intnuncials. The cell body label seen in the dorsal horn after injections into the thoracic cord

are likely glycinergic interneurons.

Selectivity of 3H glycine uptake and retrograde transport was also evaluated by comparing results of retrograde transport of the nonspecific tracer HRP-WGA and retrograde transport of 3H glycine. Iontophoretic injections of 3H glycine were made into the VLL of 4 rats as described in 3.2b. In a separate animal, a large pressure injection of HRP-WGA was made into the VLL as described in 3.2d. 3H glycine injections of the VLL lead to retrograde labelling of fewer afferent nuclear groups than the case of HRP-WGA injection; in addition, 3H labelled nuclei were found only ipsilateral to the injection site in contrast to HRP injections which labelled nuclei both ipsilaterally and contralaterally to a greater extent.

Taken together the above experiments suggest that the uptake and retrograde transport of 3H glycine into cell bodies is dependent upon selective reuptake into the glycinergic terminals. This uptake reflects the specific high affinity transport system seen in in-vitro preparations of glycine containing neurons. Applied in vivo, specific reuptake and retrograde transport of glycine is a useful tool in identifying the existence of glycinergic pathways.

3.3. Glycine Immunocytochemistry

A polyclonal antibody raised against a glycine-bovine serum albumin (GLY-BSA) conjugate (a gift of Dr. P. Pasik) was used to localize glycine neurons within the auditory brainstem and brainstem RF of the rat.

Antibody dilutions of 1/1000, 1/1500 and 1/2000 were applied to rat brainstem sections and visualized with the peroxidase-anti-peroxidase (PAP) technique to determine suitable staining parameters for specific antibody staining. Dense bilateral staining of most neurons in the MNTB was observed at all concentrations, confirming the observations of Campistron (1986) in the rat and Wenthold (1987) in the rat and guinea pig.

A second series of experiments were performed on sections of the rat brainstem including the caudal pole of the NVII to the substantia nigra at rostral levels. An antisera dilution of 1/1750 with the PAP technique was used based on the results of staining patterns at higher and lower dilutions. As mentioned above, dense immunoreactivity was observed in the MNTB (Photo p. 130). Immunostained cells were mostly round or oval, many with an eccentric nucleus as described for the principal cell population of the MNTB. The nucleus of these cells was darkly stained. Many elongate and multipolar cells of the MNTB were also immunostained.

Moderate staining was observed in some cells of VLL, LSO and LVPO/MVPO (Photo p. 130). Glycine-positive fibers traversed these auditory nuclei and immunoreactive puncta suggestive of terminals were scattered throughout. The source of glycinergic input to these cells may be via fibers of the trapezoid (Tz) (the Tz contains fibers from cell bodies in the contralateral CN and SOC) which were also immunostained. Very little immunoreactivity was detected in the MSO located medial to the LSO, or in the IC, located rostral to the VLL.

Outside of the auditory brainstem, the RF of the medulla and pons showed immunostained somata throughout the brainstem, most notably a small cluster of medium sized cells in the medial RPOo. Cells in the pontine nuclei were densely immunoreactive, especially in the lateral aspects of the nucleus which at some levels is coextensive with the RPO. GLY-positive fibers were also seen to traverse the base of the pons at this level.

Immunoreactivity in the cervical spinal cord was less dense than that observed in the MNTB. Moderate to low antibody staining of terminals and fibers however was observed in the dorsolateral tracts of the spinal cord adjacent to Laminas I - IV. Cell body staining within the gray matter was low.

Control sections were treated with antisera diluted to 1/1750 which had been preadsorbed with GLY-BSA conjugate and co-precipitated with an anti-BSA antibody. Specific

immunostaining was not detected in control sections; however, background labelling, likely due to the presence of endogenous peroxidase within the section was present.

IV. DISCUSSION

4.1a. 3H Glycine Axonal Transport

The use of 3H glycine as a tool for identifying glycinergic pathways is a relativley new technique, first demonstrated in the pigeon visual system by Hunt and collegues (1977). Hunt showed by radioautography that 3H glycine injected into the nucleus isthmus parvocellularis of the pigeon was retrogradely transported into a subpopulation of neurons in the optic tectum. Previous neurophysiological and pharmacological data (Barth and Felix 1974, Henke et al 1976, Zukin et al 1975) had suggested that glycine might be the transmitter of this visual pathway; the specific high affinity transport of 3H glycine allowed neuroanatomical confirmation of this data. This technique has most recently been applied to the mammalian CNS. Iontophoretic injection of 3H glycine into the abducens nucleus in the cat selectively labelled cell bodies in the medial vestibular nucleus, prepositus hypoglossi and the pontomedullary RF (Spencer and Baker 1985). These glycinergic neurons may be involved in the performance of horizonatal saccadic eye movements and the vestibulo-ocular reflex.

Within the central auditory system and RF, only a few neurophysiological studies have provided evidence for glycinergic pathways despite the presence of high glycine levels, high density of glycine receptors and glycine immunostaining in these areas. One report however has

shown electrophysiologically that glycine might be the transmitter of MNTB neurons projecting to the LSO of the SOC; glycine iontophoretically applied to the LSO causes reduced firing that is counteracted by strychnine and not by bicuculline (Moore and Caspary 1983). Recently, an immunohistochemical study has identified glycinergic cell bodies in the MNTB and immunoreactive terminals in the LSO (Wenthold 1987c). This pathway has now been confirmed neuroanatomically in the present study using the technique of 3H glycine retrograde transport. Thus 3H glycine retrograde transport appears to be a valuable tool in the identification of glycinergic pathways.

Glycinergic pathways within the auditory system and RF in the present study were identified using the iontophoresis of 3H glycine in a manner similar to that used for iontophoresis of proline and leucine (Schubert and Hollander 1975) and glycine (Spencer and Baker 1985). The concentration of glycine deposited by iontophoresis is linear with respect to time as shown in Figure III. The amount of 3H glycine deposited at 30 minutes is calculated to be 31 uM, which is within the range of the the K_m for the high afffinity, temperature dependent uptake of glycine in in-vitro spinal cord synaptosomal and slice preparations (Johnston and Iverson 1971). The concentration of 50uCi/ul was used in tracing glycinergic pathways in this study; this was sufficient for autoradiographic visualization.

4.1b. Transport Selectivity

The selectivity of the uptake and retrograde transport of 3H glycine in glycinergic neurons is further supported by data from three different types of experiments in this work: 1) injections of 3H glycine into cerebellum, the DLL and the spinal cord; 2) comparison of 3H glycine labelling patterns with the non - specific retrograde tracer HRP-WGA; and 3) comparison of 3H glycine labelled cells and glycine immunostained cells in the auditory brainstem and RF.

1.) 3H Glycine Injections into the Cerebellum, DLL, and Spinal Cord.

3H glycine injections into the cerebellum or DLL of the rat resulted in dense labelling of neurons and glia at the site of injection; no labelled somata or terminals however were detected outside the injection site in either case. The density of glycinergic post-synaptic receptors in DLL and the cerebellum are low as measured by 3H strychnine autoradiography (Zarbin et al 1981, Frostholm and Rotter 1985), and 3H glycine uptake rates (Johnston and Iverson 1971) and glycine levels are low in the cerebellum (Aprison et al 1969). These data support the conclusion that the lack of 3H glycine retrograde transport following 3H glycine injections into the

cerebellum or DLL are due to the absence of glycinergic terminals and high affinity uptake sites in these areas. The high density of silver grains over cells at the site of injection may represent accumulation of glycine via the low affinity, nonspecific transport system for small amino acids, including glycine. It is also possible that some labelled cells at the injection site may represent glycinergic interneurons; however, the high density of grains at the site of injection obviated an accurate determination of specific somatal labelling in these regions.

Injections of 3H glycine into the thoracic spinal cord resulted in a high density of silver grains in Lamina I - III of the dorsal horn and over the Lamina VI and VII of the ventral horn in the cervical and thoracic spinal cord (Photo p. 128). An increased density of grains was also found in the ventral tracks of the cord. No cell body or terminal labelling was found rostral to the first cervical segment of the cord. This indicates that long, descending pathways terminating in the spinal cord are not glycinergic. The spinal cord of vertebrates contains high levels of glycine that are markedly diminished after aortic occlusion (Davidoff et al 1967), due to the loss of glycinergic interneurons. The retrograde label seen in the dorsal horn following 3H glycine injections into the thoracic cord may therefore represent specifically labelled glycinergic interneurons.

In conclusion, negative results of specific distant cell body labelling following 3H glycine injections into the cerebellum, DLL and spinal cord illustrate that 3H glycine retrograde transport is a selective process which is absolutely dependent on the presence of glycinergic nerve terminals with a high affinity uptake mechanism.

2.) Comparison of 3H glycine labelling patterns following 3H glycine or HRP-WGA Injections into the VLL

A comparison of somatal labelling after injections of 3H glycine or HRP-WGA into the VLL further supports the thesis that 3H glycine is a specific retrograde marker. 3H glycine injection into VLL labelled a subpopulation of the nuclear groups that are labelled with HRP-WGA injection into VLL; in addition, after 3H glycine injections, labelled somata are found mostly ipsilateral to the site of injection in contrast to HRP injection, which results in bilateral somata labelling. Thus 3H glycine labels a subpopulation of VLL afferents. Glendenning and colleagues (1981) have traced most VLL afferents to contralateral VCN (AVCN and PVCN; the contralateral LSO projects to VLL via collaterals of LSO projections to the PL zone (Kudo 1981). The injection of HRP into the VLL in the present study confirms these observations. 3H glycine injections into the VLL in the

present study labelled the ipsilateral LSO and CN, but failed to label the contralateral LSO or CN. Thus 3H glycine, by virtue of selective uptake mechanisms present on glycinergic terminals, is taken up and transported into a subset of VLL afferents.

In summary, based on the comparison of 3H glycine and HRP injections into VLL it is clear that 3H glycine specifically labels glycinergic projections from MNTB, LVPO/MVPO, and RPOo to the VLL; while in addition to these four nuclear groups, HRP labelled cells are found in many other nuclei, such as the AVCN and DCN.

3.) Comparison of 3H glycine labelled cells and stained cells in the auditory brainstem and RF.

A polyclonal antibody raised against a glycine-BSA conjugate was used as a third method to localize glycine neurons within the auditory brainstem and RF. Immunostained areas were compared to areas labelled after 3H glycine injections.

The cells in the MNTB of the rat auditory brainstem were densely immunostained, consistent with my 3H glycine retrograde transport studies which localized reduced silver grains to MNTB somata. In both 3H glycine autoradiography and glycine immunohistochemistry, the principal cell population of the MNTB was most densely

labelled with only scant labelling of multipolar and elongate cell populations. LVPO/MVPO was also labelled after both 3H glycine autoradiography and glycine immunohistochemistry. Similar findings have been demonstrated by others with glycine antibodies staining the MNTB, LVPO, and MVPO in the guinea pig (Helfert et al 1987) and MNTB in the rat (Campistron et al 1986). The MSO, located between the MNTB and the LSO was consistently GLY-negative by immunocytochemistry in the present study and as reported in other immunohistochemical studies using glycine antibodies (Helfert et al 1987). Corrollary to this, virtually no 3H glycine labelled cell bodies were found in MSO following 3H glycine injections into the VLL or the LSO.

Scattered cell bodies immunostained with the glycine antisera were found at all levels of the RF of the pons and medulla. This result is consistent with the work of Campistron et al (1986) who observed glyine immunostaining in the rat RF. As might be expected, injections of 3H glycine in the RF indicate that glycinergic interneurons are present in the RF throughought the rat brainstem (Table XIV). Particularly prominent with immunostaining were a cluster of medium sized GLY- positive cells in the RPOo. Two cases of 3H glycine injections into VLL also resulted in accumulation of silver grains over medium sized cells of the RPOo.

The AVCN and the granule cell layer of the CN appeared

to be GLY-positive in the present study. GLY-positive AVCN staining has been shown in the guinea pig and cat (Helfert et al 1987, Adams 1987). Glycinergic terminals, although not clearly observed with the present antisera, have been localized to the VCN (AVCN and PVCN) and DCN with antibodies to conjugated glycine in the guinea pig (Altshuler 1986). Postsynaptic receptors in VCN and DCN have been demonstrated in the gerbil with 3H strychnine autoradiography (Sanes et al 1987). The origin of the glycinergic terminals in AVCN however is unclear. Wenthold (1987) suggests that the medium sized glycinergic cells of the AVCN project to the contralateral CN in the guinea pig; 3H glycine injections into the rat AVCN conducted in the present study however, do not support this finding. No labelling outside of the confines of the AVCN were observed; cell body labelling within the AVCN could only be attributed to possible glycinergic interneurons. Further experiments in both the rat and the guinea pig are needed to clarify whether this putative pathway actually exists.

Thus, glycine immunocytochemistry and 3H glycine autoradiography are complementary techniques for the localization of glycinergic cell bodies in the rat CNS. The results from these two methods, considered in conjunction with the data obtained from the use of the nonspecific marker HRP-WGA, strongly suggest that 3H glycine retrograde transport is an ideal tool for the

identification of glycinergic pathways in the CNS.

4.2. Glycinergic Pathways in the Auditory System and Adjacent RF of the Rat.

The results of retrograde transport of 3H glycine in combination with HRP retrograde transport and glycine immunohistochemistry indicate that the following pathways are glycinergic (see Figure I p. 97):

4.2a. MNTB $\longrightarrow$ VLL

An ipsilateral glycinergic pathway from the MNTB to the VLL has been neuroanatomically identified in the present study. 3H glycine injected into VLL was retrogradely transported to cell bodies in MNTB. Spangler et al (1985) had demonstrated, by 3H the technique of leucine anterograde transport, projections from MNTB to the VLL. These projections are ipsilateral and the principal cell population of the MNTB contributes most of the afferents to VLL as opposed to the stellate and elongate cell population. A high density of glycinergic post-synaptic receptors are present in the VLL of the gerbil, mouse and rat (Sanes et al 1987, Frostholm and Rotter 1985, Zarbin et al 1981). The MNTB principal cells

immunostain densely with glycine antisera in the present study as reported by others (Helfert 1987, Campistron et al 1986). There are no electrophysiological studies to date on the role of this glycinergic pathway in the processing of afferent acoustic information.

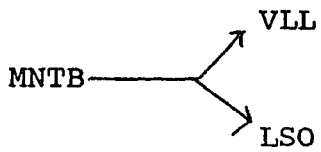
4.2b MNTB ⟶ LSO

3H glycine injections into the LSO labelled cell bodies in the ipsilateral MNTB, providing neuroanatomical evidence that the pathway from the ipsilateral MNTB to the LSO is in part glycinergic. Glycine immunohistochemistry in the guinea pig (Helfert et al 1987) has demonstrated glycinergic terminals and scattered cell bodies in the LSO. The presence of 3H strychnine labelled postsynaptic receptors in the LSO also supports the existence of glycinergic terminals in the LSO of the gerbil, mouse and rat (Sanes et al 1987, Frostholm and Rotter 1985, Zarbin et al 1981).

The glycinergic pathway from MNTB to LSO defined neuroanatomically in the present study was suggested by the physiological study of Moore and Caspary (1983). They found that iontophoretic deposition of glycine onto monaurally activated LSO cells reduced the firing of LSO units. This effect could be blocked by the glycine antagonist strychnine, suggesting that terminals from MNTB may release glycine into the LSO. This glycinergic

pathway (MNTB to LSO) is thought to mediate the inhibition of binaural afferent acoustic information. Glycine-mediated inhibition in the LSO mediates the detection and integration of the spatial and temporal qualities of sound.

4.2c.



3H glycine injection into the LSO and HRP injection into VLL results in MNTB neurons labelled with both HRP and tritium. This experiment demonstrates that collaterals of glycinergic MNTB neurons may project to both the ipsilateral VLL and LSO. In support of this data, all 3H glycine injections into VLL also lead to terminal label in the ipsilateral LSO coincident with labelled MNTB neurons. Since the VLL does not project to LSO, terminal labelling observed in the LSO does not appear to represent a simple anterograde transport of 3H glycine from VLL to LSO. Rather it likely represents retrograde transport of 3H glycine from VLL to MNTB followed by anterograde transport via MNTB collaterals projecting to LSO. In addition, 3H glycine injected into the LSO also resulted in numerous labelled MNTB somata. Furthermore, based on electrophysiological studies, glycinergic projections from MNTB to LSO have been

proposed (Moore and Caspary 1983, Harrison and Warr 1962). Glycine terminals in LSO and VLL have been identified immunohistochemically with glycine antisera (Helfert et al 1987), and glycine receptors are also present in VLL and LSO as shown by 3H strychnine autoradiography (Sanes et al 1987). Finally, the MNTB has been shown with retrograde transport of HRP to project to both the LSO and the VLL in the cat (Spangler et al 1985). Glycinergic collaterals from MNTB cells to the VLL and LSO likely relay contralateral acoustic input to the LSO for binaural processing and simultaneously provide the VLL with contralateral monaural tonal and temporal response patterns from the AVCN.

4.2d. LVPO/MVPO ⟶ VLL ; LVPO/MVPO ⟶ LSO

3H glycine injections into the VLL or the LSO lead to cell body labelling in the LVPO/MVPO. Glendenning et al (1981) has shown with HRP histochemistry that a few cells of the MVPO send afferents to the VLL and ILL in the cat. The LSO receives bilateral projections from the LVPO, and glycine immunoreactive neurons in the LVPO/MVPO have been demonstrated in the guinea pig (Helfert et al 1987). No electrophysiological data exists however regarding the role of glycine on LVPO/MVPO units. However, since the MVPO, which receives input from the contralateral CN (Brodal 1981) is known to project to the ipsilateral AVCN

(Kudo 1981), the MVPO may be a relay nucleus linking both CN.

4.2e. AVCN $\xrightarrow{?}$ VLL

3H glycine injections into VLL in the present study lead to labelling of the ipsilateral AVCN. Due to the density of label in the AVCN however, the assignment of labelled cell bodies to this nucleus was difficult. Strominger (1969) however, has shown that there is an ipsilateral projection from the AVCN to the ventrocaudal VLL in the macaque, and glycine-positive AVCN cell bodies have been demonstrated in the guinea pig and cat (Helfert et al 1987, Adams 1987). Furthermore, anterograde transport of 3H leucine from the VLL does not label terminals in the ipsilateral CN. These studies, combined with the specific retrograde nature of 3H glycine uptake and transport suggest that there is a glycinergic projection from the ipsilateral AVCN to VLL. This projection likely provides the VLL with information on spatial and temporal acoustic information from the ipsilateral ear.

4.2f. RPOo $\longrightarrow$ VLL

3H glycine injections into VLL indicate that there

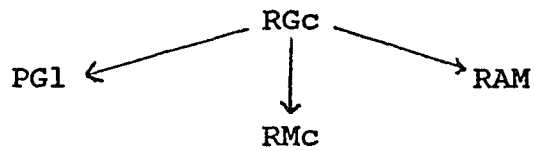
are glycinergic projections, most likely bilateral with an ipsilateral predominance, from RPOo to VLL. The present immunocytochemical studies also show GLY-positive cell bodies within the RPOo. In addition, some labelling of RPOo cells were seen after HRP injections into the VLL. Functionally, in the AGS model, lesioning of the RPOo abolishes all phases of AGS seizures in rats (Kesner 1966, Burnham 1985). Glycinergic projections from the RPOo to VLL then, may modulate motor output pathways in response to sound stimulation.

4.2g. LSO ⟶ RPOc ; LSO ⟶ PL

Based on the present study, glycinergic pathways linking the LSO and the RF are also proposed. 3H glycine injections into the lateral RPOc or the VLL and adjacent PL both result in labelling of neurons in the LSO and adjacent A5 region. LSO neurons are known to project to the VLL and PL zone ipsilaterally (Glendenning 1981, Spangler 1985), and glycinergic cell bodies have been demonstrated in the LSO of the guinea pig (Helfert et al 1987) and in the A5 area (Adams and Wenthold 1987). Glycinergic LSO projections to the RPOc and the VLL/PL may be part of the ASR circuit proposed by Davis (1982). Reticulospinal pathways may be directly influenced by glycinergic projections from LSO to RPOc; LSO neurons may relay to the RPOc information on acoustic information from

both CN; in addition, glycinergic LSO projections to VLL/PL may provide binaural information to ascending auditory pathways that relay at VLL.

4.2h.



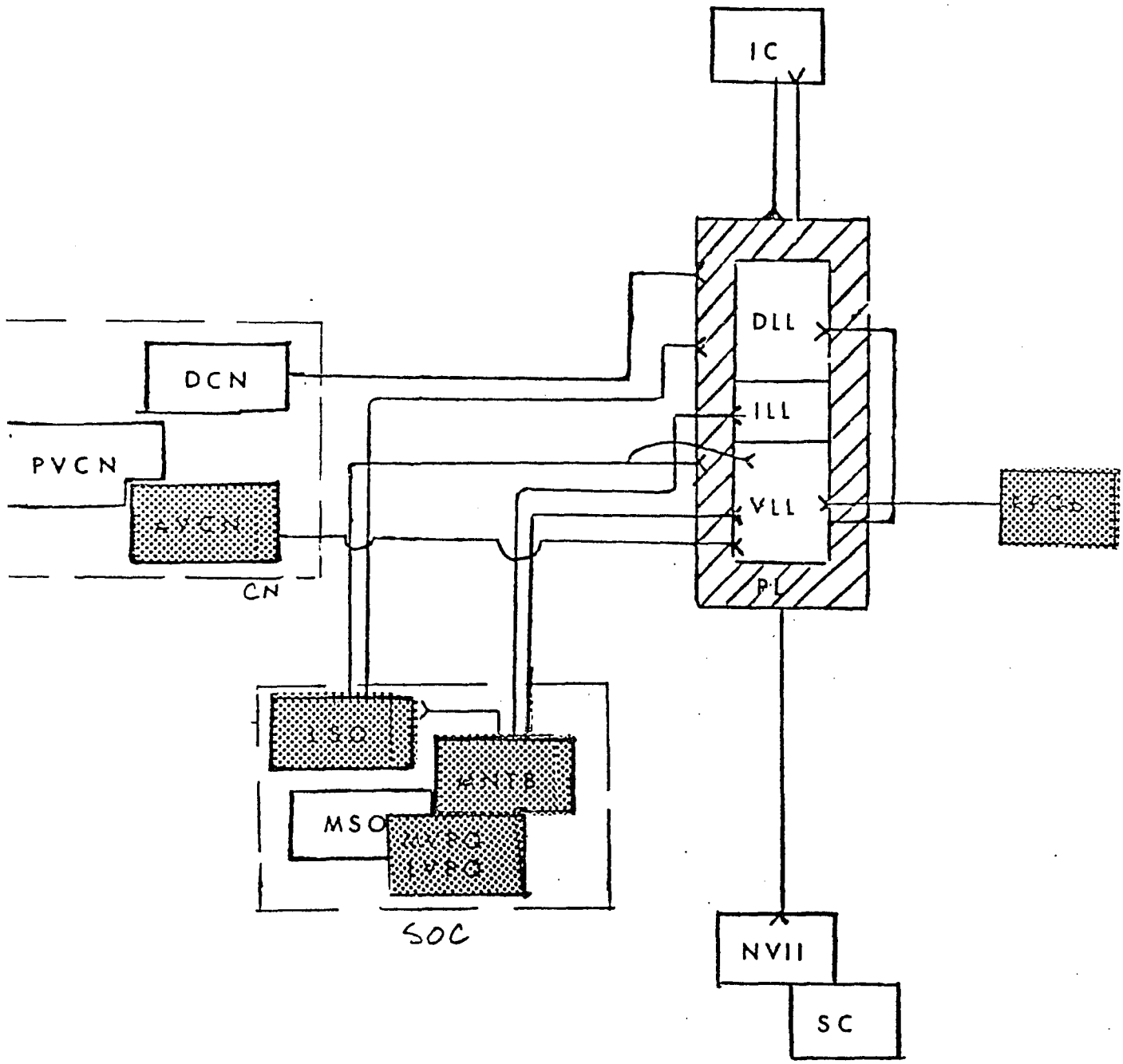
Glycinergic pathways linking the RF are short axon projections to RGc that have their cell bodies in RF nuclei implicated in the descending control of nociception - i.e. the RGc, RAM, PGl, and RMc. Given this, glycine may play a role in mediating nociception by linking nuclei of the brainstem nociceptive system with the medullary reticulospinal pathway originating in the RGc. Spinoreticular pathways afferent to the RGc and RGc afferents from the RAM may be part of a brainstem feedback loop to coordinate motor responses to sensory stimuli.

In summary, my results confirm with the techniques of 3H glycine retrograde transport, glycine immunohistochemistry and HRP retrograde transport, the suspected pathway from the ipsilateral MNTB to the LSO as well as identifying a number of possible new glycinergic pathways within the brainstem auditory system (see Figure I) and RF:

Number

1. from the medial nucleus of the trapezoid body to the lateral superior olive;
2. from the lateroventral and medioventral superior; olivary nuclei to the ventral nucleus of the trapezoid body;
3. from the lateroventral and medioventral superior; olivary nuclei to the lateral superior olive;
4. from the nucleus reticularis pontis oralis to the ventral nucleus of the lateral lemniscus;
5. from the lateral superior olive to the paralemniscal zone;
6. from the nucleus paragigantocellularis lateralis to the nucleus reticularis gigantocellularis;
7. from the nucleus reticularis magnocellularis to the nucleus reticularis gigantocellularis;
8. from the raphe magnus to the nucleus reticularis gigantocellularis;
9. from the medial nucleus of the trapezoid body to the ventral nucleus of the lateral lemniscus and the lateral superior olive;
10. from the anteroventral cochlear nucleus to the ventral nucleus of the lateral lemniscus.

Figure I . Glycinergic Pathways in the Brainstem Auditory System and RF



V. CONCLUSIONS

The use of specific uptake and transport of 3H glycine as a tool for the identification of glycinergic pathways in the rat auditory system and reticular formation is described in the present study. The uptake and transport of 3H glycine is a selective process, occurring in both the retrograde and anterograde directions. In contrast to the techniques of glycine immunocytochemistry or HRP histochemistry, the use of 3H glycine as a neuronal marker identifies both the neurotransmitter as well as the neuronal connectivity of central nervous system pathways.

The identification of the chemical neuroanatomy of glycine in the auditory system and reticular formation in the present study may be an important first step in a new direction for the reseach and treatment of hearing and hearing related disorders. Two areas of future research directions directly relevant to the present study is research in the area of age related hearing loss and audiogenic seizure disorders.

Age related hearing loss, of central and peripheral origin, has been well documented in the medical literature for decades, and has generally been considered a normal consequence of the aging process. Recent literature on the central mechanisms of hearing in relation to the aging process suggest that deficits in glycinergic neurotransmission may play a role in hearing loss. Neurons in the medial nucleus of the trapezoid body project to the lateral superior olive to integrate

information (Moore and Caspary 1983) related to the temporal and spatial qualities of sound from both ears (binaural inhibition); the present work has identified this pathway as glycinergic. Acoustic information integrated at the level of the lateral superior olive is then transmitted to rostral auditory centers. Casey (1987) has found that in aged rats there is a deficit of the surface area and density of synaptic terminals on lateral superior olive somata; in addition aged rats (24 months) have decreased numbers of MNTB neurons (Casey and Feldman (1982). These studies, taken together suggest that glycinergic projections mediating binaural inhibition at the level of the lateral superior olive may decrease in number with aging. Future research would determine whether these deficits can be correlated with decreases in glycine levels in the medial nucleus of the trapezoid body and/or changes in glycine receptor number or affinity at the level of the medial nucleus of the trapezoid body. In this regard it has been demonstrated that senescent rats (aged 24 months) have reduced glycine levels in the medulla and spinal cord compared to their younger counterparts. In addition, these older rats have reduced levels of glycine receptors as measured by 3H strychnine binding (Hunter et al 1985,1988). Neurochemical analysis of the MNTB to LSO pathway in the aged rat would be of interest in this regard. Physiologically, the consequences of the decrease of glycinergic input to the lateral superior olive via the medial nucleus of the

trapezoid may be increased acoustic input to rostral auditory centers; the way in which this may translate into hearing loss in aged animals would be an interesting subject for study.

Deficits in glycinergic neurotransmission and the possible consequence of increased central auditory input and audiologic abnormalities may be experimentally examined using the audiogenic seizure model. Hunter et al (1985, 1988) have shown that decreases in glycinergic neurotransmission in the aged rat may be correlated with an increased susceptibility to sound sensitive strychnine seizures; these seizures may be relevant to other models for seizures initiated by sound stimuli. These models, termed 'audiogenic' are inbred or outbred strains of animals (rats, mice and baboons among other mammals) that possess an inherited predisposition toward seizures in response to sound stimuli. Although reminiscent of sound sensitive seizures seen in rats given the glycine antagonist strychnine, only one study to date has examined the role of glycinergic neurotransmission in the audiogenic seizure model. Abel and Lajtha (1984) have found that in audiogenic seizure prone mice (strain DBA/2J) the administration of glycine significantly reduces the incidence and severity of sound initiated seizures in these aninmals. Given the anatomic evidence for glycinergic neuotransmission in the central auditory system as well as the reticular formation (an area from

where seizures may be experimentally induced with the glycine antagonist strychnine) presented here and in the literature, it would be of interest to further explore the role of glycine in this animal model. The availibility of a genetic model for such a study could prove useful for an examination of the genetic components of the many hearing related disorders documented clinically.

In conclusion, it is hoped that the present study serves to stimulate research leading to treatment and prevention of hearing and hearing related disorders, and that the technique presented for the chemical identification of neuronal pathways finds more widespread use as a basic tool in neurobiology.

VI. TABLES

Table I. EXPERIMENTAL SUBJECTS

Animal	Identification number	Injection Site	Method	Survival (hours)
Rat	VLL/1	VLL	ionto/3H glycine	6
Rat	VLL/2	VLL	ionto/3H glycine	6
Rat	VLL/3	VLL	ionto/3H glycine	6
Rat	VLL/6	VLL	ionto/3H glycine	6
Rat	4	NV	ionto/3H glycine	6
Rat	5	mcp	ionto/3H glycine	6
Rat	LSO/3	LSO	ionto/3H glycine	6
Rat	LSO/4	LSO	ionto/3H glycine	18
		VLL	pressure/HRP	
Rat	LSO/5	VLL	ionto/3H glycine	6
Rat	1	Pon N.	ionto/3H glycine	6
Rat	2	RPOc	ionto/3H glycine	6
Rat	RGc/1	RGc	ionto/3H glycine	6
Rat	RGc/2	RGc	ionto/3H glycine	6
Rat	RGc/3	RGc	ionto/3H glycine	18
Rat	RGc/4	RGc	ionto/3H glycine	12
Rat	RGc/5	RGc	pressure/3H glycine	18
Rat	6	RPc	ionto/3H glycine	12
Rat	--	SC	ionto/3H glycine	18
Rat	--	SC	ionto/3H glycine	18
Rat	--	Cb	ionto/3H glycine	6
Rat	--	Cb	ionto/3H glycine	6
Rat	--	AVCN	ionto/3H glycine	6
Rat	--	AVCN	ionto/3H glycine	6
Rat	--	DLL	ionto/3H glycine	6
Rat	--	DLL	ionto/3H glycine	6
Monkey	--	--	immuno/glycine antisera	*
Rat	--	--	immuno/glycine antisera	*

--- no identification number given.
* Survival time not applicable.

AVCN, anteroventral cochlear nucleus; Cb, cerebellum; DLL, dorsal nucleus of the lateral lemniscus; ionto, iontophoreseis; LSO, lateral superior olive; mcp, middle cerebellar peduncle; NV, trigeminal nucleus; Pon N., nucleus of the pons; pressure, pressure injection; RGc, nucleus reticularis gigantocellularis; RPC, nucleus reticularis parvocellularis; RPOc, nucleus reticularis pontis caudalis; VLL, ventral nucleus of the lateral lemniscus; SC, spinal cord.

Table II. IONTOPHORESIS OF 3H GLYCINE* VS. TIME

uA Current	Time (min)	Counts Per Minute (cpm) Supernatant	Pellet	Total
0	0	0	0	0
1	5	60840	3169	64009
1	10	58560	2618	61178
1	15	80120	4920	85040
1	20	121520	6447	127967
1	30	197000	15439	212439

* Fresh slabs of brain tissue were placed in a shallow dish containing 1 ml of isotonic saline. 3H glycine at a concentration of 25 uCi/ul was iontophoresed into the tissue slab at a constant current of 1 uA at time periods of 5 to 30 minutes. Slabs were homogenized in 0.1 N HCl and the supernatant and solubilized pellet were subjected to scintillation spectroscopy.

Table III. SUMMARY TABLE - TOTALS OF LABELLED CELL BODIES PRESENT* FOLLOWING UNILATERAL 3H GLYCINE INJECTIONS INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS (VLL) - AUTORADIOGRAPHY

Nucleus	Case VLL/1	Case VLL/2	Case VLL/3	Case VLL/6
MNTB	62	463	157	485
LVPO/ MVPO	4	12	39	93
LSO	0	30	45	6
RPO	0	5	0	11
RPOo	25	28+	0	0
Pon N	0	130	5	0
DLL	0	10	0	0

* All cell body labelling was found ipsilateral to the site of injection except where indicated.
+ label present ipsilateral and contralateral to the site of injection.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; DLL, dorsal nucleus of the lateral lemniscus; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; Pon N., nucleus of the pons; RPOo, Nucleus reticularis pontis oralis.

Table IV. SUMMARY TABLE - TERMINAL LABELLING* PRESENT IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL 3H GLYCINE INJECTIONS INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS (VLL)- AUTORADIOGRAPHY

Case VLL/1	Case VLL/2	Case VLL/3	Case VLL/6
Pontine N.	Pontine N.+	Pontine N.+	Pontine N.+
VLL	VLL	VLL	VLL
	AVCN	AVCN	AVCN
	LSO	LSO	LSO
	DMPO		DMPO
	DLL		NV
	RPO		RPOI
			NVII

* All terminal labelling was found ipsilateral to the site of injection of 3H glycine except where indicated.

+ label present ipsilateral and contralateral to the site of injection.

AVCN, Anteroventral cochlear nucleus; DMPO, dorsomedial periolivary nucleus; DLL, dorsal nucleus of the lateral lemniscus; LSO, lateral superior olivary nucleus; NV, trigeminal nucleus; NVII, facial nerve; Pontine N., nucleus of the pons; RPO, rostral periolivary nucleus; VLL, ventral nucleus of the lateral lemniscus.

Table V. SUMMARY TABLE - TOTALS OF LABELLED CELL BODIES PRESENT* FOLLOWING UNILATERAL 3H GLYCINE INJECTIONS INTO THE LATERAL SUPERIOR OLIVE (LSO) - AUTORADIOGRAPHY

Nucleus	Case LSO/3	Case LSO/4	Case LSO/5
MNTB	353	372	327
LVPO/ MVPO	12	23	12
MSO	5	0	2
RPO	0	9	1
DMPO	0	3	0
SPO	0	32	0
A5	0	4	0
Pon N	/	0	0

* All cell body labelling was found ipsilateral to the site of injection.

/ cell body label difficult to count due to high density of labelled terminals in close proximity to injection site.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; MVPO, medioventral periolivary nucleus; Pon N., nucleus of the pons; RPO, rostral periolivary nucleus; SPO, superior periolivary nucleus.

Table VI. SUMMARY TABLE - TERMINAL LABELLING* PRESENT IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL 3H GLYCINE INJECTIONS INTO THE LATERAL SUPERIOR OLIVARY NUCLEUS (LSO) - AUTORADIOGRAPHY

Case LSO/3	Case LSO/4	Case LSO/5
Pontine N.	Pontine N.	Pontine N.
RPO	RPO	RPO
NVII	NVII	LVPO/MVPO
VLL	DCN	MSO
DMPO	LL	
RPc	DLL	
	KF	
	A5	
	NV	

* All terminal labelling was found ipsilateral to the site of injection of 3H glycine.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; DCN, dorsal cochlear nucleus; DLL, dorsal nucleus of the lateral lemniscus; DMPO, dorsomedial periolivary nucleus; KF, Kolliker Fuxe auditory nucleus; LL, lateral lemniscus; LSO, lateral superior olivary nucleus; NV, trigeminal nucleus; NVII, facial nerve; Pontine N., nucleus of the pons; MSO, medial superior olive; RPc, Nucleus reticularis parvocellularis; RPO, rostral periolivary nucleus; VLL, ventral nucleus of the lateral lemniscus.

Table VII. SUMMARY - TOTALS OF LABELLED CELL BODIES PRESENT FOLLOWING UNILATERAL INJECTION OF 3H GLYCINE INTO THE LATERAL SUPERIOR OLIVE (LSO) AND INJECTION OF HRP INTO THE IPSILATERAL VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS (VLL).

NUCLEUS	(3H)	(HRP)	VLL/6*
MNTB	372	628	485
MNTBi	0	0	0
c			
LVPO/MVPO	23	134	93
LVPO/MVPOi	0	44	0
c			
RPO	9	15	0
RPOi	0	57	0
c			
DMPO	3	23	0
DMPOi	0	20	0
c			
SPO	32	25	0
SPOi	0	5	0
c			
A5	4	15	0
A5i	0	3	0
c			
AVCN	0	3	0
AVCNi	0	1044	0
c			
DLL	0	0	0
DLLi	0	513	0
c			
PL	0	0	0
PLi	0	78	0
c			
SP50	0	70	0
SP50i	0	61	0
c			
DCN	0	0	0
DCNi	0	34	0
c			
NVIII	0	0	0
NVIIIi	0	18	0
c			
RMc	0	12	0
RMci	0	11	0
c			
RPOo	0	2	0
RPOoi	0	9	0
c			
MSO	0	47	0
MSOi	0	39	0
c			
RGc	0	1	0
TZ i	0	7	0
i			
LSO	15**	147**	16
LSOi	0	187**	0
c			

* included for comparison: represents 3H glycine into VLL in a separate animal.

** Data not included in calculation of % total label due to proximity to injection site.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; c, contralateral to injection site;DCN, dorsal periolovary nucleus; DLL, dorsal nucleus of the lateral lemniscus; i, ipsilateral to injection site; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; MVPO, medioventral periolivary nucleus; NVIII, cochlear nerve; PL, paralemniscal zone; RGc, nucleus reticularis gigantocellularis; RMc, nucleus reticularis magnocellularis; RPO, rostral periolivary nucleus; RPOo, Nucleus reticularis pontis oralis; SPVO, superior trigeminal nucleus; TZ, trapezoid body; .

Table VIII. TOTALS OF LABELLED CELLS* IN BRAINSTEM NUCLEI FOLLOWING IONTOPHORESIS OF 3H GLYCINE INTO THE NUCLEUS RETICULARIS GIGANTOCELLULARIS (RGc) - AUTORADIOGRAPHY

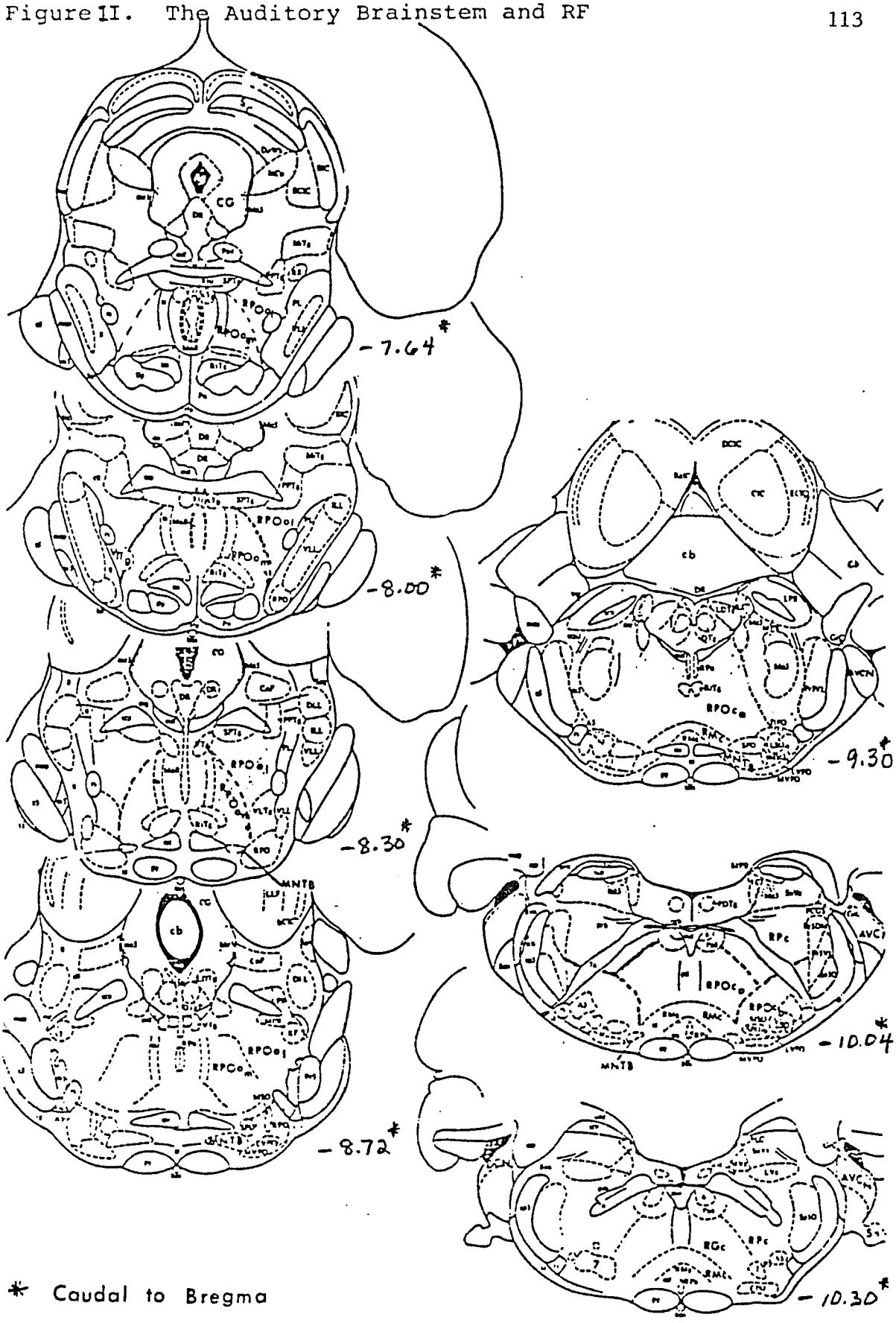
	LABELLED AREA	# LABELLED CELLS	ipsi/ contra	R/C DISTANCE FROM INJECTION (mm)	
RGc/1					
	RGc	2	c	0.320	C
	"	40	i	0.320 - 0.512	C
	LRt	8	i	0.384 - 0.960	C
	PGl	40	i	0.128 - 0.320	C
	"	80	i	0.512 - 0.896	C
	RMc	40	i	0.128 - 0.320	R
	RPc	17	i	0.384 - 1.280	R
	Sp5I	2	i	1.024	R
	RAM	6	m	0.128 - 0.192	R
RGc/2					
	RGc	6	i	1.152 - 2.112	R
	RMc	15	i	0.960 - 1.472	R
	RAP	6	m	0.960 - 1.216	R
	PGl	10	i	0.960	R
	RAM	2	m	2.048	R
RGc/4					
	RGc	10	c	0.576	C
	RAP	16	m	0.512 - 0.704	C
	"	60	m	0.064 - 0.384	C
	"	14	m	1.536 - 1.728	R
	PGl	25	i	1.536 - 1.728	R
	RMc	20	i	0.320 - 0.576	R
	"	4	c	1.536	R
RGc/3					
	RGc	27	i	1.040 - 2.800	L
	"	36	i	0.000 - 0.320	L
	RMc	5	i	0.960 - 1.440	L
		7	i	0.320	L
	RAM	6	m	0.000	-
	Spinal cord	Lamina I-III, VIII -X		Terminals	
RGc/5					
	RGc	3	i	0.240 - 0.320	R
	RPc	1	i	0.640	R
#6					
	RPc	20	i	0.000 - 0.160	C
	Sp50	40	i	0.240 - 0.400	C
	VII	8	i	0.320 - 0.480	C
	A5	3	i	0.560	R
	"	8	i	0.000	-

ipsi	label found ipsilateral to injection site.
contra	label found contralateral to injection site.
m	label found medially in tissue section.
R/C	rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive; LRt, lateral reticular nucleus; PGl, nucleus paragigantocellularis lateralis; RAM, raphe magnus; RAP, raphe pontis; RGc, nucleus reticularis gigantocellularis; RMc, nucleus reticularis magnocellularis; RPc, nucleus reticularis parvocellularis; SP5I, inferior aspect of the spinal trigeminal nucleus; SP5O, oral aspect of the spinal trigeminal nucleus; VII, facial nucleus.

VII. FIGURES

Figure II. The Auditory Brainstem and RF



* Caudal to Bregma

Figure III. Iontophoretic Tracer Deposit

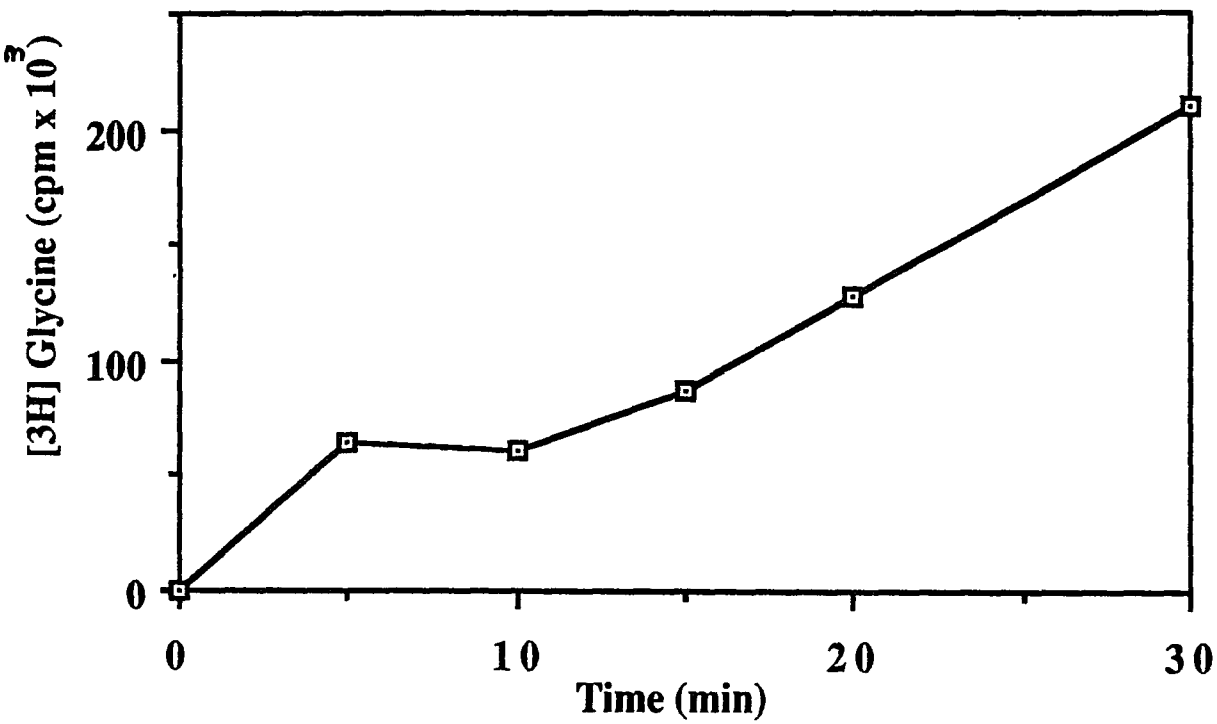


Figure IV. VLL Injection Site.

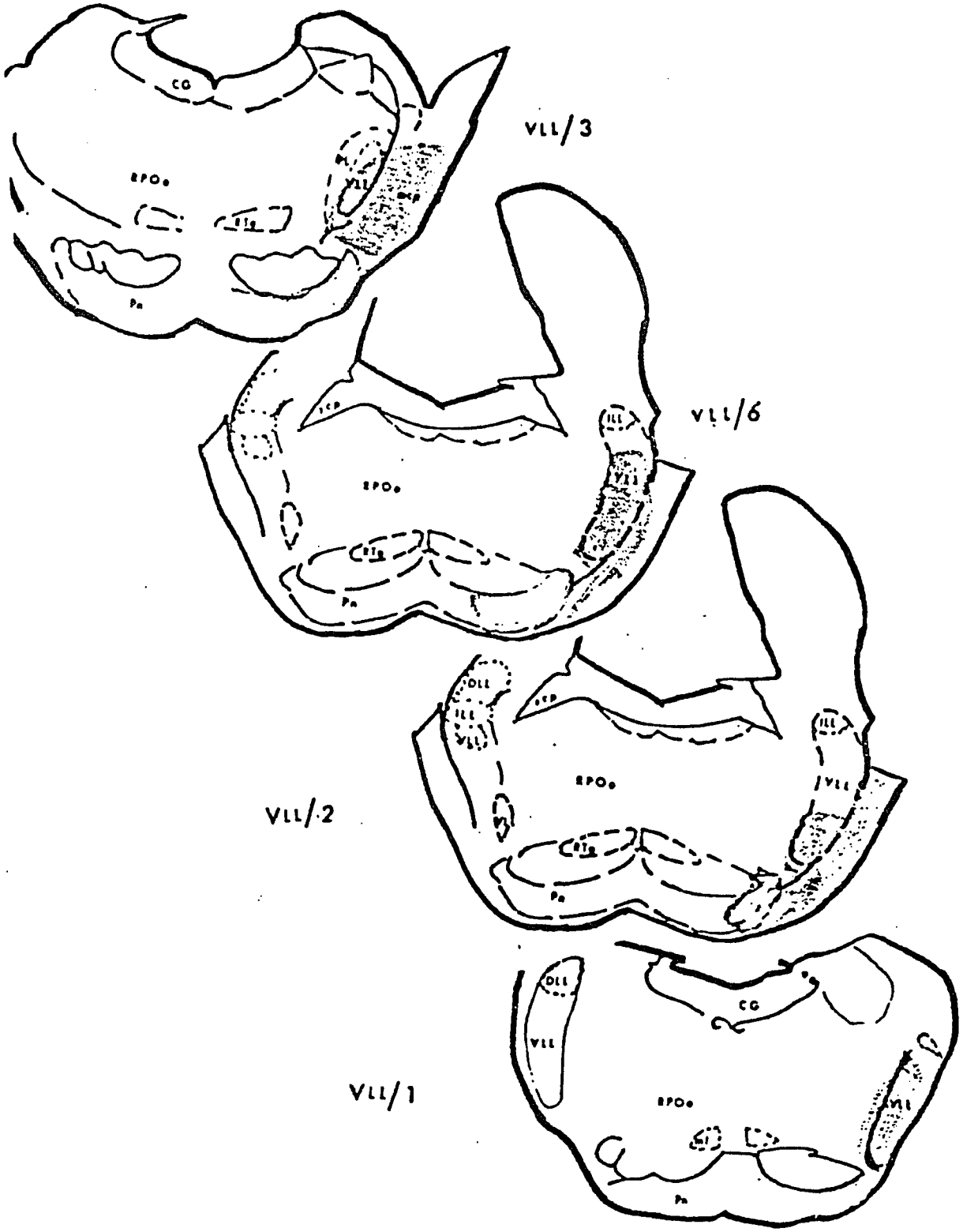


Figure V. LSO Injection Site.

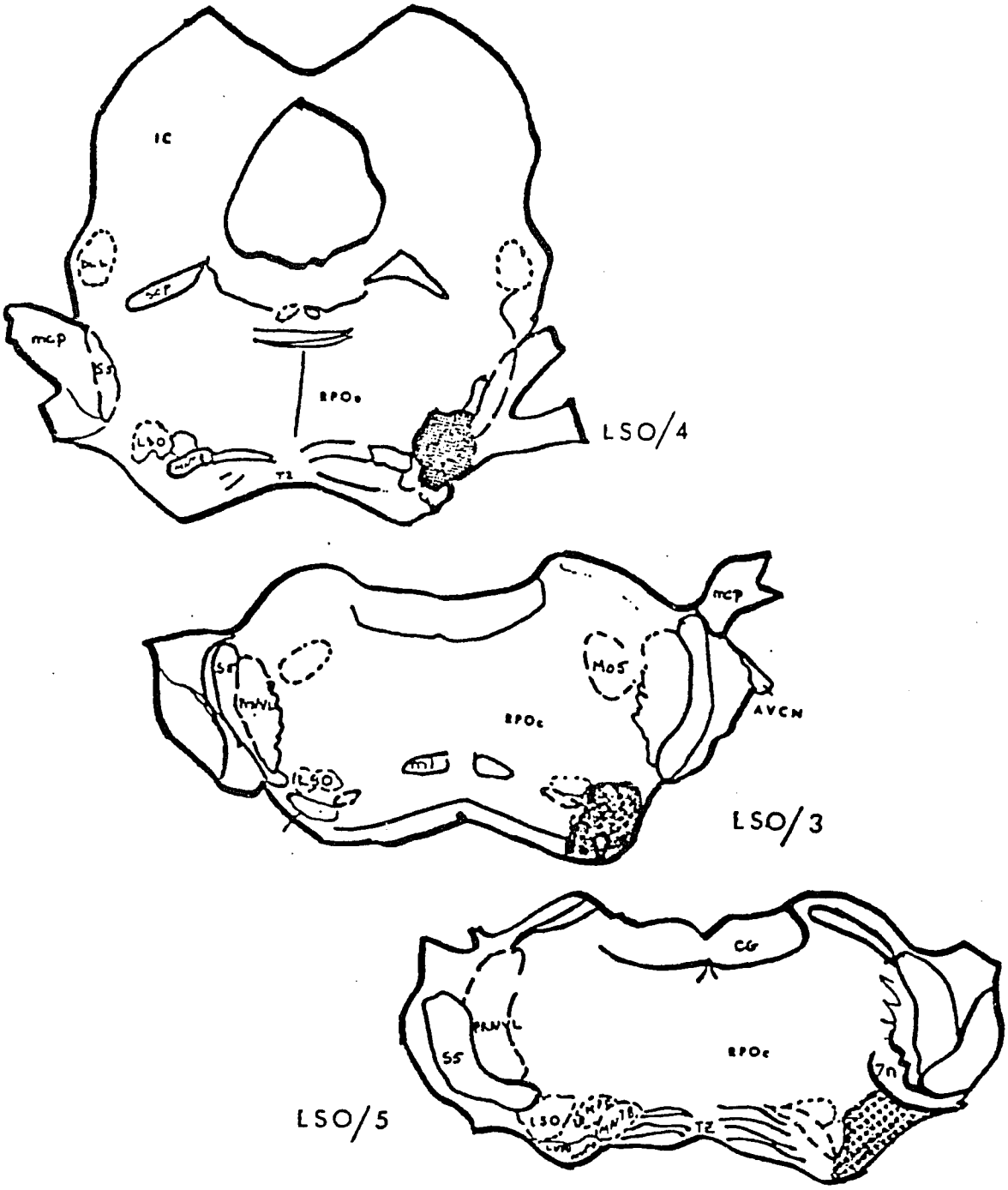
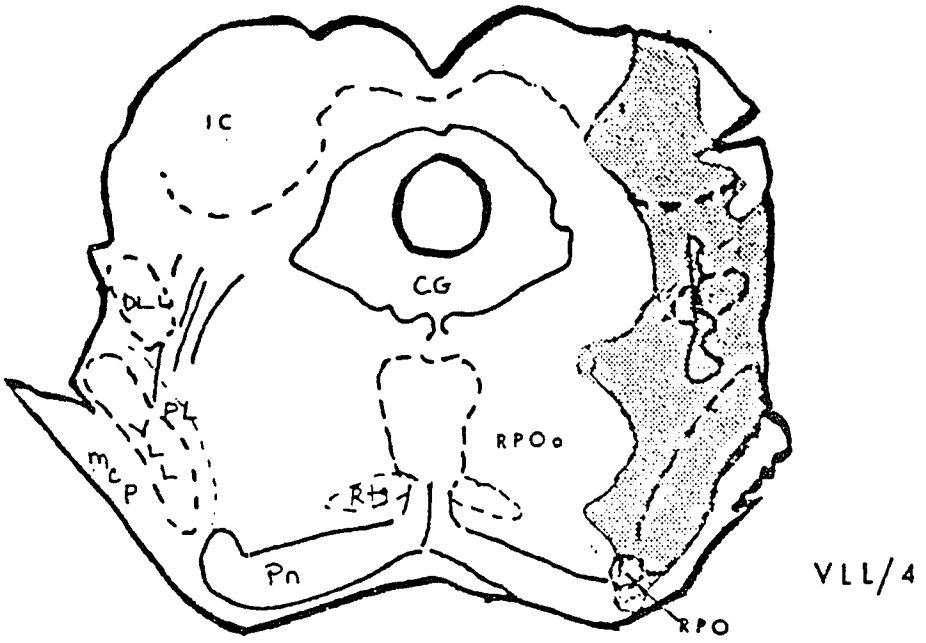
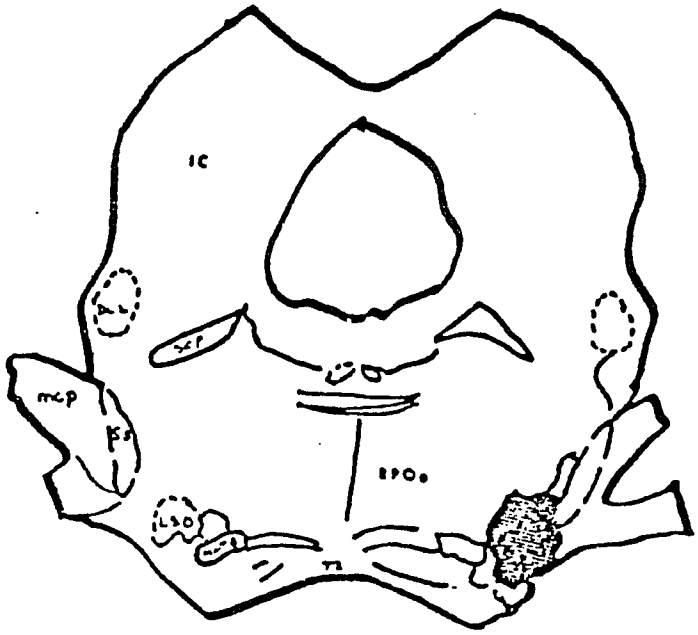


Figure VI. Case LSO/4 (tritium) and Case VLL/4 (HRP) Injection Sites

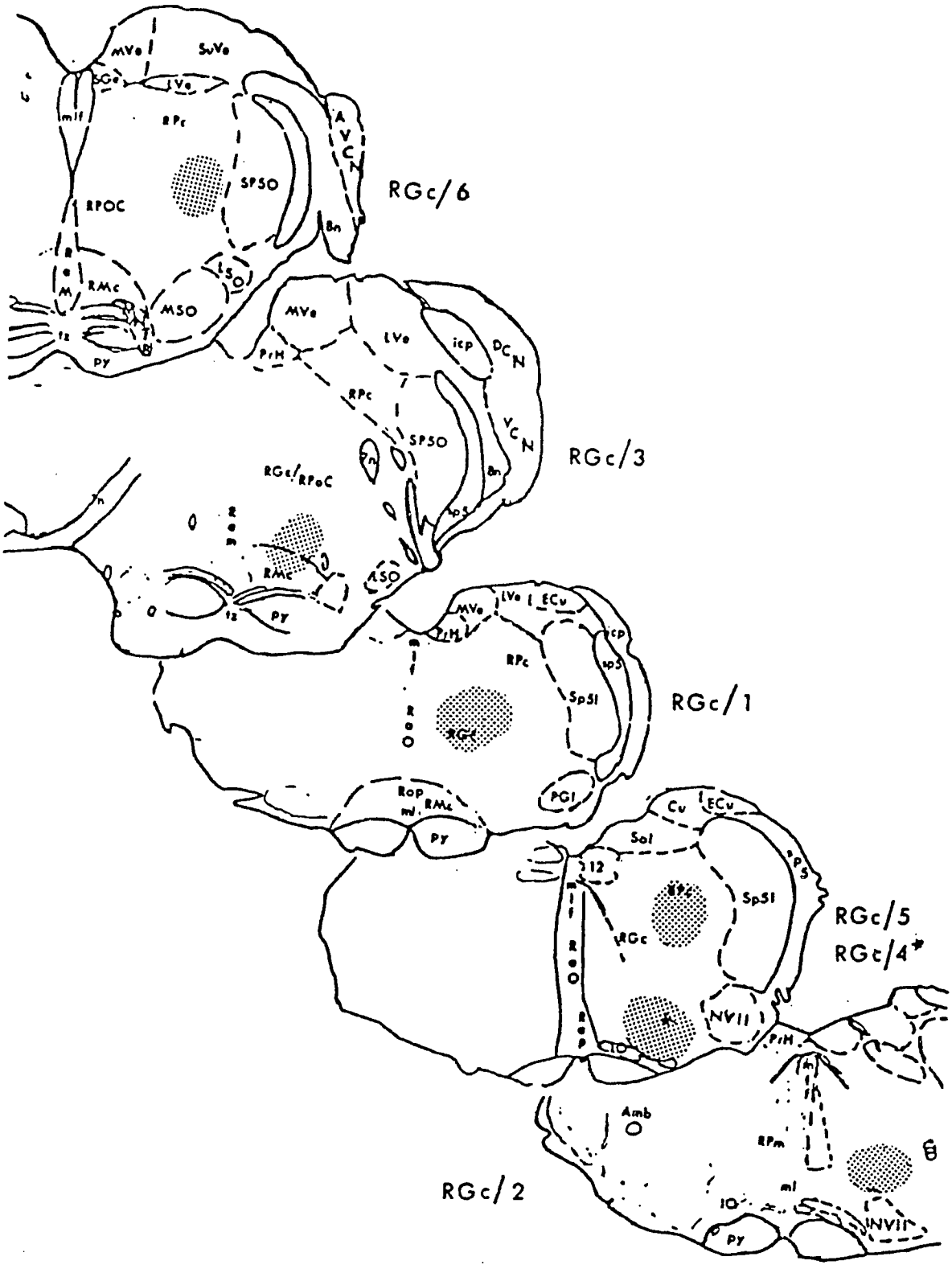


VLL/4



LSO/4

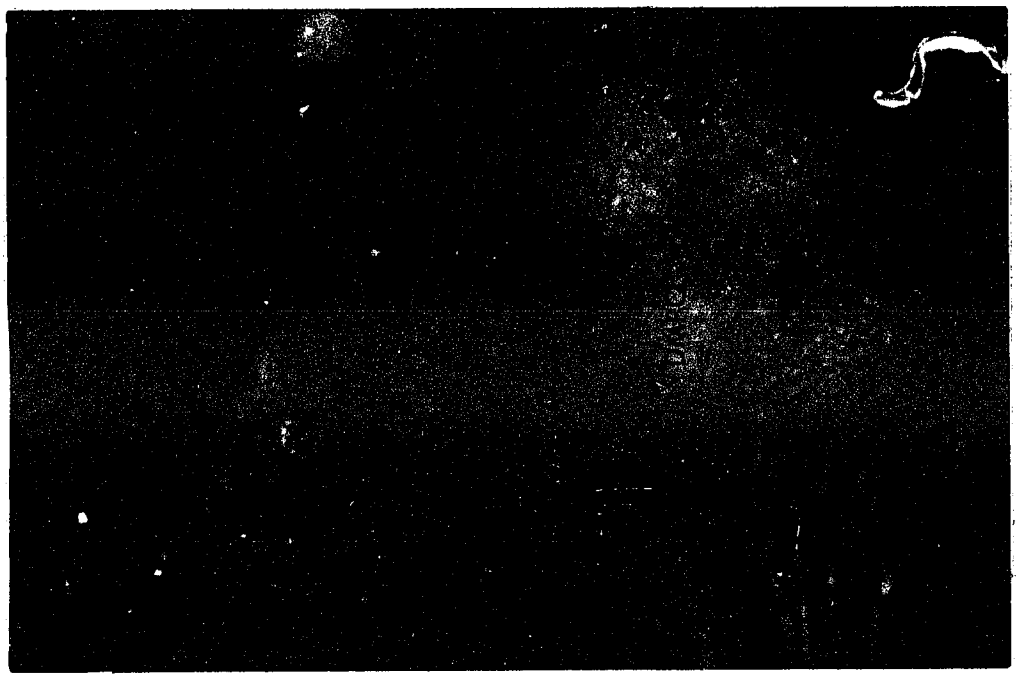
Figure VII. RGc Injection Site.

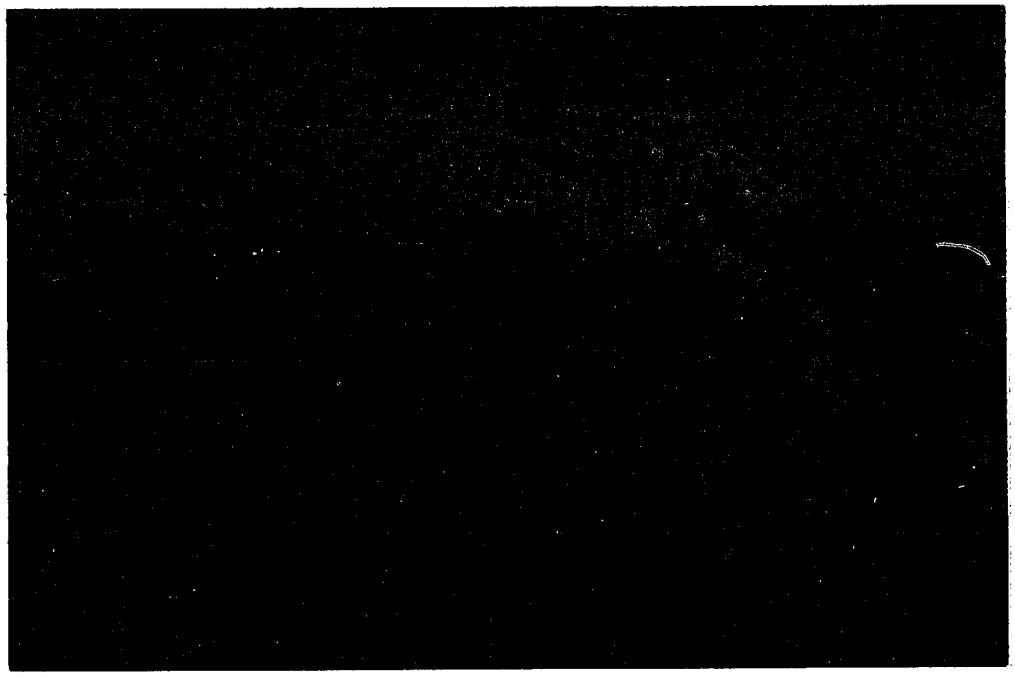


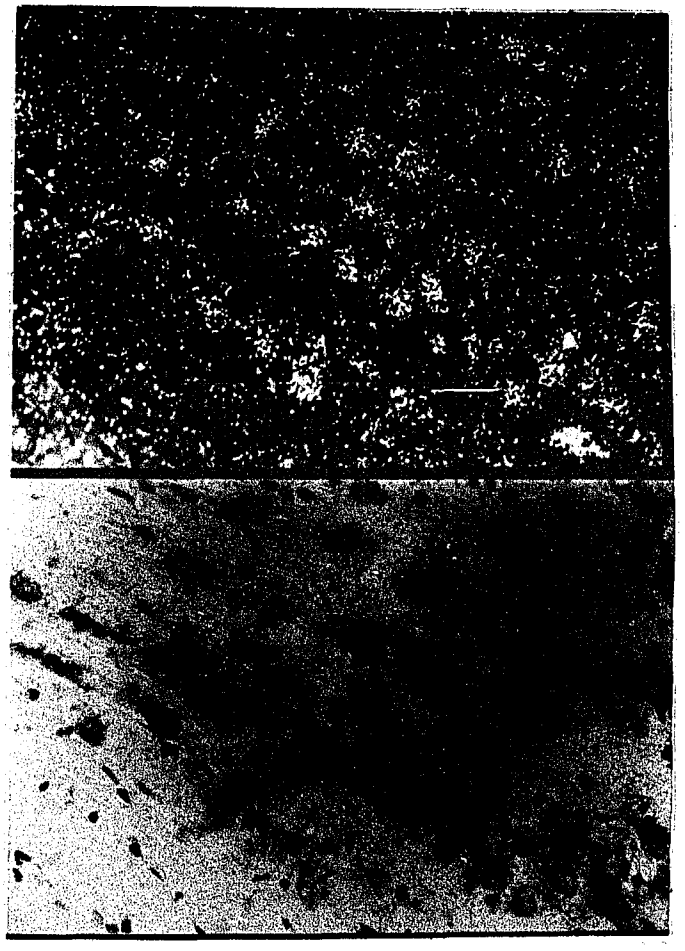
VII. PHOTOMICROGRAPHS

Facing page: Left: Darkfield micrograph of ^{3}H glycine injection site into VLL.

Right: Darkfield micrograph of retrogradely labelled cell bodies in MNTB after ^{3}H glycine injections into VLL. Terminal label is present in the LSO and DMPO.





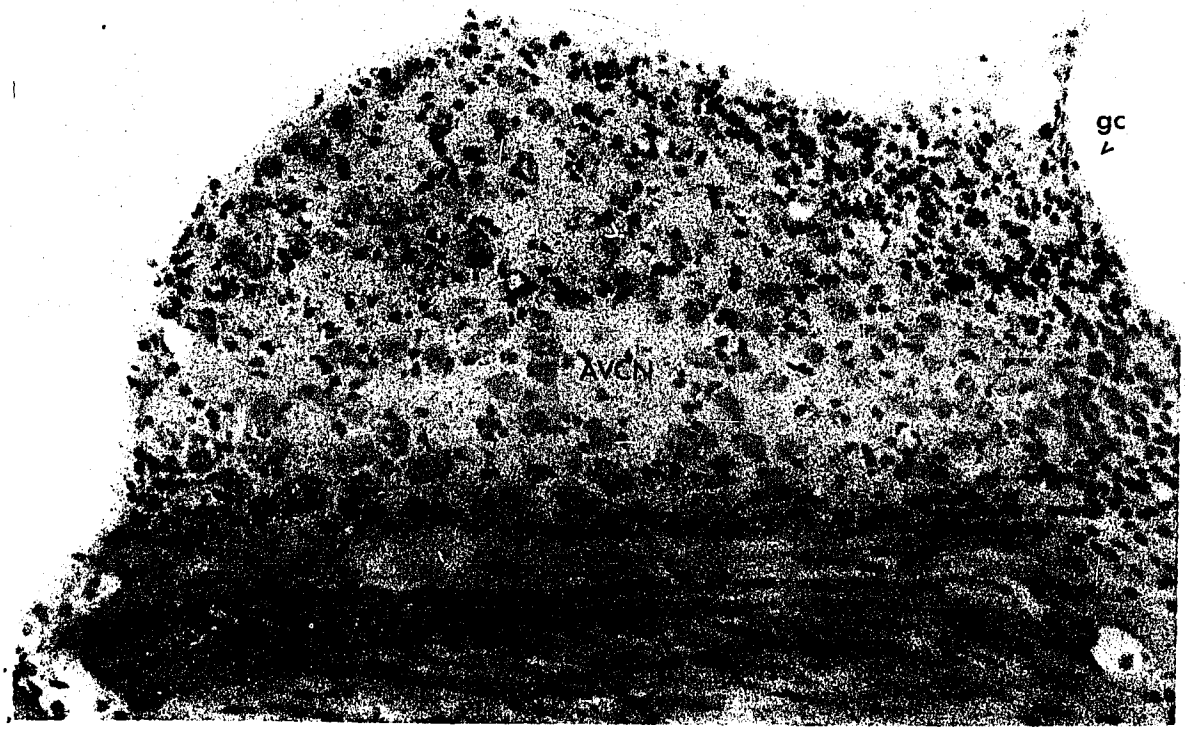


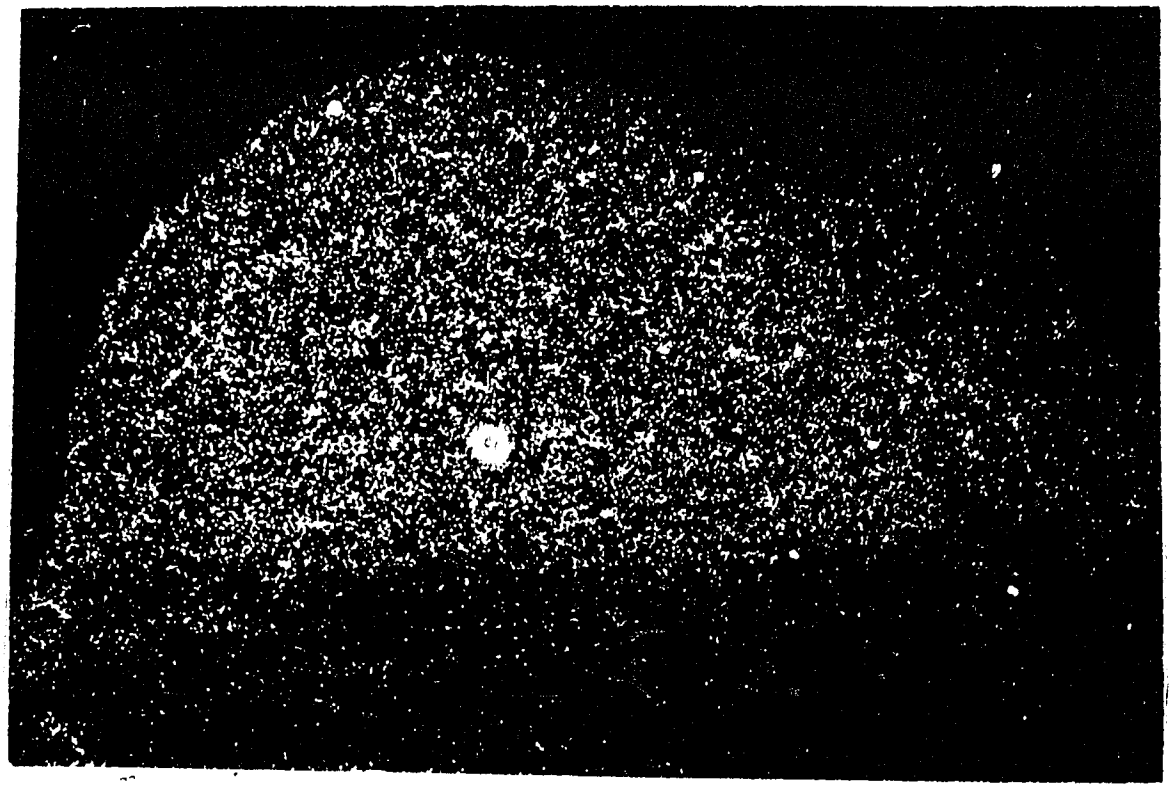
Top: Darkfield micrograph of reduced silver grains in MNTB retrogradely labelled after ^{3}H glycine i injections into VLL or LSO.

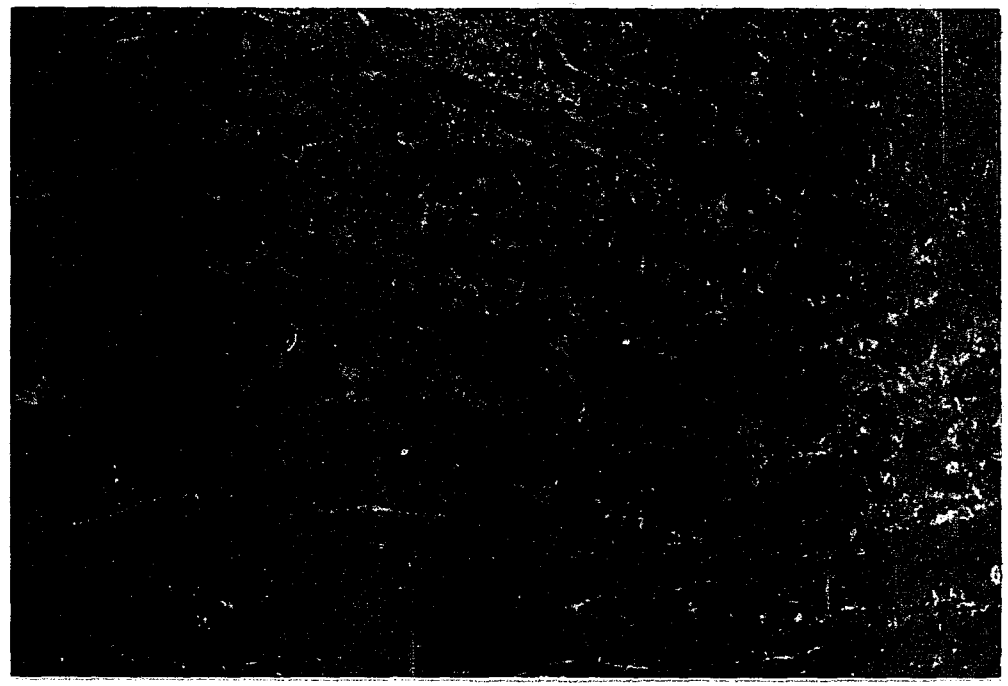
Bottom: Brightfield micrograph of top photo. Dark grains are seen overlying the principal cell of the MNTB.

Facing page: Top: Brightfield micrograph of the ipsilateral AVCN labelled after ^{3}H glycine injections in VLL.

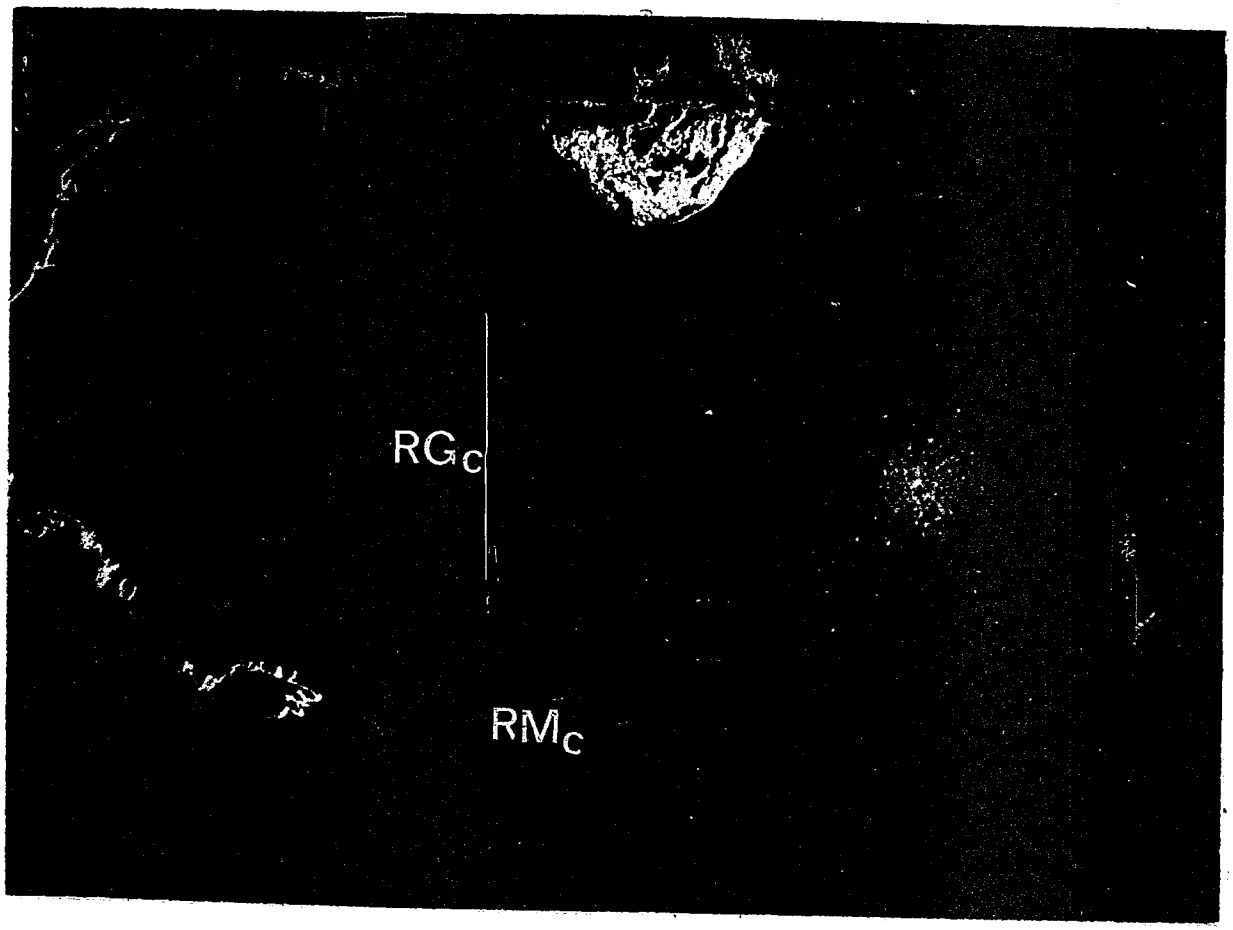
Bottom: Darkfield micrograph of top photo. Grains overly the AVCN and the granuel cell layer.


gc
AVCN

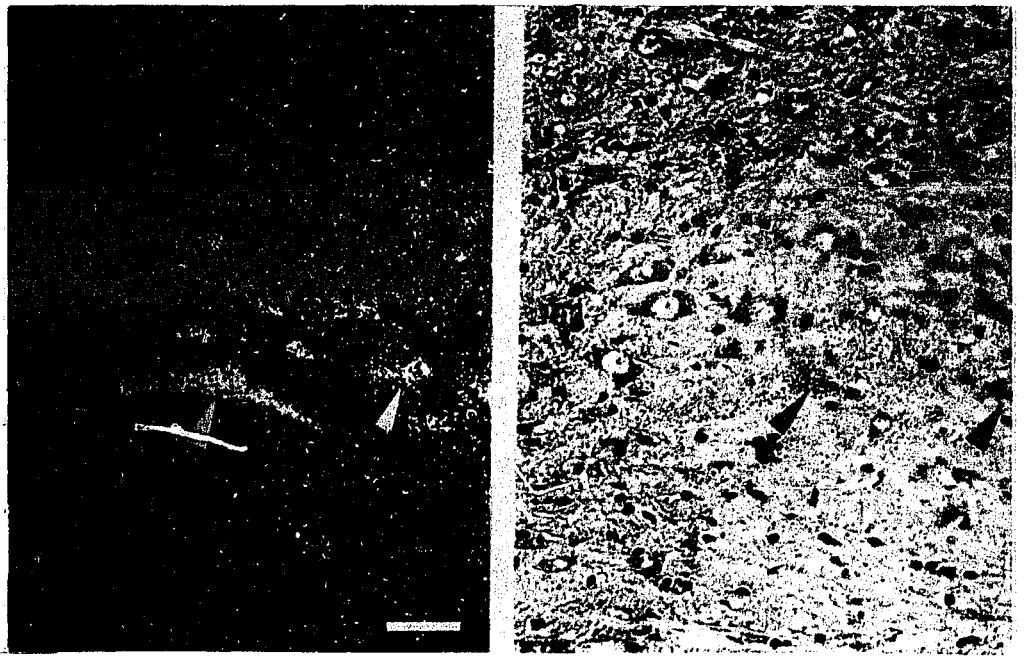




MNTB cell bodies labelled with HRP histochemistry after
HRP-WGA unjection into the ventral nucleus of the lateral lemniscus (VLL).

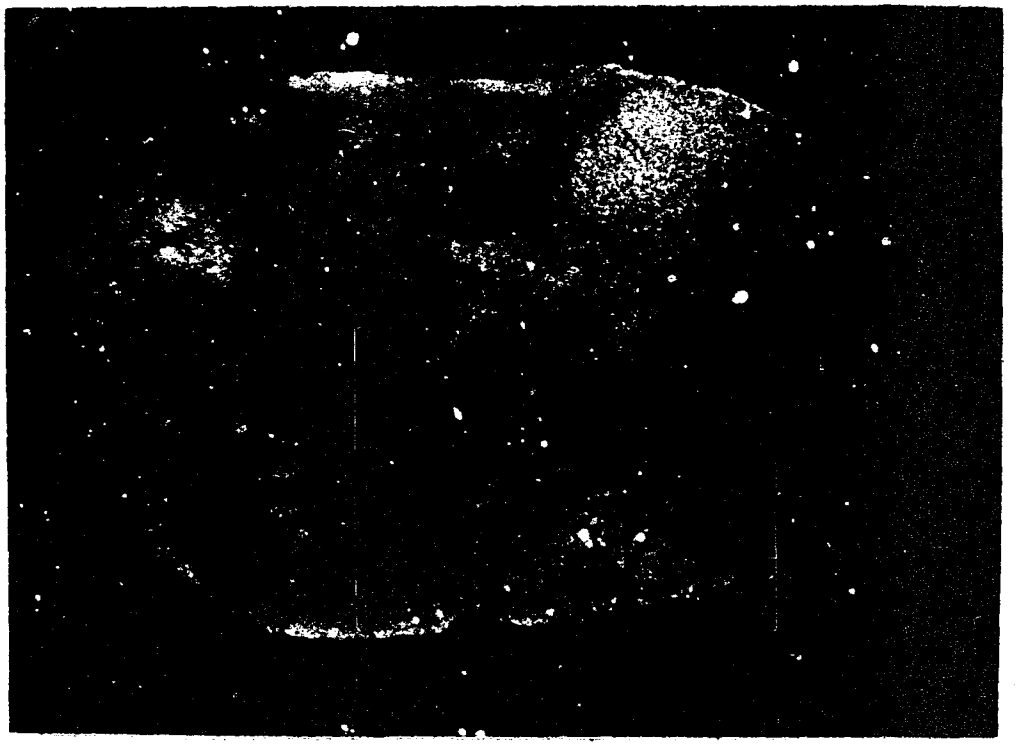


Darkfield micrograph of ^{3}H glycine injection into RGc.



Left: Darkfield micrograph of reduced silver grains in RMc after ^{3}H glycine injection into RGc.

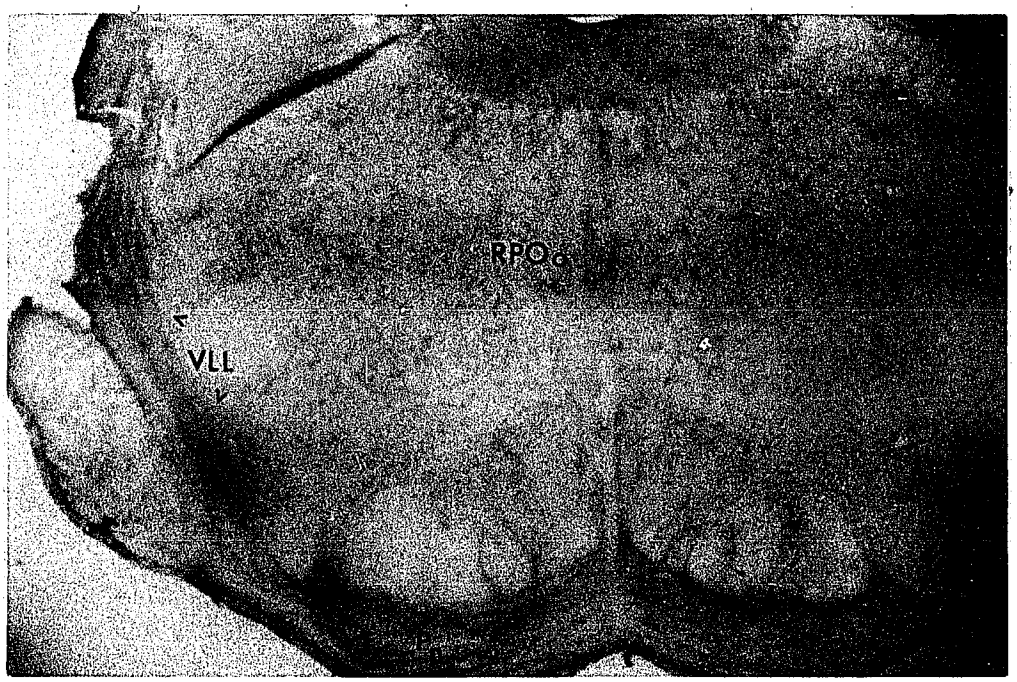
Right: Brightfield micrograph of photo at left. Dark grains are seen overlying cells in RMc.

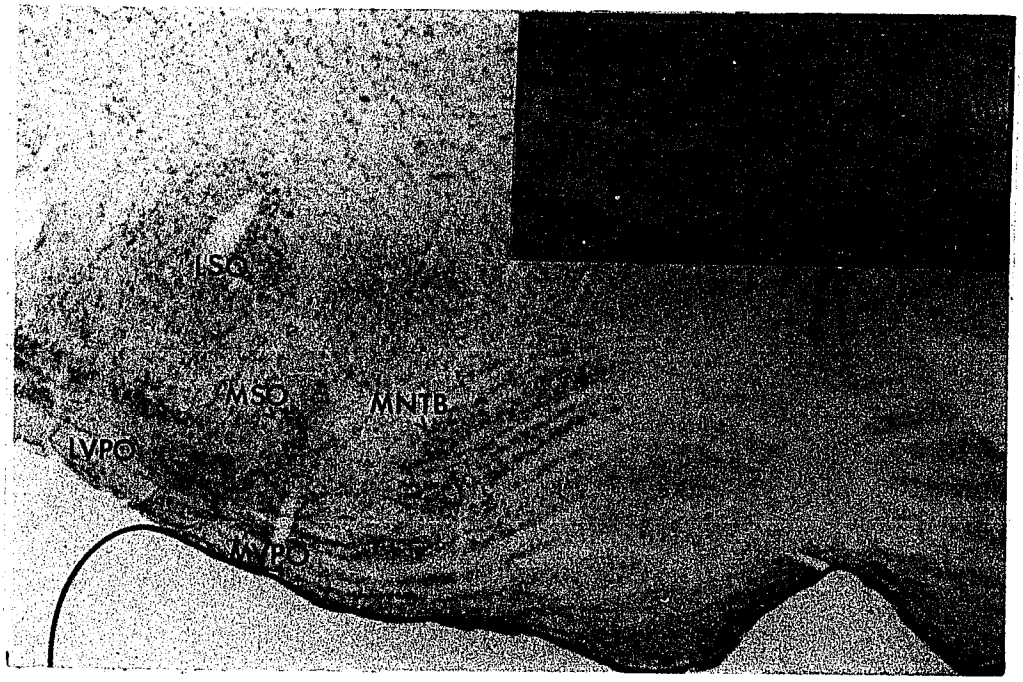


/
Darkfield micrograph of labelling in the cervical spinal cord after ^{3}H glycine injection into the thoracic cord. The ipsilateral dorsal horn is densely labelled. Moderate label is also seen in the ventral tracks of the cord.

Facing page: Top: Brightfield micrograph of immunostained glycine terminals and cell bodies in VLL with antisera against glycine. Glycine positive cell bodies are also present in the pontine nuclei.

Bottom: Brightfied micrograph of immunostained cell bodies in the MNTB, MVPO and LVPO following incubation with glycine antisera. The LSO also had positively stained terminals and scattered cell bodies. The MSO was not stained. INSERT: High magnification of immunostained principal cells in the MNTB.


RPO
VLL


LSO
MSO
MNTB
LVPO
MVPO

IX. APPENDICES

APPENDIX I. STEREOTAXIC COORDINATES

AREA	Anterior/ Posterior*	Lateral	Ventral
Anteroventral Cochlear Nucleus (AVCN)	-9.8	-4.1	-7.5
Dorsal Nucleus of the Lateral Lemnsicus (DLL)	-8.8	-2.8	-6.0
Lateral Superior Olive (LSO)	-9.5	-1.8	-10.0
N. Reticularis Gigantocellularis (RGc)	-11.8	-1.0	-8.0
Spinal Chord	Left Thoracic Chord : T 7-9		
Ventral Nucleus of the Lateral Lemniscus (VLL)	-7.8 -8.0	-2.4 -2.4	-7.5 -7.0

* in relation to the skull surface landmark Bregma.

All coordinates are based upon the atlas of G. Paxinos and C. Watson (1982) Academic Press, New York.

APPENDIX II. ROSTRO-CAUDAL DIMENSIONS OF 3H GLYCINE AND HRP INJECTIONS

INJECTION CASE	CAUDAL* SPREAD (mm)	ROSTRAL* SPREAD (mm)
VLL/1	1.000	0.243
VLL/2	1.000	0.576
VLL/3	0.864	0.576
VLL/6	0.800	0.340
VLL/4	1.120	0.640
LSO/3	1.440	0.480
LSO/4	1.040	0.320
LSO/5	0.600	0.480
RGc/1	0.896	0.576
RGc/2	0.128	0.256
RGc/3	0.384	0.384
RGc/4	0.576	0.576
RGc/5	0.640	0.320

* Represents the caudal and rostral distance from the injection site in each case.

LSO, lateral superior olive; VLL, ventral nucleus of the lateral lemniscus; RGc, nucleus reticularis gigantocellularis.

Appendix III. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INJECTION INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS - CASE VLL/1 - AUTORADIOGRAPHY

LABELLED AREA	#LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE	
VLL	16	YES	0.000	
Pontine N.	0	YES	0.000	
RPOo	2	NO	0.000	C
VLL	47	YES	.288 - .576	C
RPOo	16	NO	.288 - .576	C
VLL	40	YES	.720 - 1.008	C
RPOo	7	NO	.720 - .864	C
MNTB	55	NO	1.872 - 2.304	C
MNTB	62	NO	2.448 - 2.592	C
LVPO/MVPO	4	NO	2.448	C

* All labelled cells and terminals were found ipsilateral to the injection site.

R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; Pontine N., nucleus of the pons; RPOo, Nucleus reticularis pontis oralis; VLL, ventral nucleus of the lateral lemniscus.

Appendix IV. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INJECTION INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS - CASE VLL/2 - AUTORADIOGRAPHY

LABELLED AREA	#LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE
DLL	10	YES	0 - .144 C
Pontine N.	8 /	YES	0 - .144 C
Pontine N.	27 +	YES +	0 - .144 C
Raphe	2 |	NO	.144 C
RPO	5 /	YES	.144 C
RPOo	6	NO	.288 - .576 C
RPOo	22 +	NO +	.432 - .576 C
Pontine N.	55	YES	.288 - .720 C
VLL	40	YES	.576 - .720 C
Pontine N.	40	YES	1.008 C
VLL	60	YES	.864 - 1.008 C
NV	5	NO	1.008 - 1.152 C
LSO	8	YES	1.008 - 1.152 C
LVPO/MVPO	8	NO	1.152 C
MNTB	83	NO	.864 - 1.296 C
NV	1	NO	1.728 C
LSO	21	YES	1.584 - 1.872 C
AVCN	0 /	YES	1.584 - 1.872 C
LVPO/MVPO	4	NO	1.872 C
MNTB	230	NO	1.440 - 1.872 C
MNTB	150	NO	2.160 - 2.448 C

* All labelled cells and terminals were found ipsilateral to the injection site unless otherwise noted.
\+ label contralateral to the injection site.
| label present at midline.
/ number of labelled cells difficult to count due to densely labelled terminals within nucleus.
R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; AVCN, anteroventral cochlear nucleus; DLL, dorsal nucleus of the lateral lemniscus; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; NV, trigeminal nucleus; Pontine N., nucleus of the pons; raphe, raphe magnus; RPOo, Nucleus reticularis pontis oralis;VLL, ventral nucleus of lateral lemniscus.

Appendix V. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INJECTION INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS - CASE VLL/3 - AUTORADIOGRAPHY

LABELLED AREA	#LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE (MM)
Pontine N.	3 /	YES	.720 C
Pontine N.	2 /+	YES +	.720 - .864 C
VLL	85	YES	.720 - 1.152 C
LSO	19	YES	1.584 - 1.728 C
LVPO/MVPO	4	NO	1.728 C
MNTB	40	NO	1.584 - 1.728 C
AVCN	0 /	YES	1.584 - 1.728 C
LSO	21	YES	2.016 - 2.448 C
LVPO/MVPO	35	NO	2.016 - 2.304 C
MNTB	92	NO	2.016 - 2.448 C
AVCN	0 /	YES	2.016 - 2.448 C
LSO	5	YES	2.592 C
MNTB	25	NO	2.592 - 2.736 C

* All labelled cells and terminals were found ipsilateral to the injection site unless otherwise noted.
\+ label contralateral to the injection site.
/ number of labelled cells difficult to count due to densely labelled terminals within nucleus.
R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

AVCN, anteroventral cochlear nucleus; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; Pontine N., nucleus of the pons; RPOo, Nucleus reticularis pontis oralis; VLL, ventral nucleus of the lateral lemniscus.

Appendix VI. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INJECTION INTO THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS - CASE VLL/6 - AUTORADIOGRAPHY

LABELLED AREA	#LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE (MM)
NVII	0	YES	3.520 - 3.120 C
DCN	0	YES	3.360 - 3.120 C
MNTB	89	NO	2.640 - 1.920 C
NV	0	YES	2.640 - 2.080 C
DMPO	0	YES	2.640 C
LVPO/MVPO	12	NO	2.400 - 1.920 C
LSO_d	2	YES	2.400 C
MNTB	66	NO	2.240 - 2.080 C
LVPO/MVPO	21	NO	2.240 - 2.080 C
LSO_l	10	YES	2.080 C
LSO_d	4	YES	2.080 C
A5	8	NO	1.920 C
MNTB	130	NO	1.840 - 1.680 C
LVPO/MVPO	31	NO	1.840 - 1.680 C
A5	6	NO	1.840 C
NV	0	YES	1.840 - 1.680 C
AVCN	0 /	YES	1.760 - 1.680 C
MNTB	100	NO	1.600 - 1.400 C
LVPO/MVPO	13	NO	1.600 - 1.400 C
A5	9	NO	1.400 C
MNTB	100	NO	1.360 - 1.200 C
A5	14	NO	1.360 - 1.200 C
LVPO/MVPO	16	NO	1.280 - 1.200 C
RPO	11	YES	1.280 - 1.200 C
Pontine N.	0 /	YES	0.000

* All labelled cells and terminals were found ipsilateral to the injection site unless otherwise noted.
\+ label contralateral to the injection site.
/ number of labelled cells difficult to count due to densely labelled terminals within nucleus.
R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; DLL, dorsal nucleus of the lateral lemniscus; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; Pontine N., nucleus of

Appendix VII. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INTO THE LATERAL SUPERIOR OLIVE - CASE LSO/3 - AUTORADIOGRAPHY

LABELLED AREA	# LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE (MM)
MNTB	51	NO	1.600 - 1.280 C
NVII	0	YES	1.600 - 1.280 C
DMPO	0	YES	1.600 - 1.280 C
MNTB	159	NO	1.120 - 0.640 C
LVPO/MVPO	7	NO	1.120 - 0.640 C
MSO	5	NO	1.120 - 0.640 C
MNTB	133	NO	0.480 - 0.160 C
LVPO/MVPO	5	NO	0.480 - 0.160 C
RPc	0	YES	0.480 - 0.160 C
MNTB	10	NO	0.000
RPO	0	YES	0.000 - 0.480 R
PonN	0 /	YES	0.000 - 0.480 R
VLL	0	YES	0.640 - 1.120 R
PonN	0 /	YES	0.640 - 1.120 R

* All labelled cells and terminals were found ipsilateral to the injection site.

/ number of labelled cells difficult to count due to the density of labelled terminals and the proximity to injection site.

R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

DMPO, dorsomedial periolivary nucleus; LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; MSO, medial superior olivary nucleus; NVII, facial nucleus; Pontine N., nucleus of the pons; RPc, nucleus reticularis parvocellularis; RPO, rostral periolivary nucleus; RPOo, Nucleus reticularis pontis oralis; VLL, ventral nucleus of the lateral lemniscus.

Appendix VIII. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INTO THE LATERAL SUPERIOR OLIVE - CASE LSO/4 - AUTORADIOGRAPHY

LABELLED AREA	# LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE (MM)
NVII	0	YES	2.240 - 1.920 C
DCN	0	YES	2.240 - 1.920 C
NVII	0	YES	1.840 - 1.520 C
DCN	0	YES	1.840 - 1.520 C
MNTB	67	NO	1.120 - 0.800 C
SPO	7	NO	1.120 - 0.800 C
LVPO/MVPO	8	NO	1.120 - 0.800 C
PrNVL	3	YES	1.120 - 0.800 C
LSO	0 /	YES	1.120 - 0.800 C
MNTB	163	NO	0.720 - 0.400 C
PrNVL	10	YES	0.720 - 0.400 C
SPO	6	NO	0.720 - 0.400 C
LSO	15	YES	0.720 - 0.400 C
LVPO/MVPO	7	NO	0.720 - 0.400 C
MoNV	0	YES	0.720 - 0.400 C
MNTB	135	NO	0.320 - 0.080 C
DMPO	3	NO	0.320 - 0.080 C
LVPO/MVPO	8	NO	0.320 - 0.080 C
A5	4	YES	0.320 - 0.080 C
SPO	4	NO	0.320 - 0.080 C
PrNVL	2	YES	0.320 - 0.080 C
LL	0	YES	0.320 - 0.080 C
KF	0	YES	0.320 - 0.080 C
DLL	0	YES	0.320 - 0.080 C
RPO	0 /	YES	0.320 - 0.080 C
MNTB	7	NO	0.000
Pon N	0 /	YES	0.000
RPO	9	YES	0.000
DLL	0	YES	0.000
DLL	0	YES	0.160 - 0.320 R
Pon N	0 /	YES	0.160 - 0.320 R
RPO	0 /	YES	0.160 - 0.320 R

* All labelled cells and terminals were found ipsilateral to the injection site.
/ number of labelled cells difficult to count due to densely labelled terminals within nucleus.
R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; DLL, dorsal nucleus of the lateral lemniscus; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MoNV, motor trigeminal nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; Pontine N., nucleus of the pons; PRNVL, principal trigeminal nucleus, lateral aspect;
RPOo, Nucleus reticularis pontis oralis.

Appendix IX. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS AND TERMINALS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL IONTOPHORESIS OF 3H GLYCINE INTO THE LATERAL SUPERIOR OLIVE - CASE LSO/5 - AUTORADIOGRAPHY

LABELLED AREA	# LABELLED CELLS	LABELLED TERMINALS	R/C DIST FROM INJ. SITE (MM)
MNTB	15	NO	0.960 - 0.840 C
RPO	1	YES	0.960 - 0.840 C
MNTB	52	NO	0.720 - 0.480 C
MSO	2	YES	0.720 - 0.480 C
LVPO/MVPO	2	YES	0.720 - 0.480 C
MNTB	85	NO	0.360 - 0.120 C
LVPO/MVPO	10	NO	0.360 - 0.120 C
MNTB	22	NO	0.000
MNTB	66	NO	0.120 - 0.360 R
PonN	0 /	YES	0.480 - 0.840 R
RPO	0 /	YES	0.480 - 0.840 R

* All labelled cells and terminals were found ipsilateral to the injection site.
/ number of labelled cells difficult to count due to densely labelled terminals within nucleus.
R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

LVPO, lateroventral periolivary nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; Pontine N., nucleus of the pons; MSO, medial superior olive; RPO, rostral periolivary nucleus.

Appendix X. ROSTRO-CAUDAL DISTRIBUTION OF LABELLED CELLS* IN BRAINSTEM NUCLEI FOLLOWING UNILATERAL PRESSURE INJECTION OF HRP-WGA INTO THE LATERAL SUPERIOR OLIVE - CASE LSO/4 - HISTOCHEMISTRY

LABELLED INJ. AREA	#LABELLED CELLS	ipsi/contra	R/C DIST FROM SITE (MM)
AVCN	204	c	2.720 - 2.400 C
DCN	34	c	2.720 - 2.400 C
RMc	2	c	2.720 - 2.400 C
SP5O	9	c	2.720 - 2.400 C
AVCN	395	c	2.320 - 2.000 C
AVCN	3	i	2.320 - 2.000 C
SP5O	32	c	2.320 - 2.000 C
RGc	1	i	2.320 - 2.000 C
RMc	8	c	2.230 - 2.000 C
RMc	1	i	2.320 - 2.000 C
AVCN	305	c	1.920 - 1.600 C
LSO/A5	13	i	1.920 - 1.600 C
RMc	4	i	1.920 - 1.600 C
SP5O	12	c	1.920 - 1.600 C
CN Nerve	18	c	1.920 - 1.600 C
TZ	7	i	1.920 - 1.600 C
LVPO/MVPO	30	i	1.920 - 1.600 C
LSO	99	c	1.920 - 1.600 C
LSO	23	i	1.920 - 1.600 C
SPO	5	i	1.920 - 1.600 C
MNTB	60	i	1.920 - 1.600 C
MSO	8	c	1.920 - 1.600 C
AVCN	140	c	1.520 - 1.200 C
MNTB	277	i	1.520 - 1.200 C
DMPO	23	i	1.520 - 1.200 C
DMPO	20	c	1.520 - 1.200 C
LVPO/MVPO	42	i	1.520 - 1.200 C
LVPO/MVPO	13	c	1.520 - 1.200 C
LSO	88	i	1.520 - 1.200 C
LSO	118	c	1.520 - 1.200 C
A5	3	c	1.520 - 1.200 C
SP5O	8	c	1.520 - 1.200 C
MSO	35	i	1.520 - 1.200 C
MSO	22	c	1.520 - 1.200 C
MNTB	285	i	1.120 - 0.800 C
LVPO/MVPO	55	i	1.120 - 0.800 C
LVPO/MVPO	24	c	1.120 - 0.800 C
A5	15	i	1.120 - 0.800 C
MSO	12	i	1.120 - 0.800 C
MSO	9	c	1.120 - 0.800 C
SPO	27	i	1.120 - 0.800 C
SPO	5	c	1.120 - 0.800 C
RMc	8	c	1.120 - 0.800 C
RPO	15	i	1.120 - 0.800 C
RPO	12	c	1.120 - 0.800 C
MNTB	6	i	0.720 - 0.400 C
DLL	176	c	0.720 - 0.400 C
RPO	42	c	0.720 - 0.400 C
PL	14	c	0.720 - 0.400 C
PLm	7	c	0.720 - 0.400 C
LVPO/MVPO	7	i	0.720 - 0.400 C
LVPO/MVPO	7	c	0.720 - 0.400 C
DLL	132	c	0.320 - 0.080 C
DLLm	10	c	0.320 - 0.080 C
RPO	3	c	0.320 - 0.080 C
PL	26	c	0.320 - 0.080 C
PLm	5	c	0.320 - 0.080 C
RPOo	4	c	0.000
RPOol	4	c	0.000
DLL	30	c	0.000
PL	4	c	0.000
DLL	97	c	0.080 - 0.400 R
PL	22	c	0.080 - 0.400 R
RPOo	2	i	0.080 - 0.400 R
RPOol	1	c	0.080 - 0.400 R
DLL	68	c	0.400 - 0.800 R

i ipsilateral to injection site
c contralateral to injection site
R/C rostral or caudal distance of cell body or terminal label from the injection site as defined within coronal sections of the brainstem.

A5, Noradrenergic cell group lying dorsal and lateral to the lateral superior olive in the rat brainstem; AVCN, anteroventral cochlear nucleus; DLL, dorsal nucleus of the lateral lemniscus; DLLm, medial aspect of DLL; LSO, lateral superior olive; LVPO, lateroventral periolivary nucleus; MoNV, motor trigeminal nucleus; MNTB, medial nucleus of the trapezoid body; MVPO, medioventral periolivary nucleus; MSO, medial superior olive; PL, paralemniscal zone; PLm, paralemniscal zone, medial aspect; Pontine N., nucleus of the pons; PRNVL, principal trigeminal nucleus, lateral aspect; RMc nucleus reticularis magnocellularis; RPO, rostral periolivary nucleus; RPOo, Nucleus reticularis pontis oralis; RPOol, lateral aspect of RPOo; SPO, superior periolivary nucleus

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