PROCESSING OF INTERAURAL LEVEL DIFFERENCES IN THE AUDITORY BRAINSTEM OF THE BARN OWL

Thesis by

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ABSTRACT:

Nervous systems process information about the environment in order to generate adaptive behavior. Sensory information that is obtained through different modalities, gleaned from different interactions of the animal with its surroundings, generated by different neural algorithms, or used to infer different distal stimulus properties, is often processed by distinct pathways in the brain. The auditory system compares sounds at the two ears in order to derive the location of the source. In the barn owl, *Tyto alba*, this is accomplished by interaural comparisons of the time that a sound reaches each ear, and of the level (intensity) at each ear. Interaural time differences code for horizontal positions of sound sources while interaural level differences, due to a vertical asymmetry in the owl's ears, can encode the vertical position of a sound. These two cues together can assign unique locations to sound sources in space. The barn owl processes time- and level differences in separate neural channels that converge in the inferior colliculus. This structure is the first site of neurons with spatially restricted auditory receptive fields, and with a neural map of auditory space. Downstream projections from here provide the sensory input for accurate sound localization by saccadic head movements.

I report that the owl's two auditory processing streams are also segregated histochemically. The pathway that computes level differences stains especially strongly for the enzyme acetylcholinesterase, which may underlay processing of scalar (intensity) information over large dynamic ranges. This staining is complementary to immunohistochemical staining for calbindin, which has been shown previously to stain the pathway that processes interaural time differences.

In further hodological and physiological experiments, I describe the algorithms that generate tuned responses in the inferior colliculus that encode vertical sound source position. This study shows that a lemniscal nucleus, nucleus ventralis lemnisci lateralis pars posterior (VLVp), projects bilaterally to a subdivision of the inferior colliculus (the shell of ICc). This projection appears to preserve tonotopy, and to provide inhibition by sounds of large interaural level difference. This probably GABAergic mechanism leads to the synthesis of neuronal responses in the inferior colliculus that are narrowly tuned to interaural level difference. My methodological strategy was to increase or decrease activity in VLVp by injection of blockers or agonists of GABA-A receptors, and then to record downstream in the inferior colliculus any changes in response tuning that resulted. The study suggests that the bilateral inhibition by VLVp is sufficient to explain the peaked responses to level differences of collicular neurons. Excitatory input to the inferior colliculus is conveyed by fibers of the lateral lemniscus, and may arise from a number of stations, including lemniscal and cochlear nuclei. The circuits I describe determine the tuning of cells to interaural level differences, but are independent of and have no effect on the tuning to interaural time differences, and further support that time and level are processed separately in the owl's brainstem.

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CHAPTER 1:

GENERAL INTRODUCTION

"If we knew thoroughly the nervous system of Shakespeare, and as thoroughly all his environing conditions, we should be able to show why at a certain period of his life his hand came to trace on certain sheets of paper those crabbed little black marks which we for shortness' sake call the manuscript of Hamlet."

--William James (1890), The Principles of Psychology, pp. 136-7.

The function of perception is to guide action. One of the most striking features in the design of nervous systems is the amount of processing interposed between sensory transduction of signals from the environment, and motor output based on such signals. If one takes a broad and generalized perspective of the evolution of neural systems, the most profound increase in complexity appears to have taken place at a level central to sensory processing, yet still upstream from architectures that are clearly motor systems.

This picture of functional architecture is most appropriate to mammalian, and especially primate, neural systems. Those layers of information processing that are both too far downstream to be called sensory, yet too far upstream to be clearly motor, are usually relegated to the somewhat fuzzy category of

"cognitive processes." There is not one well-defined neural system involving cognition in which the path from sensory input to motor output has been successfully traced. Not surprisingly, some theorists have taken the disparaging view that only perception will be amenable to scientific investigation and analysis; cognition is too diffuse and unbounded a capacity to be thus understood (Fodor 1983).

Nonetheless, very impressive headway has been made in tracing sensory processing more and more centrally, especially in the primate visual system, which I will focus on repeatedly for comparison and contrast in this introduction. Much less impressive is our understanding of the more central processes that funnel into motor outputs. In part, much of the difficulty in tracing routes of information processing from the periphery either upstream or downstream lies in the lack of clear architectures for understanding the direction of the flow of information. Feedback, parallel and multi-track routes of information flow, and distributed and sparse connectivity are some of the features found especially in the nervous systems of more complex organisms that make their analysis exceedingly difficult.

In a few cases, the link between sensory and motor systems has been completed. Such systems are "reflexive," in the sense that they do not involve cognition, and in the sense that, at least to first-order, their behavior is predictable. Sensorimotor transformations, in order to be analyzable without recourse to processes outside the system, need to be "modular", in the sense that Fodor (1983) and also Liberman (Liberman and Mattingly 1989; Mattingly and Liberman 1988) and others have proposed. Such systems are informationally closed ("informationally encapsulated") and cannot draw upon more central processing ("cognitively impenetrable"). The point here is that a modular sensorimotor system can be understood in relative isolation from the

details of the rest of the nervous system. This is not to say that such modular systems cannot be inferential; indeed, what distinguishes perception from true reflex arcs is the ability to infer distal properties of stimuli from proximal effects on sensory receptors. But, in some sense, all modular perceptual processes are like reflexes in that they are rather rigid: no amount of effort on my part can persuade my visual system not to succumb to the Mueller-Lyer illusion, for example; no amount of knowledge will result in me not blinking my eyes if an object rapidly approaches close to my face, and so forth.

Two examples of the behaviors I have in mind are reflexes involved in startle behavior (which are usually not oriented, but perform at least some gross discrimination of the stimulus), and reflexes involved in orienting the animal in space (which are often not involved in recognition). Orienting behaviors of animals received much attention at the beginning of this century in the work of Jacques Loeb (1912), who, predating the Behaviorists by two decades, developed a theory of how animal behavior (in his view nearly all animal behavior) was to be regarded as sequences and combinations of various so-called *tropisms*: reflexive and spatially ordered movements. Many other ways of describing the spatial behavior of animals have been elaborated since (see Schoene (1984) for discussion).

Spatial orientation is often an essential prerequisite to obtaining optimal sensory input. Faced with a stimulus in the periphery, an animal's first response will often be a very fast orienting movement that aligns its sensory organs with the stimulus so as to permit a good analysis of the sensory input. Most animals will turn to face a visual, auditory, or tactile stimulus. In addition to allowing better sensory analysis, the animal's body axis becomes aligned with the stimulus, facilitating, for example, an attack. Reflexes in which an auditory or

visual stimulus elicits a turn of the head, or a movement of the eyes, are especially well understood.

The optic tectum of the barn owl, and the superior colliculus of mammals, are both involved in auditory and visual spatial orientation. Parallel with what David Marr (1982) wrote about vision, one could say that to hear is "to know what is where" by listening. It is striking that, as in vision (Goodale and Milner 1992; Ungerleider and Mishkin 1982), audition consists in processing the quality of the stimulus (what it is; recognizing it) at many stages separately from processing its location in the environment.

The strategy of neuroethology has been to select a system whose output, that is, whose influence on behavior, is especially well characterized. This importantly includes an understanding of the relevance of the behavior in a natural setting. By selecting behaviors that are both well described and naturally important, one hopes to select a subserving neural system whose design principles will make sense in light of the task of producing the appropriate motor output. Some vertebrate examples that epitomize this approach are echolocation in the bat, electroreception in weakly electric fish, singing in songbirds, and spatial audition in the barn owl.

It is important to realize that, while emphasizing an understanding of naturally relevant behaviors, neuroethology is emphatically not Behaviorist. We can agree with B.F. Skinner when he writes, "We cannot account for the behavior of any system while staying wholly inside it; eventually we must turn to forces operating on the organism from without." But neuroethologists (and, these days, most everyone else) will disagree when Skinner says in the same paragraph that, "The objection to inner states is not that they do not exist, but that they are not relevant in a functional analysis" (Skinner 1953, p.35). Inner

states--in our case, neurophysiological states--matter as much as inputs and behavior.

How Important are Single Neurons?

While there is virtually unanimous agreement that complex sensory systems have some modularity in terms of processing and representing different physical parameters, there is some disagreement on the relevance of single neurons within this larger processing scheme. Horace Barlow (1972) articulated the idea of the so-called "cardinal cell": a single, high-level neuron encodes a specific aspect of some stimulus; such a neuron would fire if and only if a specific feature was detected in the environment. In the extreme and clearly unrealistic case, one could imagine a single neuron encoding a particular object (an example that is now cliche: there would be a specific neuron in my brain whose sole task it was to encode the presence of my grandmother). In such a scheme, the information about attributes of stimuli would be conveyed by "labelled lines" in the brain: if a specific connection between neural centers is active, this means information about a specific feature that has been detected is being transmitted.

Ever since Karl Lashley defended the notion that neurons function by "mass action" (Lashley 1950), distributed models of how the brain might work have been increasing in popularity. With the recent explosion of work in parallel distributed processing (Rumelhart and McClelland 1986), a lot of theorists favor rather more holographic models of brain function, which feature large

populations of relatively broadly-tuned cells. Although such models do have distinct advantages over extremely punctate models (for example, they can generalize and are relatively fault-tolerant), they would, on the face of it, appear to be harder to control neurally.

What one in fact finds is generally an architecture intermediate to the two extremes outlined above (this is also an attractive position on theoretical grounds; see, for example, Feldman 1989). There is now ample evidence from a number of different systems that single neurons with very complex stimulus selectivities do exist. Some examples are neurons in the forebrain of songbirds that respond best to the bird's own song, neurons in the auditory cortex of bats that respond to very specific combinations of emitted and echoed sound, neurons in electric fish that are highly tuned to comparisons of dynamic properties of electric fields, neurons in the hippocampi of rats that fire only when the animal is in a certain position in space, and neurons in the owl that respond only to sounds coming from a particular location. Some of the most striking mammalian examples are cells in the inferotemporal cortex of primates and sheep (and, to a lesser degree, also in the amygdala, putamen, and frontal cortex) that respond to the sight of very complex objects, such as faces of conspecifics. The faith of single-cell neurophysiology is that the response properties of all such cells can inform us about neural function. Recent evidence that bears this out is found in experiments by Newsome and colleagues (Newsome, Britten et al. 1989; Salzman, Britten et al. 1990), who showed that very small numbers of neurons can code for and affect perception of the direction of visual motion. Such cases can provide an opportunity to investigate neural representations and computations through the transparent contribution made by single cells.

It is important to point out that the complex neurons of the above examples are embedded in circuits. There is little support for the notion that isolated cells are necessary either in perception or in action, as the now outdated concept of "command neurons" from invertebrate studies would have had us believe. In reflex behaviors, small circuits of several interneurons generally appear to function together as a "command circuit" (Larimer 1988; Konishi 1990). A similarly extended view holds at the apical levels of hierarchical sensory processing. In the encoding of faces, for example, stimuli appear to be encoded by the activity pattern over a relatively small ensemble of neurons with fairly restricted response properties (Tanaka, Saito et al. 1991; Gross 1992; Young and Yamane 1992). There is evidence that this is the case in the representation of faces and words in humans also: Heit and colleagues (1988) found neurons in human deep forebrain structures that respond best to a particular face or word, but each face or word will activate many cells.

It is perhaps wise to concede that neocortical processing, while not entirely holographic, is more complex than Hubel and Wiesel's initial successes (Hubel and Wiesel 1959; Hubel and Wiesel 1962) would have led us to believe. Given the recent, successful studies of functional circuits in sensory cortex (Douglas and Martin 1991a), a more circuit-oriented approach may be in order (Douglas and Martin 1991b). It is not clear to what extent this will be necessary in preparations other than neocortex, however. Work from electric fish, and from the auditory system of the barn owl, suggests that a strategic way to elucidate neural circuits and the algorithms they implement is through an analysis of single neurons (Konishi 1991).

Acoustics and Audition in Mammals

The best understood auditory system of any mammal, in terms of both neural processing and of neuroethological function, is that of the bat. Of the roughly 700 bat species that use echolocation to navigate or catch prey, the Old World genus *Rhinolophus*, and the New World *Pteronotus parnellii* have been studied most intensively. Several features appear to have independently evolved in these two cases (Simmons and Stein 1980): together with similar sonar signals (long characteristic frequencies), both *Rhinolophus* and *Pteronotus* feature a specialized behavior known as Doppler-shift compensation. When a bat is rapidly approaching an echoic object in flight, the frequency of the echo will be higher than that of the emitted sound due to Doppler-shifting. In order to maintain highest acuity in processing the echo signal, the bat lowers its emission frequency such that the echo returns at the frequency representation that is overrepresented in the bat's nervous system (its "acoustic fovea").

Compensation for Doppler-shift is one specialized behavior in echolocating bats that has recently been traced from sensory input to motor output. Metzner (1989) found neurons in the paralemniscal zone of *Rhinolophus* that project to motor nuclei involved in emitting sound. Some neurons in this zone, aptly called "auditory-vocal" cells, respond to particular combinations of the bat's vocalizations and the echo that normally elicit a Doppler-shift compensation by the bat.

Different physical properties of the bat's cry and of the echo yield different information about the environment. The duration of the sonar signal, its frequency, its harmonic structure, and its temporal structure (frequency modulation) all are processed as distinct cues. The work of Suga and colleagues (see, for example, Suga 1984, 1988, 1989) has shown that these cues are mapped in separate and specialized regions of neocortex in

Rhinolophus. The cartography of the bat's auditory cortex exhibits striking similarities to the visual cortex of primates in that both have many neural regions that map parameters that are processed separately. The advantage of a neuroethological system like bat biosonar is that it provides an especially powerful interpretation of neural processing in relation to the animal's behavior, and that it provides opportunities for comparative studies involving different species whose different behavioral specializations are reflected in accordingly different neural processing strategies (Simmons and Grinnell 1988; Volman 1990).

The auditory system of the bat is quite exceptional, however. Echolocation is rare in other mammals, which do not have the specialized auditory cortex of the bat. Localization of sounds in the environment crucially involves circuits in the brainstem. These circuits need to process auditory cues that can be used to compute sound source position in space. I will briefly discuss the acoustic cues available, some broad aspects of mammalian brainstem auditory processing, and then point out some comparisons that bear on the processing of sounds by the barn owl. There are many excellent reviews of the auditory brainstem of mammals, for example, Irvine (1986), and several of the chapters in Edelman, Gall et al. (1988).

Two binaural cues an animal can use to localize sounds are disparities in the level of the sound, or in the time a sound or its components arrive at each ear. The classic duplex theory of mammalian sound localization (Rayleigh 1907; Stevens and Newman 1936) proposed on psychophysical and acoustic grounds that level differences could be used to localize sounds in azimuth at high frequencies, and that temporal differences could aid in localization of lower frequencies. Interaural level differences (ILD) increase as the frequency

increases, i.e., as the wavelength of the sound becomes small relative to head size; diffraction of the sound around the head is then minimal and the ear oriented away from the sound will be effectively shadowed by the head. One reason high-frequency sensitivity may have arisen in many animals is to aid in using ILD as an effective cue in sound localization (Masterton, Heffner et al. 1968). Phase differences (one aspect of time differences; see below) are a better cue at low frequencies, both because the auditory nerve cannot phase-lock (encode the phase by timing of nerve impulses) and because phase becomes an ambiguous cue at high frequencies. Such a duplex use of ILD and interaural time differences (ITD) over different frequency ranges results in psychophysical performances of sound localization that typically display distinctly poor performance for a mid-range of frequencies, where neither ITD nor ILD can be used best (see Heffner and Heffner (1987) for an especially striking example).

There is evidence of segregated processing pathways in the auditory brainstem of mammals, discussed, for example, by Warr (1982) and reviewed extensively by Irvine (1986). What exactly these pathways process is, however, much less clear than it is in the case of the barn owl. The cochlear nuclei of mammals are much more complex than in birds. There are three broad subdivisions (and many more cell classes, both on cytoarchitectonic and physiological grounds). The dorsal cochlear nucleus, which probably has no analogue in birds, projects to the ventral nucleus of the lateral lemniscus, and to the inferior colliculus, and is not obviously involved in the processing of binaural disparities. One subdivision of the ventral cochlear nucleus is the posteroventral cochlear nucleus (PVCN), which has projections to the periolivary nuclei, structures that in turn feed back to the cochlear nuclei (and to the cochlea itself). Thus, the PVCN appears to be involved in centrifugal

control, although it also contributes to ascending projections to the lateral lemniscal nuclei and the inferior colliculus. The anterior subdivision of the ventral cochlear nucleus (AVCN) is the only nucleus in which some cell types may correspond to cell types in the owl. The anterior AVCN contains so-called bushy cells, which receive large, specialized auditory nerve synapses, the calyces of Held, and whose discharge is phase-locked to low-frequency sounds. The bushy cells of the AVCN project to the medial superior olive (MSO) and the ventral nucleus of the lateral lemniscus, both of which contain cells whose response is selective for interaural phase disparities. These regions involved in processing phase information represent predominantly low frequencies, consistent with the acoustic limitations on the use of phase disparities discussed above.

The bushy/globular cells within the posterior AVCN project to the contralateral medial nucleus of the trapezoid body; spherical/bushy cells from the ipsilateral AVCN project to the lateral superior olive (LSO; Glendenning, Hutson et al. 1985). This circuit will receive some attention in this introduction because it bears resemblance to the circuit in the owl that processes ILD. The mammalian LSO contains cells whose responses are sensitive to ILD, and also to transient time differences, both of which may be encoded by similar mechanisms. Predominantly higher frequencies are represented in this system.

The terminology used for classifying binaural response properties that I will use follows that of Goldberg and Brown (1968, 1969; see also Irvine 1986), which has been adopted for the owl (Moiseff and Konishi 1983). The monaural responses to stimuli at the contralateral and ipsilateral ears are given, in that order, with an optional extension that describes binaural interaction. For example, an "EI" cell would be excited (E) by contralateral sounds, and inhibited (I) by ipsilateral sounds. A cell designated "EE/F" would be excited by either

ear, but show binaural facilitation. A cell of response type "OO/F" would respond only to binaural stimuli; such cells are common in the superior colliculus of mammals (Wise and Irvine 1985) and in both the inferior and superior colliculi of owls.

Much older literature traditionally refers to ILD as "interaural intensity difference"; this is strictly a misnomer, since what is actually measured is the difference in sound pressure level, in dB SPL, and not the intensity, which is the square of the level. Psychophysically, the perceived loudness of low-intensity sounds increases roughly linearly with sound intensity (and with the square of the level). In this thesis I will use the term "ILD", but retain the usage of "average binaural intensity", or ABI, to refer to the level about which ILD can be symmetrically varied: for example, a level of 10dB in one ear and 50dB in the other will yield an ILD of 40dB and an ABI of 30dB. There are two different ways to measure ILD: either one ear can be held constant and the level at the other ear varied, or the level at one ear can be increased while the level at the other ear is decreased by the same amount. The former method is useful in unmasking inhibitory mechanisms; the latter is useful in that it holds the average binaural intensity constant and thus more nearly corresponds to the ILDs that would be generated by a free-field source moving from one ear to the other. Both methods have advantages and disadvantages (Irvine 1987); the ABI-constant method has been used most commonly in the owl.

The duplex model of auditory perception has held up well for pure tones (Brown, Beecher et al. 1978; Klumpp and Eady 1956), but there is now much evidence that the picture is more complicated for complex sounds, and that spectral cues that are a result of the frequency dependance of the pinna's transfer function (Calford and Pettigrew 1984) are used in localization (Butler and Belendiuk 1977). Familiarity with the spectral properties of the sound is

necessary for the utilization of such cues. In mammals, spectral cues are especially important for localization of sounds in elevation (Roffler and Butler 1968a,b), as well as perhaps in distance (Coleman 1963). A striking feature of the transfer function of the pinna of the cat, for example, is the presence of pronounced "notches" in the spectrum at certain frequencies. These notches vary systematically with the position of a spectrally complex sound source in both elevation and azimuth (Musicant, Chan et al. 1990). Physiological studies showed that the position of the cat's pinna affects the responses of binaural neurons in the inferior colliculus (Semple, Aitkin et al. 1983), and shifts auditory spatial receptive fields in the map of the superior colliculus (Middlebrooks and Knudsen 1987). At very low sound pressure levels, no binaural cues at all appear to be used: in the inferior colliculus, Semple and Kitzes (1987) found that at low sound levels the response of neurons is a function only of the level at the contralateral (excitatory) ear, and that there are monaural spatial receptive fields (Semple, Aitkin et al. 1983). Even in the superior colliculus it is not entirely clear what necessary role neurons sensitive to ILD play, since there is a map of auditory space (King and Palmer 1983; Middlebrooks and Knudsen 1984) that, at intensities near threshold, appears to be monaural (Palmer and King 1985); accurate monaural localization has also been found psychophysically (Belendiuk and Butler 1975; Butler and Belendiuk 1977).

Interaural Time Differences:

Interaural time differences can be computed on the basis of three distinct binaural disparities, which, in a natural setting, are often all three present. Transient stimuli, such as clicks, provide only one of these cues: different onset and offset times of the sound at the two ears. Sustained sounds also contain

phase differences, which are a function of both frequency and the separation between the ears. Sounds with complex spectra also provide interaural temporal differences in peaks of the ongoing sound signal. While transient temporal disparities can in theory be used at any frequency, phase disparities are unambiguous for all frontal azimuthal locations only at those frequencies at which the wavelength exceeds twice the separation between the animal's ears. Phase difference can still yield some information about very frontal horizontal locations down to wavelengths equal to the interaural separation. For frequencies higher than this, the same phase disparity will arise from multiple points along the horizontal plane because of the periodic nature of the stimulus. This gives rise to maximal unambiguous frequencies of around 1250 Hz in humans and 2850 Hz in cats. There are mechanisms that can disambiguate phase if the stimulus is broad-band, but an absolute limit to the use of phase disparity at around 4-5 kHz results from the inability of the auditory nerve to phase-lock to higher frequencies. Most mammals show a drastic decline in the ability with which their auditory nerve can phase-lock at frequencies above 2 kHz (Johnson 1980; Stevens and Newman 1936). The barn owl is exceptional in its ability to phase-lock at frequencies close to 9 kHz (Sullivan and Konishi 1984).

A class of neurons with low best frequencies were found to encode interaural phase differences in the MSO of the dog by Goldberg and Brown (1969). These neurons receive input from cells that phase-lock to cycles of the stimulus tone, and their response rates to binaural stimuli are a function of interaural phase delay.

Phase sensitivity is found in a subset of cells in the inferior colliculus. This occurs over different frequency ranges for different species: up to frequencies of about 2.1 kHz in the rabbit (Kuwada, Stanford et al. 1987), and 3.1 kHz in the

cat (Yin and Kuwada 1983). Animals with smaller heads can use higher frequencies to utilize interaural phase disparities. Psychophysical results support this idea: humans can detect interaural phase differences only up to about 1.5 kHz (Klumpp and Eady 1956), whereas smaller animals appear to use phase cues at higher frequencies: 2 kHz for cats, 4 kHz for rats, and near 5 kHz for very small mammals such as the weasel (Heffner and Heffner 1987).

How do we know that interaural phase differences are actually used by auditory systems to encode aspects of sound localization? Aside from the compelling physical covariance between sound source azimuth and phase disparity, there is evidence in the response properties of some cells in the inferior colliculus. Low-frequency neurons in that structure often have a preference for sound coming from a particular, restricted range of azimuth. Moreover, the response of cells varies cyclically as a sound moves along the azimuth (Calford, Moore et al. 1986). In humans, IPDs of tones below 1.5 kHz are a cue for horizontal position of the sound source. In dichotically presented stimuli, perceived acoustic images shift as expected with phase disparity (Klumpp and Eady 1956). Binaural beat stimuli (in which there is a very small frequency difference in two pure tones presented at each ear that results in a dynamic phase difference) elicit a perception of a sound moving from one ear to the other (Perrott and Musicant 1977).

Coding of interaural time difference has been modelled generally as a cross-correlation between the two sides (the outputs of the cochlear nuclei on each side). Usually this is implemented as a detection of coincident impulses from the two sides (Jeffress 1948); there is now substantial support for this mechanism from the MSO (Goldberg and Brown 1969) to the ICc (Kuwada, Stanford et al. 1987) in mammals, and in chicken and owl homologues of the MSO (Carr 1990; Overholt, Rubel et al. 1992).

Interaural Level Differences:

While neurons that encode phase disparities can usually be excited by either ear ("EE"), those that are sensitive to ILD tend to fall into three binaural classes: "EI", "IE", or types where there is more complicated binaural interaction, such as "OO/F" (*v.s.*). Neurons in the MSO (Goldberg and Brown 1968, 1969) and LSO (Boudreau and Tsuchitani 1968) can be of "EE", "EI" or "IE" type. Most "EE" cells are insensitive to ILD (but sensitive to ABI), while "EI" (or "IE") cells change their firing rates as a function of ILD.

Many neurons in the lateral superior olive are excited by ipsilateral sounds, but inhibited by binaural stimuli ("IE"; Boudreau and Tsuchitani 1968; Caird and Klinke 1983; Goldberg and Brown 1969; Tsuchitani 1988a,b). The underlying synaptic properties that lead to this behavior have been investigated. Finlayson and Caspary (1989) showed that ipsilateral stimuli lead to excitatory psps, and that sounds at the contralateral ear led to large ipsps *in vivo,* findings that have been confirmed in a brainslice preparation (Sanes 1990). It appears that the inhibition is due to a direct, glycinergic projection from the medial nucleus of the trapezoid body: the inhibition relies on the opening of chloride channels (Finlayson and Caspary 1989; Sanes 1990), can be blocked by application of strychnine (Moore and Caspary 1983), can be mimicked by application of glycine (Caspary, Rybak et al. 1985), and glycine receptors are found in the LSO (Sanes, Geary et al. 1987). The nature of the excitation by ipsilateral stimuli is less clear, although it may be due to a projection from a cell class of the AVCN that is glutamatergic and acts on non-NMDA receptors in the LSO (Caspary and Faingold 1989).

Neurons sensitive to ILD in the mammal's lateral superior olive are also sensitive to large onset time differences (and probably also other large transient disparities that occur during the signal; Caird and Klinke 1983). Yin and colleagues (1985) proposed a common mechanism to account for this phenomenon, which has been found in the inferior colliculus as well (Carney and Yin 1989). In this model (Yin, Hirsch et al. 1985), both cues are coded by coincident detection of synaptic inputs, such that an increase in level at one ear will cause a decrease in latency at central synapses--the same effect that would be obtained if there is an onset ITD that favours that ear. This idea has received support from studies in numerous mammalian systems *in vivo* (Brugge, Dubrovsky et al. 1969; Caird and Klinke 1987; Pollak 1988) and *in vitro* (Sanes 1990).

A trading between time and level is seen psychophysically in humans when ILD is offset by an acoustically realistic ITD lag at the louder ear. The physiological evidence to support this phenomenon is sparse (Brugge, Dubrovsky et al. 1969; Hall 1965), although there is evidence in the bat (Pollak 1988). The barn owl does not exhibit any trading of ILD for ITD at the cellular level: if anything, increasing the intensity in one ear shifts the optimal ITD of a neuron in a direction that would potentiate the presumed effect of decreased latency (Olsen, Knudsen et al. 1989; Takahashi, Moiseff et al. 1984; Volman and Konishi 1989). This may be because ITD and ILD are processed completely separately all the way up to the inferior colliculus in the owl.

While transient ITD and ILD may be processed by the same mechanism for some ranges of ITD and in some regions of the mammalian brainstem, this is probably not generally true for ongoing ITD. Most models of ILD consider it processed independently of ongoing ITD, and applied as a weighting function after coincidence detection has been processed (Blauert 1983). Two ways

detection of ILD could be achieved is by comparing the rates of inhibitory and excitatory inputs to the LSO (Colburn and Moss 1981), or by comparing the levels of neural activity in two cell populations (a convolution of the rate and the number of neurons). The former model may account for at least some of the mammalian data, while the latter appears to be at odds with the architecture of the mammal's auditory brainstem (there does not appear to be a higher structure whose function it is to compare the population rates of the superior olives on either side). A population activity comparison has, however, been proposed for the first nucleus in the owl that is sensitive to ILD (see below).

Comparisons among Mammals:

Some interesting patterns in the ability with which different mammals can localize tones have emerged from the work of Heffner and Heffner. These investigators compared the use of binaural cues, and also the anatomical substrate for binaural integration, in the brainstem of the pig (Heffner and Heffner 1989), gerbil (Heffner and Heffner 1988a), horse (Heffner and Heffner 1986), and weasel (Heffner and Heffner 1987). Acoustically, one would expect sound localization acuity to increase with increasing head size, since ITD and ILD are larger with greater ear separation and more head shadowing, respectively. This expectation is however only very roughly true. Humans and elephants can detect changes in azimuthal positions of sounds of about 1 degree (Heffner and Heffner 1982; Mills 1972), monkeys and cats to roughly 5 degrees (Casseday and Neff 1973), and small rodents to worse than 20 degrees (Heffner and Heffner 1988a). But there are notable exceptions: horses, for example (and apparently also cows) have an azimuthal auditory acuity of worse than 20 degrees. In fact, most hoofed animals appear to have rather

poor localization ability, especially at higher frequencies, where one would expect ILD to be utilized. An examination of the superior olivary complex in these species has shown that they possess an atypically small LSO, the first binaural nucleus that appears to be important in processing ILD in mammals. Perhaps there has been little selective pressure on accurate sound localizing ability in many hoofed mammals. Additionally, since these are generally large animals, level differences between the ears could be problematic in that sounds at the shadowed ear might be too quiet to be detected (Heffner and Heffner 1989). Primates, including humans, also have almost absent lateral superior olives (Moore and Moore 1971), although their localization is excellent, in part because they utilize spectral cues. The sizes of the LSO and MNTB covary in all species, consistent with their dual role in auditory processing.

A converse example is provided by the hedgehog, which lacks an MSO and cannot use ITD cues for sound localization (Masterton, Thompson et al. 1975). It is possible that in the case of both the hedgehog and hoofed mammals there was little pressure to either maintain or evolve high-acuity sound localization. Since these species are not predatory, it may be sufficient to obtain information only about the very approximate source of a threatening sound to take evasive action (run in the opposite direction; curl up). On the other hand, one might reason that predators that rely on auditory cues would be under much pressure to evolve very accurate localization mechanisms.

The least weasel, the smallest carnivore, has a localization ability well above that of rodents of comparable size (Heffner and Heffner 1987). Psychophysical studies of the weasel's ability to localize sound of different frequency fit the duplex model quite well: frequencies lower than around 4 kHz can be localized well, there is a prominent region around 8 kHz where performance falls to chance, and higher frequencies (up to near 40 kHz, at the limit of the weasel's

audiogram) are localized again. The acuity is around 12 degrees, better than some rodents with twice the ear separation. Especially telling is the anatomy of the weasel's LSO, which is unusually large (it has 4 turns, instead of the usual 3). Both medial and lateral superior olivary nuclei of the weasel are more similar to those nuclei in cats and dogs than to rodents. Heffner and Heffner (1987) suggested that predators such as carnivores have evolved exceptionally acute sound localization mechanisms that are mirrored in hypertrophied superior olivary nuclei. Some similar examples are provided by pigs (Heffner and Heffner 1989) and grasshopper mice (Heffner and Heffner 1988b), which are both atypical in that they are predatory and feature superior localizing abilities compared to other artiodactyls or rodents. All these examples suggest that the ability to localize sound well is a derived feature that reflects the species' degree of predatory behavior.

Higher Centers:

Auditory axons afferent to the inferior colliculus course in the lateral lemniscus, a broad band of fibers in the lateral brainstem. Embedded within this fiber tract are several nuclei, the nuclei of the lateral lemniscus. These have received scant attention until quite recently. Earlier studies had delineated two nuclei, a dorsal (LLD) and ventral (LLV) lateral lemniscal nucleus, and few reports of their physiology existed (Aitkin, Anderson et al. 1970; Brugge, Anderson et al. 1970). A comprehensive review of the auditory brainstem as recent as 1986 (Irvine 1986), devotes 6 pages out of more than 200 to all the nuclei of the lateral lemniscus. More recent studies of the afferent and efferent projections of these nuclei has clearly delineated three different regions: the

dorsal, intermediate, and ventral nuclei of the lateral lemniscus, termed DNLL, INLL and VNLL respectively (Brunso-Bechtold, Thompson et al. 1981; Glendenning, Brunso-Bechtold et al. 1981).

The INLL and VNLL have distinct afferent and efferent connections, but very little is known about either their physiology or their function in audition. These two nuclei are especially prominent in some animals with ultrasonic hearing. Some cells in the lateral lemniscal nuclei project directly to the medial geniculate body of the thalamus. The INLL (and the DNLL) also has prominent direct projections to the superior colliculus and to the midbrain reticular formation; the latter appears to be part of the circuit of the acoustic startle reflex (Davis, Gendelman et al. 1982).

The best studied lemniscal nucleus, the DNLL, has fortuitously turned out to also be the most relevant for purposes of comparison to circuits in the barn owl that I discuss below. The DNLL receives bilateral input from the LSO and ipsilateral input from the MSO (Brunso-Bechtold, Thompson et al. 1981; Glendenning, Brunso-Bechtold et al. 1981; Glendenning and Masterton 1983). A much more minor input comes from the AVCN (mostly contralateral) and ipsilateral VNLL (Glendenning, Brunso-Bechtold et al. 1981; Shneiderman, Oliver et al. 1988). A very interesting projection is a reciprocal commissural projection between the DNLL on each side of the brain that forms part of the commissure of Probst (Probst 1902). This crossed projection appears to be point-to-point: Shneiderman and colleagues (1988) injected a mixture of an anterograde and a retrograde neuronal tracer into the DNLL on one side and saw both retrograde and anterograde label in the same region in the opposite DNLL. The most prominent ascending projection of the DNLL is monosynaptically to the ipsilateral inferior colliculus, and polysynaptically (via

the commissure of Probst) to the contralateral inferior colliculus (Brunso-Bechtold, Thompson et al. 1981; Shneiderman, Oliver et al. 1988).

Most neurons in DNLL respond to binaural stimuli (Aitkin, Anderson et al. 1970); nearly all are excited by sound loud at the contralateral ear. Some low-frequency neurons in the nucleus are sensitive to phase disparities, but nearly all neurons tuned to higher frequencies are of response type "EO" or "EI" (Brugge, Anderson et al. 1970). These binaural response classes are also common in the inferior colliculus, and one proposal is that the DNLL provides inhibitory input both to the colliculus and to the opposite DNLL (Shneiderman, Oliver et al. 1988). There is support for the inhibitory role of DNLL from findings that many of its projection neurons appear to be GABAergic (Adams and Mugnaini 1984; Moore and Moore 1987).

The mammalian inferior colliculus can be subdivided into three cytoarchitectonically distinct regions: the central (ICc), external (ICx), and pericentral or superficial (ICp or ICs) nuclei (Berman 1968), a terminology that Knudsen (1983) introduced for the barn owl. An alternative nomenclature, still in common use for other avian species, is that of Karten and Hodos (1967); a modified version (Leibler 1975) is also used for structures other than the midbrain in the owl.

The ICc of mammals has a pronounced statified appearance. In most mammalian and avian species studied ICc is tonotopically organized. The ICx is less clearly tonotopic due to the broad tuning of its neurons, although there may be a somewhat more diffuse mapping of frequency contiguous with that seen in ICc (Aitkin, Dickhaus et al. 1978; Roth, Aitkin et al. 1978). In some species, the ICx appears also to receive somatosensory input (Aitkin, Dickhaus et al. 1978), and responses to somatosensory stimuli are common in the

superior colliculus (Meredith and Stein 1986), to which the ICx projects. The ICc appears to receive bilateral inputs from all auditory brainstem nuclei, including the ICc and ICx on the opposite side, although not all are equally strong. Particularly prominent are projections from contralateral cochlear nuclei, the ipsilateral MSO and VNLL, and bilaterally from the LSO and DNLL, although the details vary between species (ferret: Moore 1988; gerbil: Nordeen, Killackey et al. 1983; cat: Aitkin, Irvine et al. 1977; Roth, Aitkin et al. 1978; marsupial: Aitkin, Byers et al. 1986). Almost all cells in the ICc are excited by the contralateral ear, and many are binaural "EI" cells. Wenstrup and colleagues (1985) found a topographic map of ILD in the ICc of the bat, and maps of ILD also exist in the superior colliculus in many mammals (Wise and Irvine 1985). Many low-frequency cells in the ICc are sensitive to interaural phase disparities, although most of these do not phase-lock, and exhibit a heterogeneity of binaural response properties ("EE" and "EO" are both common; Kuwada, Stanford et al. 1987; Yin and Kuwada 1983). In recapitulation of the above discussions, one can very roughly consider three afferent auditory projection systems to the inferior colliculus: 1) direct projections from the cochlear nuclei; 2) direct projections from the superior olivary nuclei; 3) direct projections from the nuclei of the lateral lemniscus. One oversimplified scheme is to consider projections from the cochlear nuclei and superior olive excitatory, while some of the inputs from lateral lemniscal nuclei may provide inhibition. The functional significance of inputs to the IC, however, needs further study.

There is no clear evidence for any substantial population of neurons in the mammalian IC that have very restricted auditory receptive fields; in general, their spatial receptive fields are rather broad and what would be expected on the basis of their binaural response profile (Moore, Hutchings et al. 1984; Semple, Aitkin et al. 1983). In mammals, no map of auditory space has been

found in the inferior colliculus, although the superior colliculus does have such a map.

There are feedback projections from the IC, and from the lemniscal nuclei, back to lower brainstem auditory nuclei, but these are poorly understood. The IC projects to the superior colliculus, which also receives visual and somatosensory inputs in many animals, and whose cells exhibit integration to these three modalities (Meredith and Stein 1986). All parts of the inferior colliculus in mammals appear to project to the thalamic auditory nucleus, the medial geniculate body (Kudo and Niimi 1980), which, like the superior colliculus, appears to have a substantial population of neurons that are multimodal (Blum, Abraham et al. 1979). The owl's homologue, nucleus ovoidalis of the thalamus, is known to project to a forebrain region known as Field L (Karten 1968), but afferents to ovoidalis have yet to be fully delineated; they probably originate at least in part from the inferior colliculus (Karten 1967; L. Proctor, pers. comm.). The auditory thalamic nuclei in both owl and mammals are complex and appear to have many subdivisions that may have different connectivity. The major projection of the medial geniculate nucleus is to primary auditory cortex, in the temporal lobe, although there are also important projections to such targets as the amygdala. Both the medial geniculate and primary auditory cortex are tonotopically organized; the pathway to the amygdala appears to encode much cruder information about stimuli at high intensities.

Forebrain targets of auditory nuclei below the level of the midbrain have not been found, with one exception. Nuclei in the lateral lemniscus in mammals project to the pontine reticular formation and directly to the thalamus. Interestingly, there is a projection in birds from a lateral lemniscal nucleus (LLv)

to a region in the forebrain known as the nucleus basalis (Arends and Zeigler 1986); the function of this pathway is not known.

Owl Auditory System

Two striking features of sensory systems are the existence of maps, and the processing of information in parallel computational channels. Parallel processing streams have been found in a number of neural systems, most notably the primate visual system (DeYoe and Van Essen 1988). Proposals have recently been made for parallel and hierarchical processing schemes throughout most of cortex, including polymodal, association, and limbic regions (Felleman and Van Essen 1990). Many sensory systems have maps of either the projectional sort that preserve topography of the periphery, or of a computational sort, where what is mapped is a parameter that is computed in the nervous system (Konishi 1986). The visual, somatosensory, and auditory systems all have projectional maps (retinotopic, somatotopic and cochleotopic respectively). Examples of computational maps are maps of orientation selectivity, or direction of motion selectivity in the visual system, and maps of auditory space in the auditory system. In all such cases, the functional significance of mapping appears to be to use local neighbour relationships in the computations that such maps subserve. It appears that a relatively large population of cells is active for any given representation, and that each individual cell may be rather broadly tuned, an empirical finding that also

receives some theoretical support (Baldi and Heiligenberg 1988). The map of auditory space in the barn owl is somewhat special in that its neurons are at the apex of a processing hierarchy, are sharply tuned, and are topographically organized. Recent evidence suggests that only a small number of cells of this neural map contribute in representing any one auditory target: focal lesions disrupt sound localization within the restricted region of space that is represented at the lesion site (Wagner 1991).

The barn owl, like most animals (Pumphrey 1950), performs a rapid, open-loop (Knudsen, Blasdel et al. 1979) orienting movement towards novel or interesting sounds. In most animals this behavior also (sometimes only) involves movement of the eyes towards the source. Thus, rapid acoustic orienting may serve an important function in bringing targets into the region of the animal's highest visual acuity. This is borne out by the covariances observed between auditory acuity and the width of the field of best vision (Heffner and Heffner 1992), and between cell counts in superior olivary nuclei (specifically the MSO) and the abducens nucleus (Irving and Harrison 1967). Owls can move neither eyes nor ears, and a turn of the head thus serves to foveate a target with both vision and hearing. Under laboratory conditions, barn owls can engage very accurate aerial attacks onto sources of noise in complete darkness (Konishi 1973a,b; Payne 1962, 1971); and great grey owls have been observed in the wild to strike prey hidden under snow (Payne 1971). The auditory acuity of the barn owl is better than that of any other land animal (Erulkar 1972; Heffner and Heffner 1992; Volman and Konishi 1990), approaching 1 degree in discriminative ability (Knudsen, Blasdel et al. 1979; Payne 1971). This impressive performance arises out of algorithms that are carried out by two parallel ascending brainstem pathways (Takahashi and Konishi 1985) that originate in the cochlear nuclei (Konishi, Sullivan et al. 1985;

Sullivan and Konishi 1984). These two channels are referred to as the "time pathway" and the "intensity pathway", depending on whether they process ITD or ILD, respectively.

Barn owls use interaural phase and level disparities as acoustic cues in localizing sounds. Phase differences are utilized, and neurons can phase lock, up to 9 kHz in the owl (Sullivan 1985), higher than any other animal so far studied. Interaural phase difference is used as an accurate cue in localizing sound in azimuth at frequencies between 4 and 7 kHz; ILD is an effective cue for elevation between 5 and 8 kHz (Knudsen and Konishi 1979; Moiseff 1989b). Thus, unlike duplex perception in mammals, the owl can use both ILD and ITD cues of the same frequency: there is no dip in performance that would suggest duplex sound mechanisms operating in the owl. Plugging one ear causes the owl to make errors in both azimuth and elevation (Knudsen and Konishi 1979), and shifts the auditory spatial receptive fields of neurons (see below) in both dimensions (Knudsen and Konishi 1980). A direct demonstration of the use of these cues was provided under dichotic conditions: owls orient to sounds played through earphones that produce ITD and ILD as if there were a free-field sound source that gave rise to the same binaural disparities at the ears (Moiseff 1989a; Moiseff and Konishi 1981). The use of phase disparity to localize sounds along the horizon is analogous to the way most other animals use this cue. The use of ILD to give information about the elevation of the sound is unique to certain species of owls, and is due to a vertical asymmetry in the directionality of the ears. The position and shape of the ear opening and of the soft tissue surrounding it are such that the left ear is more sensitive to sounds coming from below the horizon, while the right ear is more sensitive to sounds coming from above the horizon (Payne 1971). This can be seen in Figure 1, a photograph of an owl from which the smaller facial feathers have been

removed. The owl's ears are directional at frequencies above 3-4 kHz (Coles and Guppy 1988; Moiseff 1989b; Payne 1971), and the facial ruff serves to amplify sounds as they reach the parabolic face (Konishi 1973a). Both behavior (Konishi 1973a) and central overrepresentation (Knudsen and Konishi 1978c) confirm that frequencies in the range of 3-9 kHz are most useful to the owl in localization.

The vertical asymmetry of the owl's ears appears to be a derived feature that has arisen independently several times in the evolution of owl species (Norberg 1977; Volman and Konishi 1990). There are several species, such as the great horned owl and the burrowing owl, that do not have asymmetric ears; such owls cannot use ILD as a cue for elevation and appear to have inferior localization in dark surroundings (Payne 1971). Volman and Konishi (1989) examined the tuning properties of neurons in the inferior colliculus of the great horned owl, and found that in this symmetrical owl auditory spatial receptive fields are not limited in elevation. In view of the utility of ears with vertical asymmetry, it is surprising that no animals other than owls appear to have evolved this feature (Volman 1990), suggesting that perhaps aspects of the owl's auditory system are pre-adapted to take advantage of the occurence of peripheral asymmetries. Support for this hypothesis comes from the finding that cells in the great horned owl's ICx, while having auditory spatial receptive fields that are unlimited in elevation, show tuning to ILD. It is thus possible that owls with symmetrical ears possess much of the same brainstem circuitry as the barn owl, but that their peripheral specializations do not support the use of ILD as a cue for elevation.

Three different parameters, ITD, ILD, and frequency, are processed and mapped in the barn owl's brainstem (Knudsen and Konishi 1978c). From these, the owl synthesizes a map of auditory space in the ICx where neurons encode

Figure 1.1 The ears of the barn owl are vertically asymmetric. This photograph of an owl's face from which the acoustically transparent feathers have been removed shows ear asymmetry. The left ear is more sensitive to sounds coming from below the horizon; the right ear is more sensitive to sounds coming from above the horizon. Photo courtesy of Mark Konishi.

unique spatial locations of distal sound sources (Knudsen and Konishi 1978b; Konishi 1986; Konishi et al. 1988). The ICx in turn projects topographically onto the owl's optic tectum (Knudsen and Knudsen 1983), which contains a bimodal map of visual and auditory space (Knudsen 1982) and is involved in controlling the amplitude and direction of saccadic head movements (Du Lac and Knudsen 1990). The owl's tectum is necessary for producing head saccades towards either visual or auditory stimuli, and the topographic pattern of neural activity within the tectum determines the direction of the saccade (Du Lac and Knudsen 1990; Knudsen, Esterly et al. 1991). Like the ICx, the tectum has maps of ITD and ILD (Olsen, Knudsen et al. 1989). The registration of visual and auditory maps in the tectum, and the topography of the auditory map in the ICx, both require retinotopic visual inputs during the young owl's development. In a series of recent experiments, Eric Knudsen and colleagues showed that baby owls that are blind reared (Knudsen 1988), or that are reared with prisms over their eyes that distort the topography of visual input to the retina (Knudsen and Knudsen 1989a; Knudsen and Knudsen 1989b) will have distorted maps of auditory space (Knudsen and Brainard 1991; Knudsen, Esterly et al. 1991), and will make predictable errors in localizing sounds when the prisms are removed (Knudsen 1990; Knudsen and Knudsen 1989b).

The direct involvement of auditory structures upstream to the tectum in determining auditory-evoked head saccades in the owl is less clear. A preliminary report (Wagner 1991) suggests that focal lesions within the ICx cause behavioral localization deficits within the region of auditory space that was represented at the site of the lesion. Further experiments, perhaps of the sort that I report in Chapter 4, need to be coupled with behavioral assays to

clearly link the components of auditory sensory processing to aspects of the behavior.

There are several advantages to studying spatial audition in the owl. To begin with, like other birds, this preparation is simple at the periphery. Unlike many mammals, birds have static, tubular ears that eliminate much of the complex and dynamic transfer functions of ears with movable pinnae (Calford and Pettigrew 1984; Musicant, Chan et al. 1990). The middle ear contains a single ossicle, as in reptiles and unlike the mammalian chain of three ossicles, into which inserts a single muscle that is a homologue of the stapedius muscle involved in reflexive attenuation at high sound intensities. Birds have a canal that connects the two middle ear cavities and that can affect binaural acoustic differences; this does not appear to play a major role in the relatively high-frequency localization that the barn owl carries out (Moiseff and Konishi 1981; Olsen, Knudsen et al. 1989). The owl has one of the longest basilar papillae (which contains the hair cells of the uncoiled cochlea) of all birds at over 10mm (Fischer, Koeppl et al. 1988; Smith, Konishi et al. 1985), the substrate of the exceptionally extended hearing range of up to 10kHz (Konishi 1973a). Hair cells of different sizes are found in the basilar papilla; it is not clear whether these can be homologized to the mammal's outer and inner hair cells. While the outer hair cells of mammals are massively innervated by efferent fibers of the olivocochlear bundle, and there also appears to be an efferent projection to the chick cochlea (Whitehead and Morest 1981), little is known about either the extent or the hodology of cochlear efference in the owl.

Another advantage of the owl is the separate processing of cues that I mentioned above. Phase and level information are conveyed in the rate and time of arrival of spikes of the eigth nerve. These two parameters are separated at the cochlear nuclei of the owl: nucleus angularis consists of cells that have a

large dynamic range over which they can encode sound level by average spike rate, and nucleus magnocellularis cells have a much narrower dynamic range over intensity but preserve temporal information (Konishi, Sullivan et al. 1985; Sullivan and Konishi 1984). There is a third and minor class of fibers of unclear function that enter with the eighth nerve from the otolith organ of the macula lagena, at the distal tip of the cochlear duct, which terminate medially in the brain stem (Boord and Karten 1974).

A third reason for studying neural processing in the owl is the simplicity of the architecture. The presence of feedback complicates the analysis of a system. It appears that some of the activity in primate primary visual cortex, for example, can be driven solely by feedback (Mignard and Malpeli 1991), and that abolition of feedback alters cellular responses in many cells (Sandell and Schiller 1982). However, only few examples indicate just how the response properties of neurons are likely to rely on feedback from higher centers: neurons in area V4, and the inferotemporal cortex, alter their receptive fields as a function of attention (Moran and Desimone 1985); and in V2 neurons will respond to subjective contours (von der Heydt, Peterhans et al. 1984; this finding might also be explained by bottom-up processing). These and other findings of "non-classical" receptive field properties (Allman, Miezen et al. 1985) have led to the revision that the response properties of neurons may be more dynamic and state-dependent than previously thought (Van Essen, Anderson et al. 1992). The mammalian auditory brainstem, like most other sensory systems, exhibits a profusion of feedback connections, although much of their function remains a mystery.

The auditory brainstem of the barn owl has some advantages in light of this discussion: no significant feedback has been found among the brainstem auditory nuclei, nor are there extensive horizontal connections. The known

exceptions to this observation are a minor feedback projection from the superior olive to nucleus magnocellularis in the time pathway (Carr, Fujita et al. 1989), and a reciprocal connection between a lemniscal nucleus (VLVp) on each side of the brain (Takahashi and Keller 1992). Aside from these cases, the owl's auditory processing architecture appears to be strictly feedforward. There appears to be no cross-talk between the channel that processes ITD and the processing of ILD, until the two streams anatomically and physiologically converge in cells of the inferior colliculus.

The owl's auditory processing includes the sharpening of response tuning (Fujita and Konishi 1991; Wagner 1990), suppression of some responses when additional stimuli are present (Fujita and Konishi 1991; Moiseff and Konishi 1983; Mori, Fujita et al. 1990; Wagner, Takahashi et al. 1987), and convergence of parallel channels (Fujita and Konishi 1989; Wagner, Takahashi et al. 1987). The entire auditory brainstem of the owl is organized tonotopically, and multiple parallel frequency channels exist.

The details of the pathway that processes ITD have been worked out in the anatomical studies by Takahashi and Konishi (Takahashi and Konishi 1988a,b; Takahashi, Wagner et al. 1989), and the circuits that compute phase disparities have been elucidated by Carr and co-workers (Carr 1990; Konishi et al. 1988; Sullivan and Konishi 1986). The cochlear nucleus magnocellularis, which receives synaptic end bulbs of Held from the eighth nerve, shows phase locking to frequencies up to 9kHz (Sullivan 1985; Sullivan and Konishi 1984) and projects bilaterally to the nucleus laminaris. There, binaural phase disparity is computed by detection of coincident afferent discharges (Carr 1990; Konishi et al. 1988) and converted to a code wherein a particular place in the nucleus represents a certain phase difference; a similar algorithm and implementation have recently been found also in the chick (Overholt, Rubel et al. 1992). From

laminaris, labelled neuronal lines now encode phase disparity in a given frequency channel (Carr 1990; Jeffress 1948); these lines project to the contralateral nucleus VLVa in the lateral lemniscus, and to the contralateral ICc. The terminal field of nucleus laminaris in the contralateral ICc is a sharply delimited central "core" that can also be identified by physiological criteria, or by immunohistochemical staining for the protein calbindin (Takahashi, Carr et al. 1987; cf. Chapter 3).

The pathway that subserves ILD processing has received much less attention, although its gross hodology has been established. This processing channel originates in the nucleus angularis, whose cells can encode sound level over a large dynamic range, but are insensitive to ITD (except at low carrier frequencies of the stimulus; Sullivan 1985; Sullivan and Konishi 1984). The intensity pathway is delineated by nuclei that contain terminal fields of nucleus angularis (Takahashi and Konishi 1988a,b): nucleus ventralis lemnisci lateralis pars posterior (VLVp), nucleus lemnisci lateralis pars ventralis (LLv), and the avian superior olivary nucleus (which bears no similarity to the mammalian nucleus by that name). At very anterior positions, VLVp and VLVa fuse; the response properties of this region have not been described. Angularis also projects directly to the ICc, where its terminals form a "shell" that surrounds the "core" terminal field of nucleus laminaris (Takahashi and Konishi 1988b). The shell of ICc can be further subdivided into a medial region, medial to the core, and a lateral shell, situated between the core and the ICx. The lateral shell of ICc additionally receives input from the time pathway via the contralateral ICc core (Takahashi, Wagner et al. 1989), and in turn provides the sole ascending auditory input to the ICx (Knudsen 1983).

The owl's brainstem auditory nuclei share many similarities with those of other birds. Other avians also have two cochlear nuclei, although a separate

processing of phase and level has been documented so far only in the chicken (Warchol and Dallos 1990). Conlee and Parks (1986) studied the connections of the auditory brainstem in the chicken, and Leibler (1975) examined the pigeon. The barn owl's lemniscal nuclei appear to be more specialized for processing different cues, and the terminal fields of nucleus laminaris and nucleus angularis are more segregated than in other birds. This is especially prominent for VLVa and VLVp, which are distinct in position, cytoarchitecture, hodology, and physiology at caudal levels in the owl (Takahashi and Konishi 1988a). In other birds, the terminal fields of nucleus laminaris and nucleus angularis appear to overlap. It would be interesting in future studies to compare the barn owl's auditory brainstem to that of an owl with symmetrical ears, such as the great horned owl.

Both interaural time and level differences are ambiguous at a single frequency (Knudsen, Esterly et al. 1991; Takahashi and Konishi 1986), but these ambiguities are resolved when a sound contains many frequency components (Brainard, Knudsen et al. 1992; Fujita and Konishi 1989; Konishi et al. 1988; Mori, Fujita et al. 1990). This appears to be accomplished by combining information that has been processed in different frequency channels at the level of the inferior colliculus. While the ICc (and all stations peripheral to it) is organized tonotopically, neurons in the ICx are very broadly tuned to frequency and respond best to noise. Their responses to time-shifted noise are not phase ambiguous: by combining responses to phase disparities at several frequencies, a unique, true ITD can be derived (Takahashi and Konishi 1986; Wagner, Takahashi et al. 1987). This mechanism also appears to operate in the great horned owl (Volman and Konishi 1989). In addition to the convergence of frequency channels between the lateral shell of ICc and the ICx, there is convergence of the time- and intensity pathways. Some of the neurons

in the lateral shell already show tuning to both ITD and ILD, and it may be that this convergence proceeds in steps as one progresses more laterally towards the ICx (J. Mazer, pers. comm.). The lateral shell of ICc, and the local projections from there to the immediately adjacent ICx, then appears to be the site where frequency channels converge, where time- and intensity channels converge, and where tuning to ILD is generated. No map of ILD has been found in the lateral shell; this appears to emerge only in the ICx.

Neurons in the ICx have binaural response properties that are not found at any lower stage of auditory processing. Three possibly related features are: 1) They are binaurally exclusive, that is, their responses are of type "OO/F": no response to monaural stimuli, and a non-linear response when binaural stimuli are present; 2) They show sharply peaked tuning to ILD, with the peak response typically within 15 dB of 0 ILD, corresponding to the range of peripherally generated ILD; 3) Their tuning to ILD does not shift with changing the average binaural intensity, nor does it appear to change with ITD. In fact many, if not most, neurons in ICx may be inhibited by monaural stimuli and should thus be classified "II/F" (Moiseff and Konishi 1983; pers. obs.); this is difficult to ascertain since most ICx neurons have a very low spontaneous discharge of which any inhibition might be seen. How tuning to ILD that is invariant with ABI and ITD emerges in the ICx is addressed in the studies of Chapter 4.

It is difficult to find mammalian homologues to the auditory nuclei in the brainstem of the owl. One clear similarity is between the MSO and nucleus laminaris, which have similar afferent hodology and are both concerned with processing phase differences. The cochlear nuclei of the owl cannot be so easily identified with cochlear nuclei in mammals. Rather, nucleus magnocellularis may correspond to the subpopulation of bushy cells in the

AVCN that phase-lock and project to the MSO. Similarly, nucleus angularis might have as its mammalian correlate the stellate cells of the AVCN and PVCN that exhibit "chopper" and "onset" firing patterns (Sullivan 1985). While the owl's inferior colliculus is homologous to the IC of mammals (Karten 1967; Knudsen 1983), the owl's lemniscal nuclei present a special problem. Are they to be identified with the level of processing of the superior olivary nuclei, or of the lemniscal nuclei in mammals? Of particular interest is the owl's nucleus VLVp, studied in Chapter 4. By afferent hodology, VLVp resembles the mammal's LSO: there is direct excitatory input from a cochlear nucleus, and indirect inhibitory input. Like VLVp, the LSO also projects to the IC, and it is excited by one ear and inhibited by the other. Much of the same features, however, also apply to the mammal's DNLL, which is situated in a more similar region of the brain than is the LSO, although it has been the subject of far fewer studies. These analogies between owl and mammal are discussed more in the discussion sections to Chapters 3 and 4.

CHAPTER 2:

METHODOLOGICAL STRATEGY

The strategy of elucidating the mechanisms that underlay synthesis of higher-level responses in the owl was to functionally dissect the neural circuits that compute high-level parameters. This approach is "top-down" in the sense that it starts with a well described locus at or near the apex of a processing hierarchy. Neurons in the owl's space map have complex and restricted stimulus-response properties that enable them to encode the spatial direction of distal sound sources. I chose to look at the next layer of processing upstream.

Prerequisite to such an approach are a knowledge of the anatomical circuitry (how do nuclei project to each other?), and a knowledge of the response properties of neurons at every stage of processing. Both of these had been worked out in part through previous research on the owl, but there were some loose ends to be tied up. One important loose end was in the anatomy of the projections of the intensity pathway into the inferior colliculus; I report those results at the beginning of Chapter 4.

Armed with hodological and physiological knowledge, I undertook to dissect the pathway that processes ILD just prior to the computation of responses that are tuned to ILD in the colliculus. Figure 2 shows that several nuclei of the intensity pathway provide input to the central nucleus of the inferior colliculus: the nucleus angularis, the nucleus of the superior olive, and LLv. The inputs of VLVp to the inferior colliculus are described in Chapter 4. Nucleus angularis is a special case in that it provides the input to all other nuclei of the intensity

Figure 2.1 The pathway that processes ILD in the owl. The intensity pathway consists of all nuclei that are terminal fields of nucleus angularis (NA): the nucleus of the superior olive (SO), nucleus ventralis lemnisci lateralis pars posterior (VLVp), nucleus lemnisci lateralis pars ventralis (LLv), and the shell of the central nucleus of the inferior colliculus (shell). Time and intensity are processed separately in the core and shell of the central nucleus of the inferior colliculus, respectively, and converge in the external nucleus (ICx). Dotted lines: efferent projections of NA. Solid lines: projections of lemniscal nuclei. The projections of VLVp are described in Chapter 4.
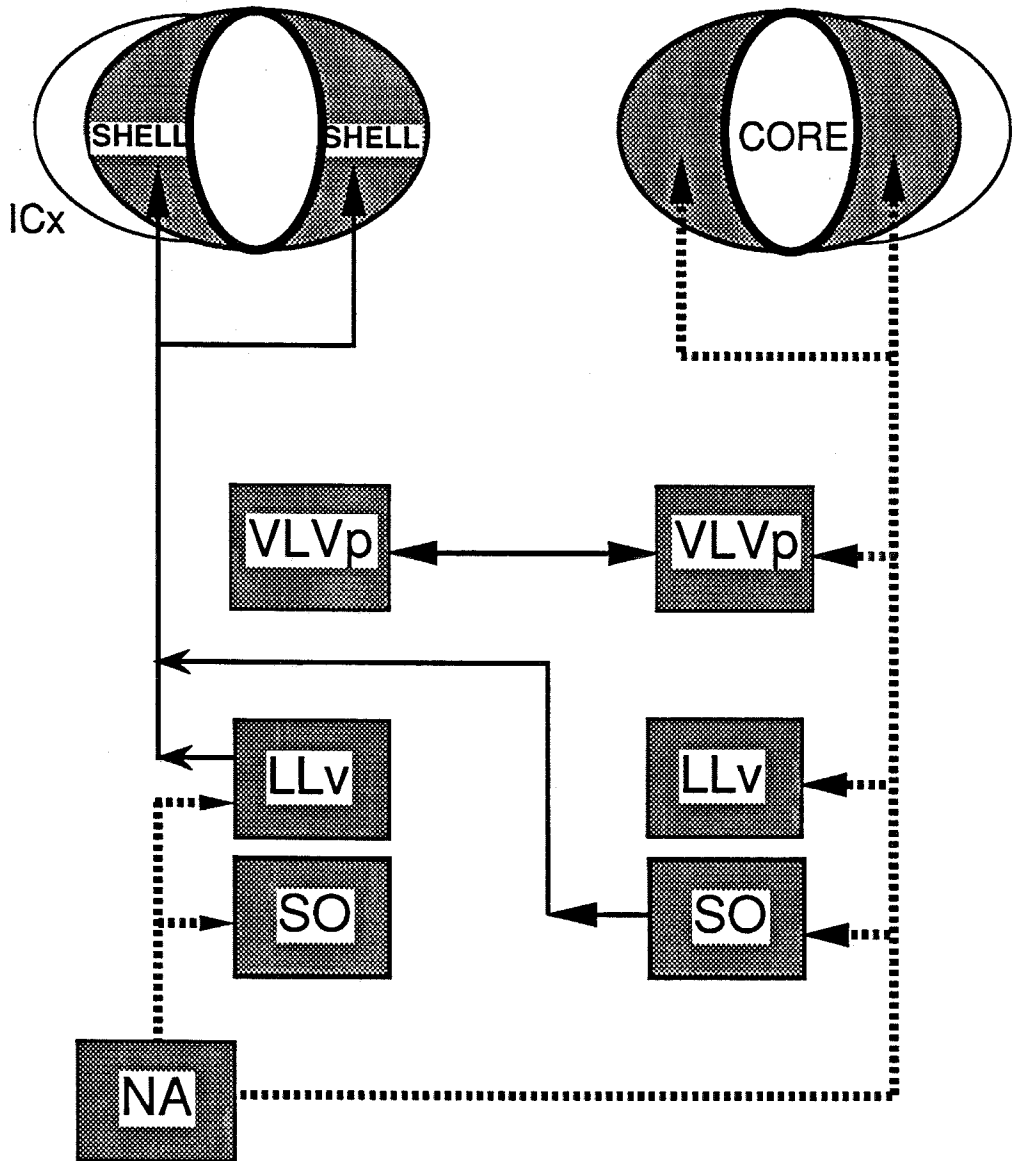
pathway. Thus, blocking neural activity in angularis is tantamout to plugging the ear on that side as far as the intensity pathway is concerned. This was shown by Takahashi, Moiseff et al. (1984), who found that anesthetization of the nucleus angularis on one side shifted neuronal tuning to ILD in the owl's space map as if the ear on that side had been plugged; tuning to ITD was unaffected since it is processed in a parallel channel.

Unfortunately, little can be learned about the direct mechanisms of ILD-tuning from Takahashi's earlier experiments, other than that they are segregated from the mechanisms that compute ITD. Although angularis does provide a direct input to the inferior colliculus, it has not been possible to selectively affect that projection without affecting the rest of the intensity pathway. There are, however, projections from second-order nuclei of the intensity pathway that are suited to further analysis. Responses within the LLv and the superior olive have only been described in one pilot study (Moiseff and Konishi 1983). Furthermore, these nuclei are located deep and close to the bone of the skull, making access difficult. The genesis of response properties in either LLv or the superior olive has not been investigated.

The best candidate for my study was nucleus VLVp, a lateral lemniscal nucleus that had a well studied physiology and connectivity, and for which there was at the time preliminary evidence of how responses in VLVp were generated. Subsequent to an initial survey (Moiseff and Konishi 1983), Manley, Koeppl et al. (1988) provided a detailed study of responses in VLVp. They found that neurons in this nucleus are all excited by sounds loud at the contralateral ear, and inhibited by sounds loud at the ipsilateral ear. There is no sensitivity to ITD, and a clear tonotopic map along the antero-posterior axis of the nucleus. The strength of inhibition by ipsilateral stimuli decreases from the

dorsal top of VLVp to the bottom, such that neurons at the top of VLVp are very strongly inhibited by binaural stimuli ("EI") while cells at the bottom respond to binaural stimuli as they would to contralateral monaural stimuli ("E0"). Takahashi and Keller (1992) recently published what was still preliminary evidence when I began my study: the inhibition in VLVp comes from the VLVp on the opposite side via the commissure of Probst. Thus, a sound at the left ear, for example, will excite neurons in the left nucleus angularis, which in turn project to the right VLVp. Neurons of the right VLVp now inhibit neurons in the left VLVp. That angularis directly excites, and polysynaptically inhibits VLVp cells in this way was shown by Takahashi and Keller (1992) by anesthetizing the VLVp on one side with lidocaine while recording from the neurons in the VLVp on the other side. The inhibition by ipsilaterally-favoured ILDs was diminished. I report similar results in Chapter 4.

Neither my results, nor those of Takahashi and Keller (1992) address the details of how neurons within VLVp could function in representing ILD. One recent model that is consistent with what is known experimentally proposes that it is the population activity within the VLVp on each side, and the fact that the VLVps are reciprocally connected, that serves to set up a sharp gradient of inhibition along the dorso-ventral axis of each VLVp (Spence and Pearson 1989). This model agrees with the experimental findings of Manley, Koppl et al. (1988), but goes further in positing a gradient of inhibition that is not uniform, but more like an edge within VLVp. Neurons at the top of VLVp would be inhibited, and, along a rather sharp boundary, the strength of this inhibition would diminish as one progressed ventrally, leading to "E0" neurons at the bottom of the nucleus. This model needs further experimental support, and it is unclear how such an edge of inhibitory strength will be used by higher centers.

The Reversible Lesion Method

There are several different ways to alter neural activity in one region in order to elucidate the functional contribution that region makes. Traditionally, lesions, either electrolytic or pharmacological, have been employed for this purpose. The obvious drawback is that lesions are irreversible. It is not usually possible to have in-session comparisons of function before and after lesion, and there are undesirable complications that include the possibility of sprouting and changes in receptor sensitivities. Nonetheless, the lesion method has been enormously successful, and is virtually the only method available in humans (where the lesions are the result of injury, not experiment, of course; Damasio and Damasio 1989). Dissection of parallel functional pathways has been accomplished elegantly in the visual system, for example, where Merigan, Maunsell, and colleagues investigated the contribution of the magnocellular and parvocellular pathways to processing different visual cues (Maunsell, Nealey et al. 1990; Merigan, Byrne et al. 1991; Merigan, Katz et al. 1991).

One reversible method of inactivation is through the use of a cryoprobe to cool tissue locally (Campeau and Davis 1990), and this has been useful in elucidating cortical functioning in visual (Schiller and Malpeli 1977; Sandell and Schiller 1982) and somatosensory systems (Turman, Ferrington et al. 1992; for a review, see Brooks 1983). One drawback is that it is difficult to cool very small regions of deeper neural tissue, especially without introducing any damage. For nuclei in the auditory brainstem of the owl, size and access recommend pharmacological approaches.

Pharmacological agents are of two types: non-specific (for example, the local anesthetics), and specific (agonists or antagonists of neurotransmitter receptors). The local anesthetic lidocaine, which blocks voltage-gated sodium

channels, has been used in the analysis of learning and reflex behavior (Chapman, Steinmetz et al. 1990), as well as more complex behaviors such as drinking (Vivas and Chiaraviglo 1992). In a series of studies of parallel processing in the visual thalamus of the cat, Malpeli (Malpeli 1983a; Malpeli, Lee et al. 1986) pioneered the reversible lesioning of selected neural channels with lidocaine. Sparks, Lee et al. (1990) also used this agent to establish population coding of saccades in the superior colliculus. A drawback to the local anesthetics is that they will also block fibers of passage, since axons, like cell bodies, rely on sodium channels for action potential initiation and propagation. Malpeli (Malpeli 1983b; Malpeli, Lee et al. 1986) also used cobalt ion, which blocks presynaptic calcium channels and has the advantage of blocking neural transmittion only where there is also synaptic transmission.

Potentiation of neural activity has been achieved in countless studies with direct electrical stimulation of brain sites, the main problem being the requirement for relatively large stimulating electrodes and the complex spread of current from the electrode tip (see, for example, Bagshaw and Evans 1976). Also, fibers of passage and neurons are both stimulated with direct current injection, and it is possible to stimulate antidromically. One clear advantage to the method is its temporal resolution: it is reversible on the timescale of milliseconds. Electrical stimulation, like the lesion method, has been very successful in studying how the superior colliculus controls eye, head, and pinna movements . A respectable literature on stimulation in humans, usually carried out during exploratory neurosurgery, has also accumulated over the years. In the barn owl, Du Lac and Knudsen (1990) showed by electrical stimulation how patterns of activity in the owl's optic tectum control head saccades.

The excitatory transmitter glutamate, or agonists for some of its receptor subtypes, has been used somewhat more rarely for generally increasing neural

activity (see Sholomenko, Funk et al. 1991b; Wickesberg and Oertel 1990 for recent examples in the avian brainstem), in part because it appears to be toxic to cells at high concentrations and may thus cause irreversible lesions that counfound an interpretation of the effect. Glutamate can also excite interneurons that are themselves inhibitory to neurons that project out of a region, so its effects need to be interpreted with caution.

More specific pharmacological agents are numerous, and depend on the transmitter systems being investigated. Exclusive use of a specific transmitter or receptor by a processing channel opens the potential to selectively affect that pathway. Unfortunately, such clean cases are rare; one notable exception was the finding that amino-phosphonobutyrate blocked the on-center channel in retinal processing (Schiller 1992).

Gamma-aminobutyric acid (GABA) is the major inhibitory transmitter in more rostral regions of the vertebrate CNS (there are rare but well-documented exceptions in very young animals and in the hippocampus, where GABA can be excitatory). Like its central excitatory analog, the glutamate receptor, the GABA receptor can be modulated in many ways. Distinct pharmacological profiles indicated two major subtypes of GABA receptor: the ionotropic GABA-A receptor, which appears to be predominantly postsynaptic, and the GABA-B receptor, which can be found both pre- and post-synaptically and which acts via second messengers. The GABA-A receptor belongs to a superfamily of recently cloned ligand-gated ion channels (Schofield and al 1987; see Betz 1990 for a review). Virtually all central neurons appear to possess GABA-A receptors (and glutamate receptors), and one potential way of modulating their activity is thus through agents that modulate the GABA-A receptor (Enna 1983). The GABA-A receptor appears to have appeared early in evolution (probably before chordates diverged from invertebrates over 500 million years ago) and

has similar structures in very different species (Harvey, Vreugdenhil et al. 1991). I know of only one minor difference that has been found between avian GABA-A receptors and those found in mammals, an alternative splicing of one of the subunits (Bateson, Lasham et al. 1991).

There are many sites to which drugs can bind at the GABA-A receptor. Recent reviews of the pharmacology can be found in Olsen (1991). GABA binds to and opens a chloride conductance at the receptor. The convulsant picrotoxin blocks this conductance by binding at a distinct site. A diverse class of allosteric modulators that appear to bind to different sites includes the anxiolytic benzodiazepines (such as Valium), the sedative barbiturates, and probably also ethanol, steroids, and perhaps general anesthetics. All these agents can potentiate GABAergic transmission, although they do so through different mechanisms and depend on the subtype of GABA-A receptor. Not all GABA-A receptor subtypes, which consist in a large diversity of heteromultimeric assemblies (reviewed in Burt and Kamatchi 1991), are affected equally by these agents (Paredes and Agmo 1992). Specific brain regions appear to express restricted varieties from the palette of GABA-A receptors available: the amygdala, for example, has a very high concentration of receptors that bind benzodiazepines, while receptors in the cerebellum can be modulated by ethanol but have a low affinity for benzodiazepines; some GABA-A receptors in the retina are completely insensitive to barbiturates, and so forth. Interestingly, both the major excitatory and inhibitory transmitter systems of the vertebrate CNS can be modulated by classical transmitters of the opposite function: the NMDA-subtype of glutamate receptor is positively modulated by the classically inhibitory transmitter glycine, and a recent report indicates that the GABA-A receptor can be positively modulated by glutamate (Stelzer and Wong 1989). It is becoming apparent that a thorough

understanding of the physiological role of GABA and its receptors will have to account for these complexities.

Two drugs that act relatively indiscriminately on all vertebrate GABA-A receptors are the channel blocker (G. Johnston in Olsen 1991) and convulsant (Welch and Henderson 1934) bicuculline (an isoquinoline alkaloid from *Dicentra cucullaria* and other plants), and the agonist muscimol (from the mushroom *Amanita muscaria* ; Krogsgaard-Larsen 1988). Both appear to bind to the same site that GABA binds to on the beta subunit of the receptor, and muscimol will open channels in the absence of GABA. Except for the non-specific lidocaine, these two drugs have been used more frequently in studies of the sort I am discussing than any other pharmacological agent (see Paredes and Agmo 1992 for a recent review).

Muscimol produces single channel conductances very similar to those produced by GABA, but the frequency of opening of the channel is increased. The main difference between GABA and muscimol is that while GABA is removed quite rapidly from the synapse (through diffusion and uptake mechanisms in neurons and glia; Iversen and Bloom 1972), muscimol is not. Behavioral deficits induced by muscimol injection typically last many hours; this was clearly seen in studies of injection of somatosensory cortex (Hikosaka, Tanaka et al. 1985), or of the superior colliculus (Hikosaka and Wurtz 1985), although a study in which motor cortex was injected saw reversibility of the action of muscimol after 60 minutes (Matsumura, Sawaguchi et al. 1991). The drug has been used with some success in dissecting the perceptual function of different auditory cortical representations in the bat (Riquimaroux, Gaioni et al. 1991).

Bicuculline is somewhat less potent than muscimol, and blocks the GABA-A receptor, probably by competing for the GABA binding site. It decreases the

probability that the channel opens, and reduces the open time. The more soluble and potent (Pong and Graham 1972) derivatives bicuculline methiodide, or methochloride are commonly used since bicuculline itself is hydrolyzed within minutes at physiological pH (Olsen, Ban et al. 1975). Numerous studies have confirmed the efficacy of bicuculline in blocking GABAergic transmission with a reversible time course on the order of 30 minutes (Feger and Robledo 1991; Matsumura, Sawaguchi et al. 1991; Sanders and Shekhar 1991; Sholomenko, Funk et al. 1991a). The drug can be used to disinhibit behavioral cassettes (Oishi and Kubota 1990) or unmask latent physiological properties (Jacobs and Donoghue 1991). Especially successful has been the use of bicuculline in elucidating the circuitry underlying visual receptive field formation in primary visual cortex: iontophoretic application abolishes "on" and "off" subdivisions of receptive fields (Sillito 1975) and orientation selectivity (Sillito, Kemp et al. 1980), although the models of visual receptive field synthesis that emerged from these studies are controversial. Bicuculline has also been used to assess GABAergic mechanisms in receptive field formation in the cat somatosensory cortex (Dykes, Landry et al. 1984) and in the electrosensory system of gymnotiform fish (Shumway and Maler 1989). Bicuculline can be used to induce reversible color-blindness when applied to the retina of some species (Wietsma and Spekreijse 1991). Both muscimol and bicuculline have been used in recent behavioral experiments on sensorimotor systems, such as the vestibulo-ocular reflex (Straube, Kurzan et al. 1991), and the superior colliculus (Hikosaka and Wurtz 1985; Sparks, Lee et al. 1990).

In the avian brainstem, Sholomenko and colleagues (Sholomenko, Funk et al. 1991a) used GABA, muscimol, bicuculline, and picrotoxin to investigate the contribution of brainstem pattern generators to locomotion. The time courses of

the effects of these drugs were very similar to those in my experiments (Chapter 4), with GABA having the most rapid, bicuculline an intermediate, and muscimol an irreversible time course.

That GABA, GABA-A receptors, and biosynthetic enzymes that correlate with the presence of GABA are found in the auditory system is well established in both mammals (Adams and Mugnaini 1984; Caspary, Rybak et al. 1985; Enna 1983; Finlayson and Caspary 1989; Helfert, Bonneau et al. 1989; Webster, Batini et al. 1990) and the brainstem (Adams and Mugnaini 1984; Caspary, Rybak et al. 1985; Code, Burd et al. 1989) and forebrain (Mueller and Scheich 1988; Stewart, Bourne et al. 1988) of birds. Mueller and Scheich (1988) demonstrated that GABAergic inhibitory mechanisms contributed to the auditory receptive field properties of cells in the forebrain of the awake chicken. Observations that barbiturate anesthesia in mammals may affect the response characteristics of cells in the inferior colliculus (Kuwada, Stanford et al. 1987) provide other, less direct evidence of the involvement of GABA in the auditory system.

GABA in the Barn Owl

In the barn owl, the existence of inhibitory mechanisms in auditory processing was first suggested in findings by Knudsen and Konishi (1978a), who found a center-surround organization to the auditory spatial receptive fields of neurons in the ICx that has also been found in the owl's optic tectum (Brainard, Knudsen et al. 1992). An immunocytochemical study by Carr and colleagues (Carr, Fujita et al. 1989), showed that the presence of GABAergic cell bodies and terminals was widespread throughout the auditory brainstem of the owl. Importantly, these authors reported that all nuclei of the lateral

lemniscus possess a high density of GABAergic cells, and that the central nucleus of the inferior colliculus contains a dense plexus of GABAergic terminals. There is a gradient in the density of GABAergic cell bodies in VLVp, from dorsal to ventral; although this gradient suggestively mirrors the gradient of inhibition that Manley, Koppl et al. (1988) found, the connection between these two findings is unclear. A recent paper by Fujita and Konishi (1991) investigated the role of GABA in the processing of ITD in the owl, and found that response selectivity, magnitude, disambiguation and temporal character could all be affected by this transmitter. These mechanisms contribute to the shaping of restricted auditory receptive fields along the horizontal dimension. Like mechanisms in other systems (for example, orientation selectivity in vision (Ferster 1986) and receptive field formation in somatosensory cortex (Gardner and Constanzos 1980)), both excitation and inhibition probably play an important role in the synthesis of responses to ITD in the owl.

I used GABA, bicuculline methiodide, muscimol, and lidocaine hydrochloride as pharmacological agents to block or induce neural activity in nuclei of the barn owl. Prior trials with cobalt ion to block synaptic transmission, or with glutamate to induce neural activity were not successful. Muscimol was used in only a few experiments because of its irreversibility and toxicity.

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CHAPTER 3:

ACETYLCHOLINESTERASE STAINING DIFFERENTIATES FUNCTIONALLY DISTINCT AUDITORY PATHWAYS IN THE BARN OWL

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ABSTRACT

The aim of this study was to examine how the functional specialization of the barn owl's auditory brainstem might correlate with histochemical compartmentalization. The barn owl uses interaural intensity and time differences to encode, respectively, the vertical and azimuthal positions of sound sources in space. These two auditory cues are processed in parallel ascending pathways that separate from each other at the level of the cochlear nuclei. Of the two cochlear nuclei, angularis showed more intense staining than nucleus magnocellularis. Nucleus angularis projects to all of the nuclei and subdivisions of nuclei that belong to the intensity processing pathway. Acetylcholinesterase (AChE) stained all regions that contain terminal fields of nucleus angularis, and thus provided discrimination between the time- and intensity pathways. Moreover, staining patterns with acetylcholinesterase were complementary to those previously reported with an anti-calbindin antibody, which stains terminal fields of nucleus laminaris, and thus stains all the nuclei and subdivisions of nuclei that belong to the time pathway. Some of the gross staining patterns observed with AChE were similar to those reported with antibodies to glutamate decarboxylase. However, AChE is a more useful marker in discriminating between these pathways than is calbindin or glutamate decarboxylase. Acetylcholinesterase staining of the intensity pathway in the owl may be related to encoding of sound intensity by spike rate over large dynamic ranges.

Histochemically distinct compartments within brain structures suggest functional differentiation. But the link between histochemical patterns and neural function has been established in only a few examples. Especially rare is knowledge of how histochemically revealed structure relates to behavior (Hess and Rockland 1983; Carr 1986). Neural pathways for which the connections, response properties of neurons, and behavioral functions of subsystems are well described offer unique opportunities for the study of the relationships between histochemical compartmentalization and functional specialization.

The auditory brainstem of the barn owl consists of two parallel pathways that process different binaural cues for sound localization. The owl uses interaural time- and intensity differences for localization in azimuth and elevation, respectively (Sullivan and Konishi 1984; Takahashi et al. 1984). The two pathways separate from each other at the level of the cochlear nuclei. The intensity pathway, shown schematically in Figure 1, originates in the cochlear nucleus angularis, which is innervated by the ipsilateral eighth nerve. The nucleus angularis projects to multiple targets, including nucleus ventralis lemnisci lateralis, pars posterior (VLVp), the nucleus of the superior olive, nucleus lemnisci lateralis, pars ventralis (LLv), and the shell of the central nucleus of the inferior colliculus (shell of ICc; Takahashi and Konishi 1988a). These nuclei all contain neurons which are sensitive to interaural intensity differences (Moiseff and Konishi 1983; Adolphs 1988; Takahashi and Konishi 1988a,b).

The time pathway originates in the cochlear nucleus magnocellularis, which projects only to nucleus laminaris, where interaural time differences are computed (Carr and Konishi 1990). Laminaris in turn projects to nucleus ventralis lemnisci lateralis, pars anterior, and to the core of ICc (Takahashi and Konishi 1988a,b), which both contain neuronal sensitivity to time differences, but

not to intensity differences (Moiseff and Konishi 1983). The time and intensity pathways converge in the lateral shell of ICc (Adolphs 1988; Takahashi et al. 1989), which provides the sole ascending auditory input to the external nucleus of the inferior colliculus (ICx), the site of a map of auditory space.

All the owl's brainstem auditory nuclei that are involved in processing time differences stain with an antibody to vitamin D-dependent calcium binding protein (calbindin; Takahashi et al. 1987). Antibodies to the neurotransmitter GABA, as well as to its biosynthetic enzyme, glutamate decarboxylase, tend to stain the intensity pathway more densely than the time pathway, although this differential staining is insufficient for unequivocal discrimination between the two pathways (Carr et al. 1989). The present report shows that acetylcholinesterase stains nucleus angularis and all the nuclei and subdivisions of nuclei that contain terminal fields of nucleus angularis, and thus marks the intensity pathway of the owl.

Fig. 3.1: **Schematic of the intensity pathway** (shaded boxes and dashed lines), **and the time pathway** (open boxes and solid lines). Boxes denote nuclei, and lines their connections. Only the terminal fields of the left NA and of the right NL are shown for clarity as they delineate, respectively, the intensity and time pathways. Nucleus SO is a terminal field of both NA and NL and has separate subdivisions that participate in each of the two pathways.

I use the terminology of Karten and Hodos (1967) as modified by Leibler (1975) for pontine and medullary nuclei. I use Knudsen's (1983) terminology for the subdivisions of the inferior colliculus.

Abbreviations:

NM   nucleus magnocellularis

NA   nucleus angularis

NL   nucleus laminaris

SO   nucleus of the superior olive

LLv   nucleus lemnisci lateralis, pars ventralis

VLVp   nucleus ventralis lemnisci lateralis, pars posterior

VLVa   nucleus ventralis lemnisci lateralis, pars anterior

ICx   external nucleus of the inferior colliculus

"Core" and "shell" refer to the subdivisions of the central nucleus of the inferior colliculus.

METHODS

Data were obtained from 6 adult barn owls of both sexes. In order to unambiguously establish the identity of certain regions that stained with AChE, I used physiological and hodological methods in conjunction with histology. After electrophysiological recording, tracers were injected in some cases, and both the response properties of neurons at the injection site as well as retrograde connectivity were then used as independent criteria in correlating subsequent staining patterns with processing function. All 6 owls were used in parallel studies of auditory physiology that are not reported here.

Tracer injections and perfusion

In order to position electrodes for injecting tracers, I used the physiological response properties of neurons to locate the target sites. I used techniques similar to ones reported earlier (Takahashi and Konishi, 1986; Wagner et al. 1987; Fujita and Konishi 1991). Owls (Tyto alba) were anesthetized with ketamine hydrochloride (100mg/ml, Ketaset, Aveco; 0.1ml/hr) and diazepam (5 mg/ml, Diazepam Injection, Steris Labs; 0.1ml/hr). The anesthetized owl was wrapped in a soft leather jacket and the skull was held in a fixed stereotaxic position in which the plane defined by the center of the ear bars and the ventral surface of the palatine ridge was tilted 45 degrees downward from the horizontal (Wagner et al. 1987). This stereotaxic plane was reproducible between different owls, and was also the plane parallel to which the brain was sectioned in subsequent histology (see below). A small craniotomy and retraction of dura allowed microelectrode and micropipette penetrations of the brain. A local anesthetic was applied to the edges of the wound during surgery. Experiments

typically lasted 12-20 hours, after which the craniotomy was closed with acrylic applied to the skull, and the scalp was sutured. An antibiotic ointment (Neosporin, Burroughs Wellcome) was applied on the wound and the owl was returned to an individual cage and carefully monitored until feeding resumed.

Two of the six owls received pressure injections of fluorescently labelled latex microspheres (Lumafluor, New City, NY). These tracers were injected through double-barrelled glass pipettes in which one barrel was filled with Wood's metal (Frank and Becker 1964) for recording from the injection site, and the other barrel (tip diameter 10-20 μm) was filled with the tracer and glued to a 1-μl Hamilton syringe with epoxy. Undiluted tracer volumes of 0.2-0.4 μl were injected into medial and/or lateral parts of the shell of ICc. Survival times of 1-2 weeks were used.

Owls were subsequently overdosed with pentobarbital (8 ml of Nembutal 50 mg/ml, Abbot Laboratories), exsanguinated with phosphate-buffered saline (pH 7.4) and fixed, in all but one case, by transcardial perfusion with 1% paraformaldehyde and 1.25% glutaraldehyde (in 0.1M phosphate buffer, pH 7.4). The fixative was then replaced by ice-cold 10% sucrose (in 0.1M phosphate buffer). Brains were removed, blocked stereotaxically in a transverse plane parallel to that of the electrode penetrations, sunk in 20% sucrose overnight, and cut frozen into 30μm-thick sections on a sliding microtome. Sections were stained with neutral red and/or processed for histochemical staining for acetylcholinesterase (AChE). One owl (#427) was overdosed and perfused as above, except that the fixative was 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. This owl brain was subsequently sectioned as above and then processed for immunohistochemical staining for calbindin.

Staining methods

Histochemical staining for AChE was carried out on 5 of the owls (owl # 402, 357, 424, 391, 398) as follows: brain sections were washed in 0.1M phosphate buffer (pH 7.4), then washed in 0.2M TRIS maleate buffer (pH 5.7), and then preincubated with 100 μM ISO-OMPA (tetraisopropylpyrophosphoramide) for 30 min. to inhibit non-specific cholinesterases. Sections were subsequently processed at room temperature according to the method of Karnovsky and Roots (1964), using acetylthiocholine iodide (Sigma) as the substrate. After the reaction product had formed (1-2 hrs.), sections were washed in buffer, and either defatted and mounted or, where so noted, further processed using the modification of Hedreen et al. (1985) for better axonal, dendritic, and perikaryal staining. Control sections were incubated as above in the presence of 1:50,000 neostigmine methylsulfate (Prostigmin; Hoffman-La Roche) to inhibit all esterase activity, and did not show any staining.

Calbindin immunohistochemistry was carried out on 1 owl (#427) after Takahashi et al. (1987). Free-floating sections were incubated at 4°C for 48 hrs. with a polyclonal antiserum kindly donated by Dr. Harvey Karten of the University of California, San Diego (1:10,000 dilution). The antiserum was the same used by Takahashi et al. (1987). Sections were subsequently processed by the avidin-biotin ABC method (Vector Labs) in conjunction with horseradish peroxidase/ diaminobenzidine histochemistry. Serial dilution controls confirmed the staining to be specific to the antibody.

Quantitation of staining

Staining for both AChE and calbindin was quantitated by recording the exposure time measured for a metering spot (100-250μ diameter) of the photographic system attached to the light microscope that was used in examining

the sections. This system was identical to that used by Takahashi et al. (1987). For each new section, the light meter was recalibrated to a reference value of light intensity.

Quantitation of staining for AChE for each of the 5 owls was carried out as follows. For each nucleus, 2 sections within 100μ of the antero-posterior middle of the nucleus were quantitated. The staining within each nucleus was comparable at all antero-posterior levels. For each section, 4 regions of the nucleus were spot-metered bilaterally with a spot diameter approximately 1/6 the cross-sectional area of the nucleus. All regions of the nucleus in that section were sampled, and both fibers and cells were included within the metering spot. Staining intensity was normalized to the staining intensity of ICx seen in each owl, since staining in ICx was uniformly dark and showed a small variance among the 8 measurements taken. For one owl (#357), 20 regions within each nucleus were spot-metered bilaterally with a spot radius of 100μ and used in comparing staining between intensity- and time pathway nuclei.

Quantitation of anti-calbindin staining was carried out for one owl (#427) in a manner identical to that for owl #357 above. Staining intensity was normalized to that seen in ICx since, like AChE, calbindin stains ICx strongly and reproducibly.

RESULTS

Inferior Colliculus

The ICc consists of a "core", which is defined by the terminal field of nucleus laminaris, stains with anti-calbindin, and has neuronal responses sensitive to time- but not to intensity-differences, and of a "shell", which is defined by the terminal field of nucleus angularis (Takahashi and Konishi 1988a). The shell of ICc can be further subdivided into a medial and a lateral part, which are situated medial to the core, and between the core and ICx, respectively. Neither of these parts of the shell stains with anti-calbindin, and both feature neuronal responses sensitive to intensity differences. While the medial shell has neurons that are insensitive to time differences (Adolphs 1988), the lateral shell's cells are sensitive because of a converging input from the contralateral core of ICc (Takahashi et al. 1989).

Figure 2 shows that calbindin and AChE stain complementary subdivisions of the ICc. The core shows strong terminal staining with anti-calbindin (top), whereas AChE (center and bottom panels) stains fibers in the shell, but spares the core. Both markers stain the ICx intensely. Independent criteria for the shell of ICc were obtained on physiological and hodological grounds by recording from the medial and lateral parts, and injecting fluorescently labelled latex microspheres into these sites. These sites were in the region which stained heavily for AChE. These additional criteria for assigning the shell of ICc were necessary because subdivisions of the ICc cannot be seen on the basis of cytoarchitecture, and because the border between ICc and ICx is often difficult to determine.

Physiological recordings showed that the injection site in the medial shell (Fig.3; 1 owl) was proximal to neurons that were sharply tuned to frequency,

tonotopically organized in the dorso-ventral dimension, excited by sounds loud at the contralateral ear, inhibited by sounds loud at the ipsilateral ear, and insensitive to interaural time differences. These properties are characteristic of medial shell neurons (Takahashi and Konishi 1983; Adolphs 1988). Likewise, retrogradely labelled structures were identical to those previously reported (the contralateral nucleus angularis, superior olive, LLv, VLVp, and medial shell of the contralateral ICc; Adolphs 1988).

The injection sites in the lateral shell (Fig.2, middle panel, and Fig.3; 2 owls) were proximal to neurons with responses characteristic of that region (Takahashi and Konishi 1983; Fujita and Konishi 1989; Takahashi et al. 1989) : neurons were sharply frequency-tuned, tonotopically organized, showed variable tuning to intensity differences, and tuning without noise-induced peak suppression to interaural time differences. Retrograde label was seen in the contralateral ICc core, bilaterally in the VLVp, and in the contralateral superior olive, LLv, and nucleus angularis. This hodology is in agreement with previous studies (Takahashi and Konishi 1988a,b; Takahashi et al. 1989). Thus, independent evidence was obtained that the region of ICc that stained heavily for AChE included the medial and lateral parts of the shell of ICc.

Fig.3.2: **Staining of the central nucleus of the inferior colliculus (ICc).** *Top*: Section stained immunohistochemically with antiserum to calbindin (CaBP). The core of ICc (arrows) stains more intensely than the shell. The normalized antero-posterior (A-P) level of the section from the caudal pole of the nucleus was calculated to be 0.5. Scale bar: 1.0mm. *Center*: Section stained for AChE. The shell stains darker than the core, complementary to the calbindin staining. A rhodamine-labelled microsphere tracer injection site is visible in the lateral shell of ICc (arrow). The external nucleus (ICx) stained strongly for both calbindin and AChE. Normalized A-P level: 0.4. *Bottom*: A section at a more anterior level through the inferior colliculus stained for AChE and photographed under dark-field illumination. The unstained (dark) core is surrounded by the stained (bright) shell. Normalized A-P level: 0.7. Orientation and scale are identical for all three panels.

CaBP

AChE
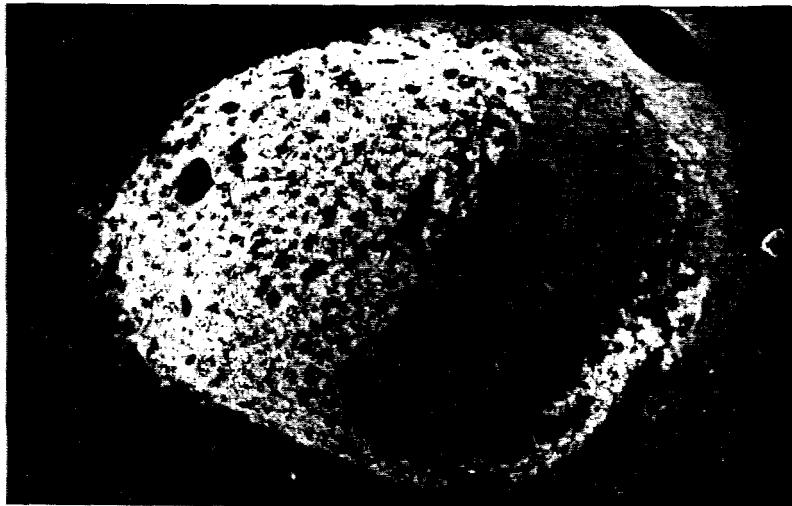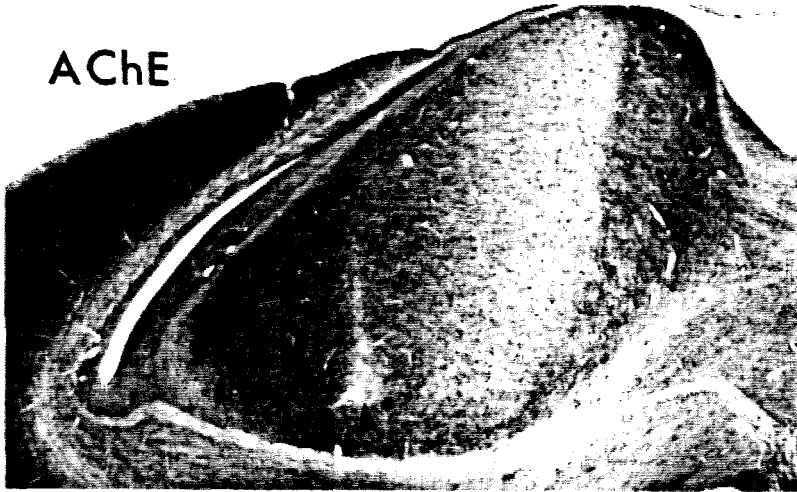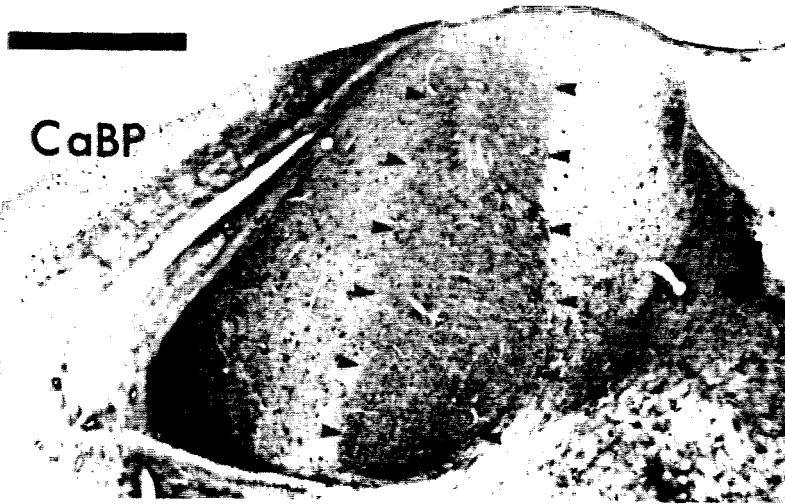
Fig.3.3: **Epifluorescence photomicrograph of the inferior colliculus showing tracer injections.** Fluorescein-labelled microspheres were injected into the medial shell of ICc (small arrow) and rhodamine-labelled spheres were injected into the lateral shell (larger arrow). Both injection sites were situated in the region of ICc that stained strongly with AChE. These injections allowed correlations to be made between physiological, hodological, and staining properties (see text for details). Normalized A-P level: 0.3. Scale bar: 500µm.

Lemniscal and Superior Olivary Nuclei

The anterior and posterior subdivisions of the nucleus ventralis lemnisci lateralis, VLVa and VLVp, are part of the time- and intensity pathways respectively. The posterior subdivision, which receives input from the contralateral nucleus angularis (Takahashi and Konishi 1988b), and projects to the shell of ICc (Adolphs 1988; T.Takahashi, pers.comm.) contains neurons that respond differentially to intensity differences and are insensitive to time differences (Moiseff and Konishi 1983; Manley et al. 1988). The anterior nucleus, VLVa, on the other hand, receives input from nucleus laminaris (Takahashi and Konishi 1988b) and its neurons are sensitive to time- but not to intensity differences (Moiseff and Konishi 1983).

Nucleus VLVa stained much more intensely with anti-calbindin than with AChE (Fig. 4A,B). This result was in agreement with the findings of Takahashi et al. (1987) that anti-calbindin stains terminal fields of nucleus laminaris. Acetylcholinesterase staining in VLVa was restricted to extremely faint staining of cell bodies. The adjacent nucleus VLVp of the intensity pathway showed generally weaker anti-calbindin staining than VLVa. However, the anti-calbindin staining was not uniform, and significant staining was seen at the dorsal pole of VLVp (Fig. 4A). Acetylcholinesterase staining in VLVp was much more intense than in VLVa, but also heterogeneous, with the strongest staining of fibers in the dorsal pole of VLVp (Fig.4B). All regions within VLVp stained more intensely with AChE than did any part of VLVa. Since VLVp and VLVa are immediately adjacent, acetylcholinesterase appears to provide a somewhat more definitive discrimination between the two nuclei than does calbindin.

Another lemniscal nucleus, nucleus lemnisci lateralis pars ventralis (LLv), is considered part of the intensity pathway. It receives bilateral input from angularis

(Takahashi and Konishi 1988b), projects to the shell of ICc (Adolphs 1988), and has neuronal responses insensitive to interaural time differences (Moiseff and Konishi 1983). Takahashi et al. (1987) did not find anti-calbindin staining in this nucleus. I could not distinguish LLv from the surrounding tissue with anti-calbindin (data not shown). However, the nucleus showed uniformly dark staining of both fibers and cell bodies with AChE. Both the main body of LLv, and its dorsal "island" can be seen to stain equally strongly (Fig.4B); both these subdivisions appear to be hodologically equivalent to each other (Takahashi and Konishi 1988b).

The nucleus of the superior olive appears to contain subdivisions that belong to either the time- or the intensity pathway (cf. Fig.1). The medial portion of the nucleus receives afferents from nucleus laminaris, while the lateral aspect of the nucleus is a terminal field of nucleus angularis (Takahashi and Konishi 1988b). The ventrolateral part of the superior olive projects most heavily to the medial shell of ICc (Adolphs 1988), a region belonging to the intensity pathway. I found that anti-calbindin stained the medial portion of the superior olive (Fig. 5, left), as would be expected, since anti-calbindin labels the terminal field of nucleus laminaris. Faint staining can also be seen in the ventral pole of the nucleus, as Takahashi et al. (1987) reported. The lateral portion of the superior olive, however, showed most intense fiber staining with AChE (Fig.5, right).

Fig.3.4: **Staining of lemniscal nuclei.** *A.*: Antiserum to calbindin (CaBP) preferentially stains the more medial, pear-shaped nucleus VLVa. The immediately adjacent and more lateral VLVp is stained more faintly, although there is significant staining at its dorsal pole. Normalized A-P levels: VLVa: 0.5; VLVp: 0.5. Scale bar: 500μm. *B.*: Acetylcholinesterase histochemistry stains VLVp, but not VLVa. The dorsal pole of VLVp (d) stains darker than the ventral pole (v). Nucleus LLv exhibits uniformly strong staining. Normalized A-P levels: VLVa: 0.4; VLVp: 0.3; LLv: 0.6. Scale bar: 500μm.

A. CaBP

d, l, m, v (orientation indicator)

VLVp
VLVa

B. AChE

d
VLVp
VLVa
v
LLv
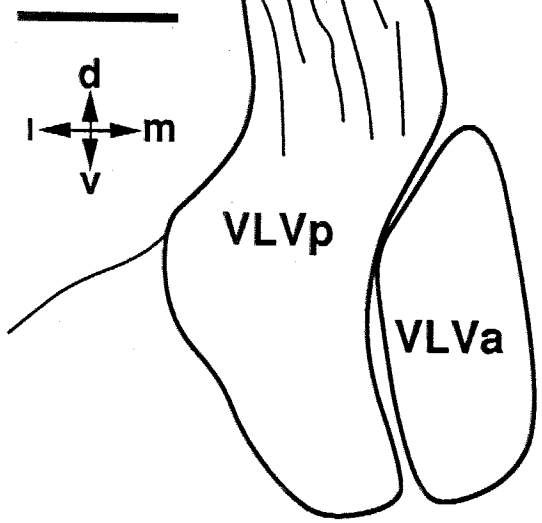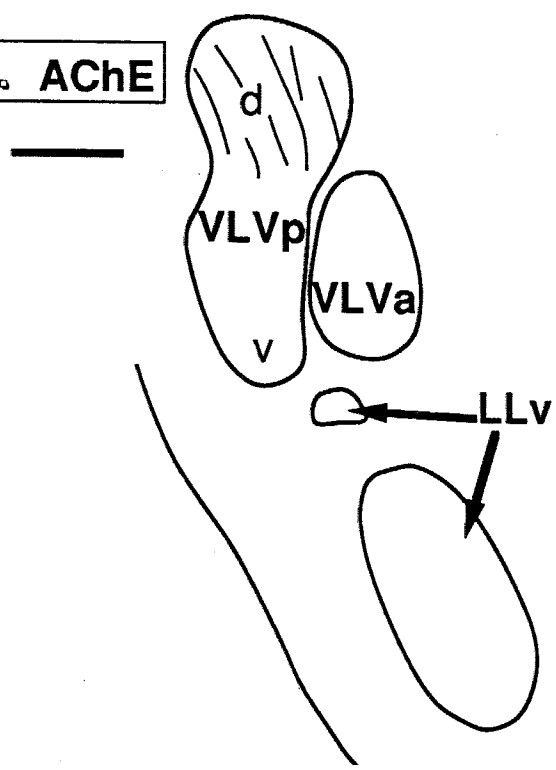
Fig. 3.5: **Staining of the superior olivary nucleus.** *Left*: Calbindin antiserum (CaBP) stains the SO in its ventral and medial aspects. Normalized A-P level: 0.5. *Right*: Acetylcholinesterase stains more lateral portions of SO. Normalized A-P level: 0.4. Scale bar: 200µm.

AChE

CaBP
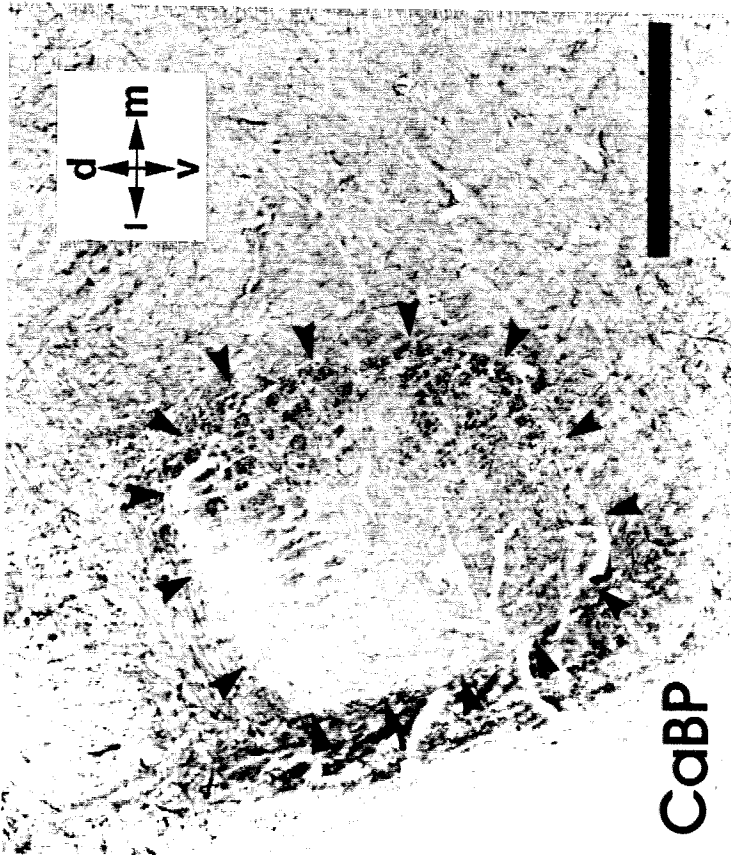
Cochlear Nuclei and Laminaris

The cochlear nuclei and nucleus laminaris showed clear differences in the intensity with which they stained for AChE. Nucleus angularis stained more strongly than either nucleus magnocellularis or nucleus laminaris (Fig.6). The pattern of staining in each nucleus also differed. In nucleus angularis, a very dense fiber plexus stained intensely and cell bodies showed variably strong staining (Fig.7, top), whereas in nucleus magnocellularis, only the somata of cells were stained (center). In nucleus laminaris there was perikaryal staining at sites of afferent terminals onto the somata, as well as weak and variable staining of cell bodies (Fig.7, bottom).

Quantitation of AChE and Calbindin

Quantitative analysis of acetylcholinesterase staining from all 5 owls showed that nuclei of the intensity pathway (nucleus angularis, the superior olive, LLv, VLVp, and the shell of ICc) all stain more intensely for AChE than nuclei of the time pathway (nucleus magnocellularis, laminaris, VLVa, and the core of ICc; Fig.8A). While the subdivisions of the superior olivary nucleus did not differ significantly when mean staining intensity across all 5 owls was compared (Fig. 8A), there was a large difference in staining intensity between the lateral and medial subdivisions of the superior olive within each owl (Fig. 8B).

The differences in staining intensity between adjacent nuclei of the time- and intensity pathways were complementary for AChE and calbindin. Figure 8B shows that the subdivisions of the superior olive, of VLV, and of ICc that process time information all stain significantly more with anti-calbindin than do those subdivisions that process intensity information. The converse is true for AChE staining: subdivisions of these nuclei that process intensity stain more than those that process time. The only inhomogeneities in staining were found in VLVp.

While anti-calbindin gives patchy strong staining of fibers in the dorsal VLVp, staining of the rest of the dorso-ventral extent of the nucleus is fainter than staining of VLVa (Fig.4A, 8B). Acetylcholinesterase also stained VLVp somewhat more strongly dorsally, but all parts of VLVp stained more than any part of VLVa. There were no qualitative differences in staining patterns seen along the antero-posterior axis of VLVp, or of any other nucleus.

With both anti-calbindin and acetylcholinesterase staining, the differences that allowed discrimination between processing pathways were attributable primarily to differences in the density and/or intensity of fiber staining within nuclei. Thus, nuclei belonging to the intensity pathway stained more for AChE even in cases where not all cells within the nucleus stained more strongly than all cells in an adjacent nucleus of the time pathway.

Fig. 3.6: **Acetylcholinesterase staining of nucleus angularis (NA), nucleus magnocellularis (NM), and nucleus laminaris (NL).** The fiber plexus within NA stains the darkest. Normalized A-P levels: NA: 0.3; NM: 0.3; NL: 0.1. Scale bar: 500μm.

AChE

NA

NL

NM
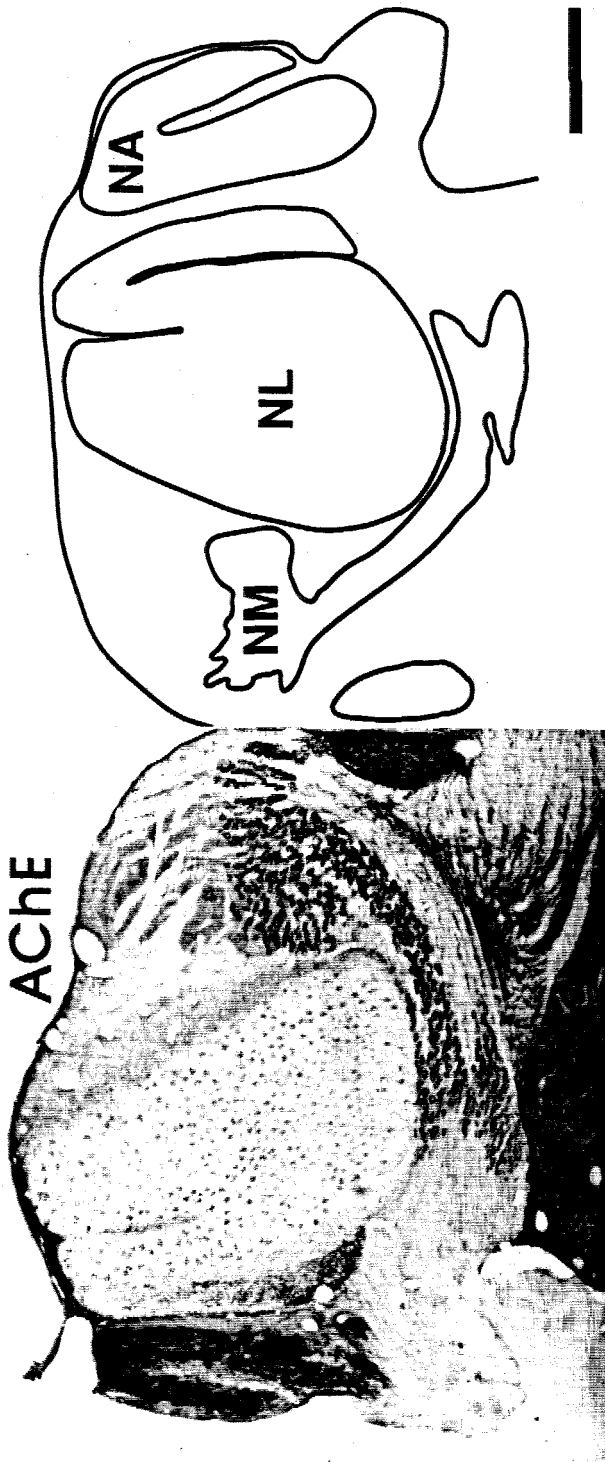
Fig. 3.7: **Pattern of AChE staining.** All three sections were stained using the modification of Hedreen et al. (1985). *Top*: Nucleus angularis (NA) consists of a densely staining fiber plexus. Some cell bodies also stain. *Center*: The somata in nucleus magnocellularis (NM) stain strongly. *Bottom*: In nucleus laminaris (NL), perisomatal staining is seen in addition to weak staining of cell bodies. Normalized A-P levels: NA: 0.3; NM:0.6; NL: 0.3. Scale bar: 200μm .
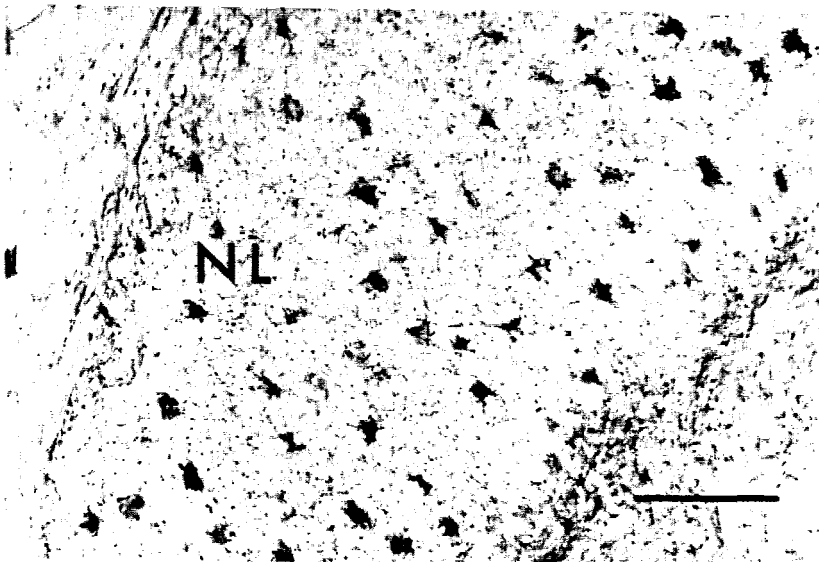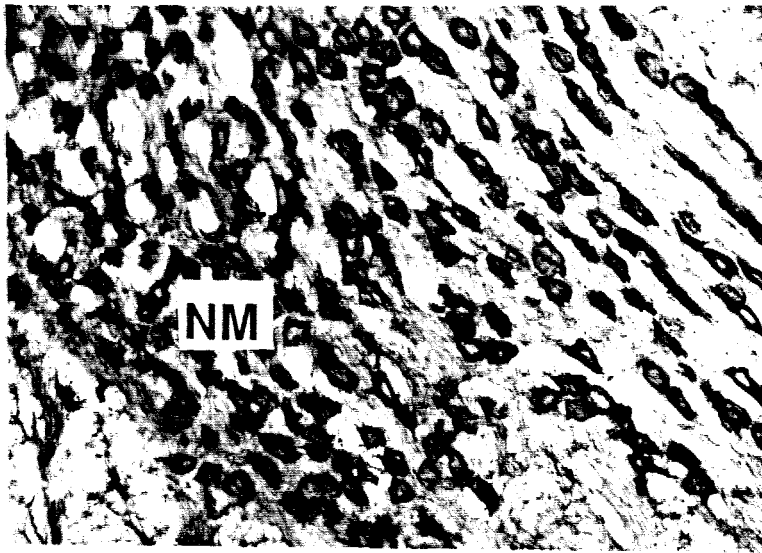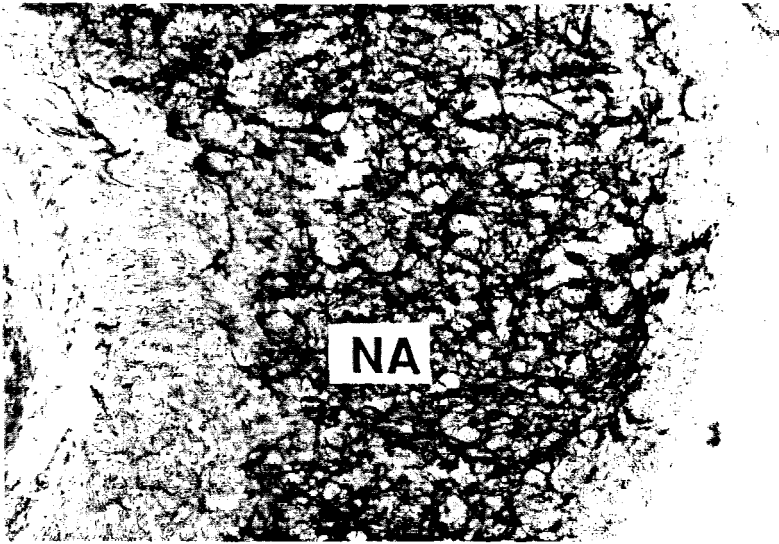
Fig. 3.8. **Quantitative staining intensities.** *A.* Mean intensity (± s.d.) of AChE staining in auditory brainstem nuclei from 5 owls. For each owl, eight measurements were taken bilaterally for each nucleus near the antero-posterior middle of the nucleus and normalized to staining of ICx. Measurements were taken at all regions of the nucleus at that section. The only inhomogeneities were seen in VLVp; this figure pools measurements taken throughout the dorso-ventral extent of VLVp. From left to right: nucleus angularis (NA); nucleus magnocellularis (NM); nucleus laminaris (NL); the lateral (L) and medial (M) subdivisions of the superior olivary nucleus (SO); nucleus lemnisci lateralis pars ventralis (LLv); the posterior (p) and anterior (a) subdivisions of nucleus ventralis lemnisci lateralis (VLV); the medial shell (M), core (C), and lateral shell (L) of ICc.

B. Complementary staining with calbindin and AChE. Differences in normalized staining intensity (± s.d.) between nuclei of the time pathway and of the intensity pathway are plotted for calbindin (owl #427) and for AChE (owl#357). For each bar, twenty measurements were taken bilaterally in each nucleus, from two different sections near the antero-posterior middle of the nucleus. Staining intensity was normalized to staining of ICx, and the differences between the means of the normalized staining intensities of different nuclei were calculated. The superior olive was divided into three regions of equal area along the medio-lateral dimension, and the extreme medial region was compared to the extreme lateral region. The only inhomogeneities were seen in VLVp with both AChE and calbindin staining; measurements pooled staining intensities throughout the dorso-ventral extent of VLVp. From left to right: medial third subtracted from lateral third of superior olivary nucleus (M-L SO); anterior subtracted from posterior subdivision of nucleus ventralis lemnisci lateralis (VLVa-p); core of ICc subtracted from medial shell of ICc (core-ms); core subtracted from lateral shell of ICc (core-ls).

A.

B.
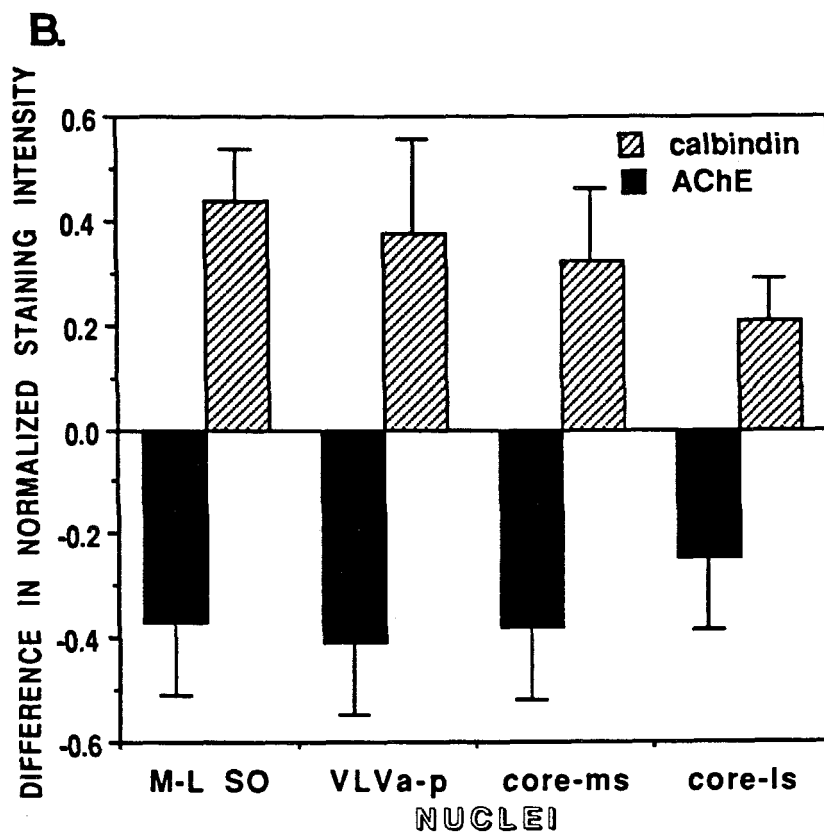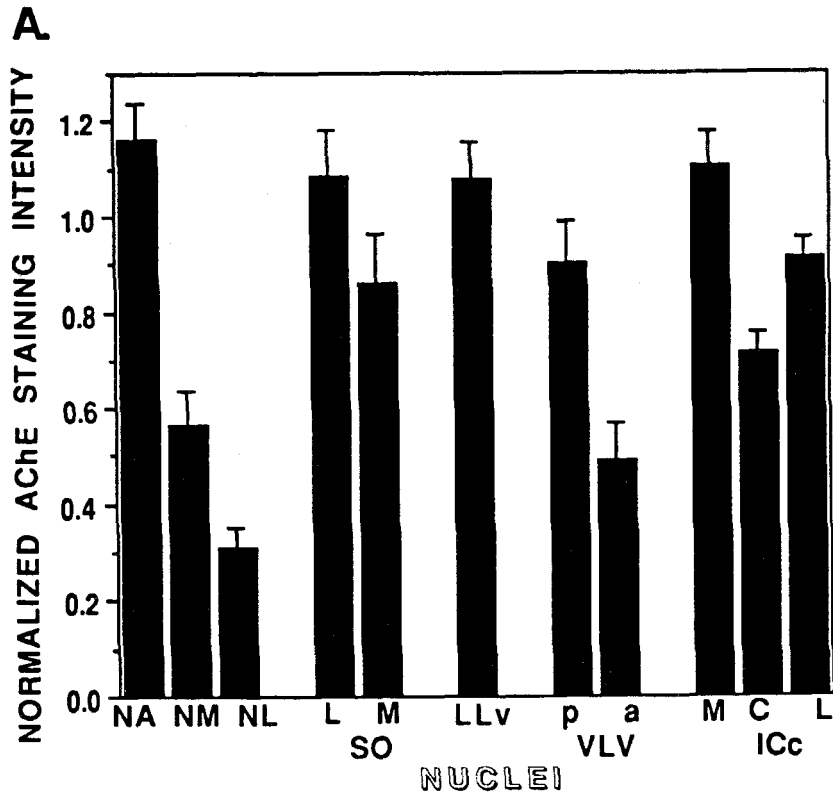
DISCUSSION

Acetylcholinesterase staining: Comparison with other systems

Acetylcholinesterase stained nuclei of the intensity pathway more strongly than adjacent nuclei that process time information at all levels in the brainstem of the barn owl up to the level of the inferior colliculus. Figures 8A and 8B summarize these findings. In the owl, all nuclei of the intensity pathway receive input from nucleus angularis. It is therefore possible that AChE stains the terminal fields of angularis, as well as fibers within angularis itself. Greenspon and Fass (1984) examined AChE staining in the cochlear nuclei of the canary. They reported strong staining of cell bodies and fibers in nucleus angularis. As in the owl, the canary's nucleus magnocellularis showed somatal staining, and nucleus laminaris showed fainter staining of terminals surrounding cell bodies. It would be interesting to see if nuclei that receive input from angularis in the canary also stain for AChE. The only bird species other than the owl for which functional differentiation of cochlear nuclei has been established is the chicken (Warchol and Dallos 1990; Carr 1991): nucleus angularis is sensitive to intensity and nucleus magnocellularis to phase. The correlations between the histochemical, hodological, and functional characteristics of the neural pathways subserving audition in other avians remains to be worked out.

Acetylcholinesterase staining has been used extensively in a number of other systems: in describing thalamic nuclear organization in mammals (Olivier et al. 1970; Graybiel and Berson 1980); in studying compartmentalization of the striatum of primates and cats (Graybiel and Ragsdale 1978); and in examining the organization of the rat brain (Koelle 1954; Shute and Lewis 1963). In birds, AChE was used to study nuclei involved in the vocal control of song (Ryan and Arnold 1981; Zuschratter and Scheich 1990), as well as in delineating

hodological subdivisions of thalamic nuclei that cannot be visualized with conventional stains (Martinez-de-la-Torre et al. 1990).

Why does acetylcholinesterase stain the intensity pathway?

There are various hypotheses that could explain why AChE stains the intensity pathway more strongly than the time pathway in the owl. Neurons that process intensity information may need to respond over a very wide dynamic range. Since sound intensity is encoded by firing rate, these neurons also typically have much higher maximal firing rates than do neurons that phase lock in the time pathway (Sullivan 1985). Sullivan and Konishi (1984) have studied neurons in nucleus angularis, the first nucleus of the intensity pathway, and in nucleus magnocellularis, the first nucleus of the time pathway. Neurons in nucleus angularis respond to a much larger range of intensity and have about twice the maximal firing rate of neurons in nucleus magnocellularis, which saturate rapidly. It is possible that the presence of AChE reflects the use of a transmitter that can enable a gain-control function and/or increase signal-to-noise in neurons that process intensity information. These functions have been attributed to cholinergic neurotransmission in mammalian cortex (Prince and Huguenard 1988) as well as in other brain regions (see below).

A finding possibly analogous to the above hypothesis has been made in the primate lateral geniculate nucleus, where parvocellular layers stain more strongly for AChE than magnocellular layers in prosimians that are nocturnal (Hess and Rockland 1983; McDonald et al. *in press*). It has been hypothesized that a cholinergic input to the parvocellular layers may facilitate information flow under conditions of low light intensity. This would be consistent with the findings that acetylcholine can increase neuronal excitability and increase signal-to-noise in information processing in the lateral geniculate nucleus (Singer 1973; Sillito et

al. 1983; McCormick and Prince 1987), as well as in probably most of sensory neocortex (Robbins and Everitt 1987).

The transmitters that might underlie the presence of AChE in the owl include acetylcholine, substance P (Millar and Chubb 1984), and the enkephalins (Chubb et al. 1983), all of which have been shown to be hydrolyzed by AChE. Several studies indicate that AChE staining is not necessarily quantitatively correlated with either the neurotransmitter acetylcholine or with its biosynthetic enzyme choline acetyltransferase (Silver 1974; Hoover et al. 1978; Mesulam et al. 1984). However, areas staining for choline acetyltransferase appear to be a subset of those that stain for AChE (Eckenstein and Sofroniew 1983; Zuschratter and Scheich 1990). Thus, although some structures do show precise correlation between AChE and choline acetyltransferase staining (Illing 1990), caution must be excercised in assigning cholinergic function to regions exhibiting AChE staining. Acetylcholine, Substance P, and enkephalins are all prime candidate neuromodulators that could subserve such functions as gain-control or improving signal-to-noise as discussed above. Preliminary studies in the optic tectum of the barn owl, which stains very strongly for AChE (pers. obs.), showed that microiontophoresis of acetylcholine changes the average firing rate of neurons in that structure over several minutes (D. Feldman, pers. comm.). However, microiontophoresis of either nicotinic or muscarinic cholinergic agonists and antagonists was without effect in preliminary studies of neurons in the ICc and in VLVp (pers. obs.; 14 neurons tested). Further iontophoretic studies, as well as immunohistochemistry for choline acetyltransferase, substance P and enkephalins are needed to test these possible transmitter systems for their involvement in the intensity pathway of the owl.

Comparison with staining for GABA

Carr et al. (1989) reported immunohistochemical staining for the inhibitory transmitter GABA, and for its biosynthetic enzyme, glutamic acid decarboxylase (GAD) in the barn owl. Staining for the two molecules is comparable in the owl, and bears some similarity to AChE staining. The nucleus angularis shows intense fiber staining for both GAD and for AChE. In both cochlear and lemniscal nuclei, GAD generally stains nuclei that process intensity information more densely than nuclei that process time information, although this pattern is not clear enough to allow discrimination between the two functional pathways with this method. It is possible that intensity processing requires more inhibition; again, one function could be in gain control. The presence of both AChE and GABA in nuclei of the intensity pathway may reflect the need to encode stimulus intensity over a very wide dynamic range. Neuromodulation and inhibition are two ways to control information processing over large ranges.

Notable differences between GAD immunohistochemical staining and staining for AChE are seen in the detailed patterns. Anti-GAD often stains both cell bodies and fibers in a nucleus, whereas AChE stains predominantly fibers. Also, GAD staining does not distinguish between different subdivisions of the superior olive, nor does it distinguish core from shell in the ICc.

Comparison with staining for calbindin

The only marker other than AChE that labels a processing pathway in the owl is an antigen with calbindin-like immunoreactivity. Takahashi et al. (1987) reported immunohistochemical staining for calbindin could be used to delineate the terminal field of nucleus laminaris, thus defining the time pathway. Studies in other species have established calbindin as a marker for functional pathways: in the rat, Celio (1990) found that entire functional systems, such as the taste pathway, are delineated by calbindin staining. Calbindin preferentially stains

neurons that fire tonically at high frequencies in the electrosensory system of electric fish (Maler et al. 1984; Losier and Matsubara 1990) and in the cat vestibular system (Sans et al. 1986). Allman and Zucker (1990) argued that calbindin staining of the interblobs in monkey primary visual cortex may reflect the processing of temporal information subserved by this region, a finding of special interest since AChE preferentially stains the cytochrome-oxidase blobs (Horton 1984). These findings may be related to the finding by Takahashi et al. (1987) that the molecule labels cells that phase-lock in the auditory system of the barn owl. The molecule's calcium-binding function may underlie these similarities (Carr 1986). It should be noted, however, that calbindin is not the only such calcium-binding molecule, and there is good evidence for the involvement of other molecules similar to calbindin in many of these systems. In particular, the homologous protein calretinin cross-reacts with many antibodies to calbindin, and is likely to be responsible for the anti-calbindin staining seen in the cochlear nuclei and laminaris in the owl, as well as in the chick (Rogers 1987; Zettel et al. 1991; C.E. Carr, pers. comm.). More central auditory nuclei, however, appear to be calretinin-negative. Like calbindin, calretinin has been proposed to subserve neuronal functions involving high temporal acuity (Rogers 1987, 1989).

In the auditory brainstem of the rat, Webster et al. (1990) found antibody staining for calbindin in afferent terminals within the lateral and medial superior olive, and in all somata of the medial nucleus of the trapezoid body. These authors postulated that calbindin may be associated more with nuclei that process intensity differences in this species. Matsubara (1990) corroborated these findings in the cat, and also found heavier terminal calbindin label in the lateral than in the medial superior olive. Zettel et al. (1991) found some calbindin-positive labelling of cells in the medial superior olive of the mustached bat, whereas in other mammals all cells of this nucleus are calbindin-negative.

The antibodies used by all these workers were reported to be specific for calbindin.

The medial nucleus of the trapezoid body and the lateral superior olive are nuclei of the mammalian central auditory pathway crucially involved in processing intensity, while the medial superior olive is concerned with the processing of time differences. However, while the medial superior olive appears to be homologous to nucleus laminaris, Takahashi and Konishi (1988b) have argued that there are no clear avian counterparts to the medial nucleus of the trapezoid body or to the lateral superior olive. The first site containing neurons sensitive to interaural intensity differences in the owl, VLVp, is more similar to the mammalian dorsal nucleus of the lateral lemniscus than to the lateral superior olive; and LLv may have as its counterpart the ventral nucleus of the lateral lemniscus in mammals (Takahashi and Konishi 1988b; Carr 1991). Resibois et al. (1990) reported that the rat dorsal and ventral nuclei of the lateral lemniscus contain calbindin (but not calretinin). I found that anti-calbindin stains the dorsal part of VLVp (Fig.4A), and may also stain LLv, which is indistinguishable from the staining of the surrounding tissue. However, it is not clear whether this staining is due to calbindin or to a similar calcium-binding molecule in the owl. In the mustached bat, nuclei receiving binaural input, such as the dorsal nucleus of the lateral lemniscus, are conspicuously calbindin-negative. Birds and mammals appear to differ in the distribution of calretinin in the auditory brainstem; further studies are needed in the owl to compare calbindin staining.

Staining patterns in VLVp

Differential AChE staining was seen along the dorso-ventral axis of VLVp. This nucleus, a member of the intensity pathway, showed particularly dark

staining dorsally (Fig. 4B). Takahashi and Konishi (1988b) reported that label after tritiated proline injections into the cochlea is strongest dorsally in VLVp. Carr et al. (1989) reported that cells staining for glutamic acid decarboxylase are more numerous in dorsal than in ventral VLVp. Additionally, Manley et al. (1988) found that neurons in the dorsal part of VLVp show stronger inhibition to some auditory stimuli than neurons in more ventral regions of the nucleus. Preliminary investigations by Takahashi (pers. comm.) suggest that the ventral VLVp projects primarily to the dorsal VLVp on the other side; this projection provides the source of inhibition to cells in the dorsal VLVp (Takahashi and Keller, 1992). These findings appear to support the idea that neurons that process intensity information may employ inhibitory and modulatory mechanisms that can be reflected in relatively high concentrations of certain neurotransmitters. The transmitter GABA, and neuromodulatory transmitters that can be hydrolyzed by AChE, are two such candidates.

Concluding Remarks

Histochemical patterns of staining for acetylcholinesterase correlate with a functionally specialized pathway in the owl which processes interaural intensity differences. This intensity pathway is comprised of all the nuclei and subdivisions of nuclei that contain terminal fields of the cochlear nucleus angularis, which itself stains intensely for AChE. It is thus possible that AChE is a marker for the terminal fields of nucleus angularis, and that discrimination between time- and intensity pathways by AChE staining results from the hodological segregation of these two processing streams. Neurons of nucleus angularis differ from neurons in the time pathway in their wide dynamic range, and in that they encode stimulus intensity by their rate of firing. These neural

processing strategies may employ neuromodulatory transmitters that utilize acetylcholinesterase.

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CHAPTER 4:

BILATERAL INHIBITION GENERATES NEURONAL RESPONSES TUNED TO INTERAURAL LEVEL DIFFERENCES IN THE AUDITORY BRAINSTEM OF THE BARN OWL.

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ABSTRACT

I investigated the mechanisms by which neurons gain selectivity for interaural level difference in the brainstem of the barn owl, *Tyto alba*. Differences in the timing and level of sounds at the ears are used by this owl to encode, respectively, azimuthal and vertical position of sound sources in space. These two cues are processed in two parallel neural pathways. Below the level of the inferior colliculus, all neurons in the pathway that processes level differences show monotonic responses to this cue. Only in the inferior colliculus, which contains a map of auditory space, are neurons sharply tuned to specific interaural level differences. How are these response properties generated from those of the nuclei that provide input to the inferior colliculus?

I show that the posterior subdivision of the nucleus ventralis lemnisci lateralis (VLVp) projects bilaterally to the lateral shell of the central nucleus of the inferior colliculus, the input stage to the map of auditory space. Manipulations of the responses in VLVp affected the responses to level differences in the inferior colliculus; responses to time differences were unaffected. By systematically increasing or decreasing neural activity in VLVp, I show that the VLVp on each side provides inhibition to the colliculus at large level differences. This results in a peaked response that is tuned to level differences in the inferior colliculus. Furthermore, the inhibition by VLVp of cells in the colliculus appears to be GABAergic and is probably direct. I suggest that this circuitry and the algorithms it supports are the neural substrates that allow the barn owl to exploit level differences for computation of sound source elevation.

Two striking features of neural sensory systems are the presence of neurons that encode complex and highly selective stimuli, and the organization of such neurons into topographic maps. In the visual and somatosensory systems of many species, maps of sensory space are generated directly by the preservation of the receptor epithelium's topography. In the auditory system, however, the only parameter whose magnitude is organized at the periphery is frequency: the cochlea contains a one-dimensional tonotopic map. A neural representation of space based on auditory cues, on the other hand, must be centrally synthesized by the computation of binaural disparities in the timing and the level of sounds (Konishi 1986). In the barn owl, a vertical asymmetry in the external ears and facial feathers generates interaural level differences (ILD) that vary systematically with the elevation of the sound source for frequencies above 3 kHz (Coles and Guppy 1988; Moiseff 1989a). Interaural time differences (ITD) are due to the separation of the ears along the horizontal axis. Together, these two cues are used by the owl to accurately localize the direction from which a sound is emanating in both elevation and azimuth. The robustness and acuity of the barn owl's auditory system enable it to catch small, rustling prey in complete darkness.

Anatomical and physiological evidence indicates that interaural level differences and time delays are processed in parallel and functionally independant pathways, referred to as the intensity pathway and the time pathway (Sullivan and Konishi 1984; Takahashi, Moiseff et al. 1984). The intensity pathway consists in all nuclei that are terminal fields of the cochlear nucleus angularis. The time pathway consists in all nuclei that are terminal fields of the first nucleus sensitive to binaural time differences, nucleus laminaris. Nucleus angularis and laminaris project to different regions of the

central nucleus of the inferior colliculus, the "shell" and "core", respectively (Takahashi and Konishi 1988a).

The time- and intensity pathways converge in the lateral shell of the central nucleus of the inferior colliculus (lateral shell of ICc; Adolphs 1988; Takahashi and Konishi 1988a; Takahashi, Wagner et al. 1989) which provides the sole ascending auditory input to the external nucleus of the inferior colliculus (ICx), the site of a map of auditory space (Knudsen 1983; Wagner, Takahashi et al. 1987). The lateral shell of ICc, and the ICx, contain neurons that selectively respond to unique combinations of time- and level differences (Moiseff and Konishi 1983; Fujita and Konishi 1989); as a consequence, these neurons have auditory receptive fields with clearly delimited vertical and horizontal borders (Knudsen and Konishi 1978).

Some cells in the lateral shell of ICc, and all cells in the ICx, are exclusively binaural and will not fire at large ILDs favoring either ear. This results in a sharp peak of the response as ILD is varied. The tuning to ILD can be used to code for elevation in bi-coordinate sound localization (Moiseff 1988a,b). Cells in ICx also have responses to ILD that are unaffected with changes in average binaural intensity (ABI), or with changes in ITD. The coding of ILD thus provides a prime example of invariant computing of a distal stimulus attribute.

The ILD pathway, shown schematically in Figure 1, originates in the nucleus angularis, a cochlear nucleus, which is innervated by the ipsilateral eighth nerve. Neurons of nucleus angularis have monotonic rate functions with sound level and do not phase lock, except to carrier frequencies at the lowest region of the owl's audiogram (Sullivan and Konishi 1984). The second-order nucleus, nucleus ventralis lemnisci lateralis, pars posterior (VLVp), receives a direct, excitatory projection from the contralateral nucleus angularis (Takahashi and Konishi 1988b; Takahashi and Keller 1992) and an inhibitory input that arrives

from the VLVp of the opposite side via the commissure of Probst (Takahashi 1988; Takahashi and Keller 1992). As a result, neurons in VLVp are sensitive to binaural stimuli: they are excited by sounds loud at the contralateral ear via a direct connection from nucleus angularis, and they are inhibited by sounds loud at the ipsilateral ear via an indirect inhibitory projection. This results in a response curve that is a sigmoid function of ILD (Moiseff and Konishi 1983; Manley, Koeppl et al. 1988).

Previous experiments on the functional role of the nuclei that process ILD have been carried out in paradigms similar to the ones presented here. Takahashi, Moiseff et al. (1984) injected the local anesthetic lidocaine into nucleus angularis while recording responses to auditory stimuli in ICx. They found that only the tuning to level differences of the ICx neuron was affected; the responses to ITD were unaffected. This showed that the two processing streams were functionally independant, and that nucleus angularis participated only in the intensity pathway.

More recent experiments (Takahashi 1988; Takahashi and Keller 1992) demonstrated that the anesthetization of one VLVp disinhibits the cells in the opposite VLVp, thus confirming that the VLVp on each side of the brain inhibit each other. But what computations take place upstream from VLVp? Where does the nucleus project, and how might it contribute to the synthesis of responses in higher stations? I address these issues in the experiments reported here.

Figure 4.1: **Schematic of the intensity pathway** (shaded boxes and dashed lines), **and the time pathway** (open boxes and solid lines). Boxes denote nuclei, and lines their connections. For the inferior colliculus, subdivisions are represented by ellipses. Only the terminal fields of the left NA and of the right NL are shown for clarity as they delineate, respectively, the intensity and time pathways. Nucleus SO is a terminal field of both NA and NL and has separate subdivisions that participate in each of the two pathways.

Abbreviations:

NM   nucleus magnocellularis

NA   nucleus angularis

NL   nucleus laminaris

SO   nucleus of the superior olive

LLv   nucleus lemnisci lateralis, pars ventralis

VLVp   nucleus ventralis lemnisci lateralis, pars posterior

VLVa   nucleus ventralis lemnisci lateralis, pars anterior

ICx   external nucleus of the inferior colliculus

"Core" and "shell" refer to the subdivisions of the central nucleus of the inferior colliculus.
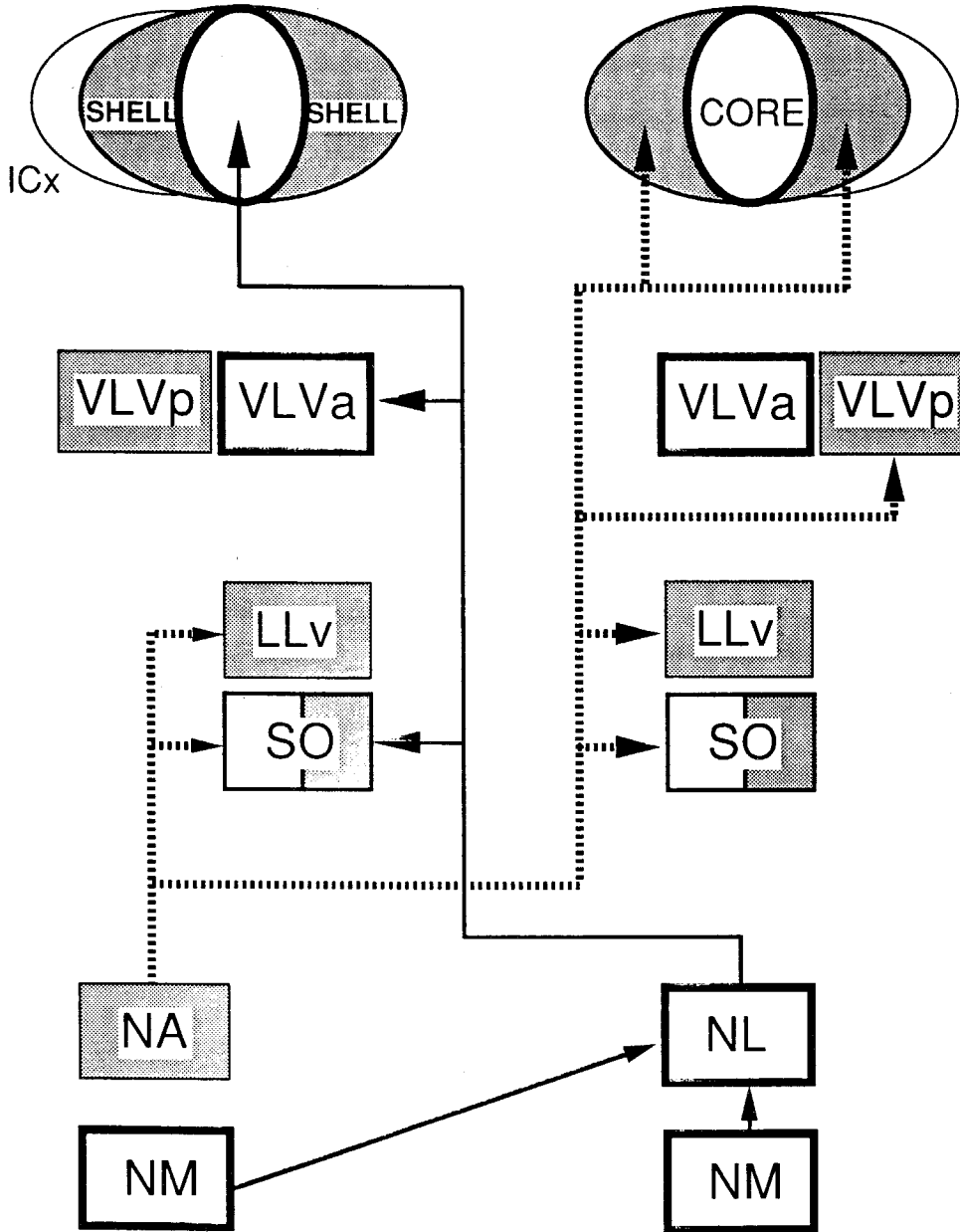
METHODS

Physiology:

The techniques used were similar to ones reported earlier (Manley, Koeppl et al. 1988). Thirteen adult barn owls (*Tyto alba*) were initially anesthetized with ketamine hydrochloride (100mg/ml, Ketaset, Aveco; 0.1ml/owl/hr) and diazepam (5 mg/ml, Diazepam Injection, Steris Labs; 0.1ml/owl/hr), and then maintained under ketamine anesthesia. The anesthetized owl was wrapped in a soft leather jacket, and the skull was held in a fixed stereotaxic position in which the plane defined by the center of the ear bars and the ventral surface of the palatine ridge was tilted 45 degrees downward from the horizontal (Wagner,Takahashi et al. 1987). A small craniotomy and retraction of dura allowed microelectrode and micropipette penetrations of the brain. A local anesthetic was applied around the edges of the scalp wound. Experiments typically lasted 12-20 hours, after which the craniotomy was closed with acrylic and the scalp was sutured. Antibiotic ointment (Neosporin, Burroughs Wellcome) was applied on the wound and the owl was returned to an individual cage. At least 3 days of recovery, during which the animal was closely monitored and fed, were allowed between experiments.

All recording was done with glass electrodes filled with Wood's metal plated with gold and platinum. Exploratory physiology used a single-barrelled glass electrode that had been filled with Wood's metal. During the experiments, triple-barrelled glass pipettes (1.2mm OD, 0.6mm ID; A-M Systems Inc., WA) were used, in which one barrel was filled with Wood's metal and plated with gold and platinum at the tip, and the other two barrels were filled with solutions of drugs for iontophoresis. Triple-barrelled electrodes had an outer tip diameter of 5-15μ with a gradual taper. After plating, the recording barrel impedance was

0.5-3.0 megaohms at 1 kHz. A backing current of -5 nA was applied to the drug barrels to prevent leakage.

For larger, pressure injections of drugs or of tracers, a glass pipette gradually tapering to a 10-20µ tip was glued to the metal tip of a 5µl Hamilton syringe with epoxy (Takahashi, Moiseff et al. 1984). Injection electrodes were positioned stereotaxically after localizing the target with a Wood's metal electrode. Multi-unit potentials could be recorded through the syringe for verification. In experiments involving multiple injections, there was at least 1 hour recovery time between injections. Up to four series of injections were performed on each side per owl. Additional injections produced lesions that prevented complete recovery of neuronal responses. The positions of the structures injected were verified histologically by these lesions. All injections, unless otherwise noted, consisted of 0.2µl of the drug in isotonic saline (pH 6-7), or of 0.1µl of a tracer (see below). The drugs injected were lidocaine hydrochloride (4% buffered, Xylocaine, Astra Pharmaceuticals), bicuculline methiodide (BMI; 5mM aqueous, pH 7.0, Sigma), GABA (0.5M aqueous, pH 7.0, Sigma), or muscimol (3mM aqueous, pH 7.0, Sigma).

Drugs were iontophoresed in physiological saline solution at acidic pH. A continuous positive current of 20nA was used for iontophoresis of BMI (5mM, pH 3.0-3.5) or GABA (0.5M, pH 3.0-3.5)(Caspary, Rybak et al. 1985; Fujita and Konishi 1991; Mueller and Scheich 1988). After iontophoresis, a negative backing current of 5 nA was applied to prevent leakage.

Auditory stimuli consisted of tone or pseudorandom noise bursts (1 per second; 100ms duration; 5ms rise-fall time) delivered through calibrated earphones (Takahashi and Konishi 1986; Wagner, Takahashi et al. 1987). Stimuli of varying ILD were presented at the neuron's preferred ITD, and the average binaural intensity (ABI) was typically set to 20 dB above the neuron's

threshold. ILD-response functions were generally obtained at a fixed ABI; only in a few cases was ILD presented by holding the sound level at the excitatory ear constant, and varying the level at the ear that inhibited responses (Irvine 1987). To establish responses to time differences, stimuli of varying ITD were presented at the ILD that the neuron preferred before injections were made. All stimuli were presented in randomized order.

Only the results from well isolated neurons are presented here. Spikes were amplified, time-stamped, and stored for subsequent analysis. All stimulus presentation and data acquisition was done either by a PDP 11/40 computer controlling a digital tone-synthesizer and a pair of digital attenuators, or by a Masscomp 5600 computer that generated tones or noise that was subsequently fed through the same pair of digital attenuators. All spike data points represent the average value of 5-10 repetitions of the measurement; error bars indicate the standard error of the mean.

Normal Histology:

Following physiological experiments, the owl was overdosed with pentobarbital (8cc i.m. of Nembutal 50mg/ml, Abbot Laboratories), exsanguinated with PBS (pH7.4) and fixed by transcardial perfusion with1% paraformaldehyde and 1.25% glutaraldehyde (in 0.1M phosphate buffer, pH 7.4). The fixative was cleared with ice-cold 10% sucrose in 0.1M phosphate buffer. Brains were removed, blocked stereotaxically in a transverse plane parallel to that of the electrode penetrations, sunk in 20% sucrose overnight, and cut frozen into 30μ-thick sections on a sliding microtome. Sections were stained with neutral red and/or processed for histochemical staining for acetylcholinesterase (Karnovsky and Roots 1964; Adolphs 1991) and examined for electrode tracks and lesion sites from the injection experiments.

Tracer Studies:

Five owls were used in studies of the projections from lemniscal nuclei to the inferior colliculus (see table 1). Each owl was also used in physiological experiments. All tracers were injected under physiological guidance. The lectin from *Phaseolus vulgaris* (PHA-L; Vector Labs; Gerfen and Sawchenko 1983) was simultaneously pressure injected (0.2μl) and iontophoresed (3-5μA pulsed on/off 5 seconds for a total duration of 20 minutes) as a 2.5% buffered aqueous solution; owls were subsequently perfused as above except that the fixative consisted of 4% paraformaldehyde with no glutaraldehyde.

Fluorescently labelled latex microspheres (Lumafluor, New City, NY; Katz, Burkhalter et al., 1984) were pressure injected in undiluted volumes of 0.2-0.4 μl; owls were perfused normally. Sections were subsequently examined for neurons retrogradely labelled with the microspheres under a fluorescence microscope equipped with a motorized stage and labelled neurons were manually digitized onto an image of the section.

The B-subunit of cholera toxin (List Laboratories; Ericson and Blomqvist 1988) was pressure injected as 0.2μl of a 1% buffered aqueous solution; owls were perfused normally. In the case of PHA-L and cholera toxin, sections were later processed with a secondary antibody to the tracer and then processed by the avidin-biotin ABC method (Vector Labs) in conjunction with horseradish peroxidase/ diaminobenzidine histochemistry.

Definition of terms

Interaural level difference, ILD, is defined as the sound pressure level (in dB SPL) at the contralateral ear minus the level at the ipsilateral ear, in order to have a uniform terminology for measurements on both sides of the brain.

Average binaural intensity, ABI, is the numerical average of the sound pressure level at each ear. ILD was varied in two ways. ILD at constant ABI decreased the level at one ear while increasing the level at the other ear by the same numerical amount (in dB). ILD at one ear fixed held the level at one ear constant while varying the level at the other ear; ABI also changed in this case. The ILD at constant ABI method was used unless otherwise noted.

RESULTS

1.Hodology

Table 1 lists the owls that were used for hodological experiments. Retrograde tracers were injected into the inferior colliculus, and anterograde tracers into VLVp, to study the connectivity between the two nuclei. The injection pipette was always positioned under physiological guidance. Multi-neuron responses were recorded through the injection pipette to confirm structures to be injected.

Figures 2-4 show that VLVp provides bilateral input to the lateral shell of ICc. The B-subunit of cholera toxin was injected into the lateral shell of ICc in two owls. One example of such an injection is shown in Figure 2. Retrogradely labelled cell bodies were clearly visible in the VLVp on both sides (Fig. 2B,C). Other structures that were retrogradely labelled (data not shown) were the contralateral nucleus angularis, the contralateral nucleus of the superior olive, the core of the contralateral ICc, and the ipsilateral nucleus lemnisci lateralis pars ventralis (LLv). All of these projections are consistent with previous reports (Takahashi and Konishi 1988a; Adolphs 1988; Takahashi, Wagner et al. 1989); a projection from VLVp to the IC is reported here for the first time. In both owls injected, the contralateral VLVp was labelled heavily throughout its dorso-ventral extent, while the ipsilateral VLVp showed sparser label predominantly at the dorsal pole of the nucleus (Fig. 2B, C). Frequency is mapped in the antero-posterior dimension of VLVp, and in the dorso-ventral dimension in the ICc. Label was found in restricted antero-posterior portions of VLVp that qualitatively appeared to correspond to the frequency representation that was injected in the lateral shell. Thus, the projection from VLVp to the lateral shell is bilateral and may be topographic for frequency. A few labelled cells were also seen in the

Table 4.1: **Owls used for Anatomy.**

Owl number	Tracer	Structure injected
438	cholera tx	lateral shell
395	cholera tx	lateral shell
391	red beads	lateral shell
391	green beads	medial shell
438	PHA-L	VLVp
451	PHA-L	VLVp
415	PHA-L	lateral shell

very ventral pole of the contralateral VLVa, a nucleus of the time pathway immediately adjacent to VLVp (Figs. 2, 4A).

In order to confirm these projections, the lectin tracer PHA-L was simultaneously injected and iontophoresed into the VLVp on one side (2 owls). This technique resulted in both anterograde label of terminal fields of VLVp neurons, and of weakly retrogradely labelled cell bodies that project to VLVp. Figures 5 and 6 show that VLVp projects bilaterally to the inferior colliculus. The pattern of anterograde label seen suggests that labelled terminal fields are situated in the shell of ICc. Both medial and lateral parts of the shell are labelled, but the core is not. Anterograde label was also seen in the contralateral VLVp (Fig. 6). In addition, weakly retrogradely labelled cell bodies could be identified in the contralateral VLVp (Fig.7), consistent with a recent report by Takahashi and Keller (1992) that the VLVp are reciprocally connected. The resolution of the PHA-L tracing suggests that the ventral portion of one VLVp projects to the dorsal portion of the VLVp on the other side (Fig. 8). Additional retrograde label was seen in the contralateral nucleus angularis (data not shown).

One owl received two injections of PHA-L into different regions of VLVp along the antero-posterior axis. This axis maps frequency, and the physiology of the injection sites confirmed that one, larger injection targeted the region of VLVp that maps 7.5 kHz, while the second, smaller injection targeted a region that maps 4.3 kHz. These two injection sites could, however, not be resolved in subsequent histology (Fig. 9A). Nonetheless, labelled terminal fields of VLVp form two distinct bands along the dorso-ventral dimension in IC, which maps frequency in that nucleus (Fig. 9B). The weaker label is in the band that represents lower frequencies (more dorsal), strong support for a projection from VLVp to the contralateral colliculus that preserves the mapping of frequency.

Anterograde label was also seen in the ipsilateral IC, but this projection was much weaker (Fig 9A). Only one dorso-ventral position in both the medial and lateral parts of the ipsilateral shell was labelled, qualitatively corresponding to the more heavily labelled frequency representation. It is possible that the ipsilateral terminal fields corresponding to the lower frequency injection were labelled too sparsely to be visible. Both sections shown in Figure 9 were also stained for the enzyme acetylcholinesterase as part of another study, increasing the background from which label might be distinguished. Label was also seen in the contralateral VLVp (data not shown).

Figure 4.2: **The inferior colliculus receives bilateral input from VLVp.**
A. The B-subunit of Cholera toxin was pressure injected into the right lateral shell of ICc under physiological guidance. The injection site (dark region) spans most of the dorso-ventral extent of the anterior lateral shell of ICc. IC: inferior colliculus; OT: optic tectum. **B.** Retrogradely labelled cells are seen throughout the contralateral VLVp (straight arrows). Few labelled cells were also seen in the extreme ventral pole of VLVa (curved arrows). The border between VLVa and VLVp is marked by arrowheads. **C.** Retrogradely labelled cells are seen on the ipsilateral side only in VLVp, mostly in the dorsal portion (straight arrows). Border between VLVa and VLVp: arrowheads. Scale bar: 500 μm. Owl 438.
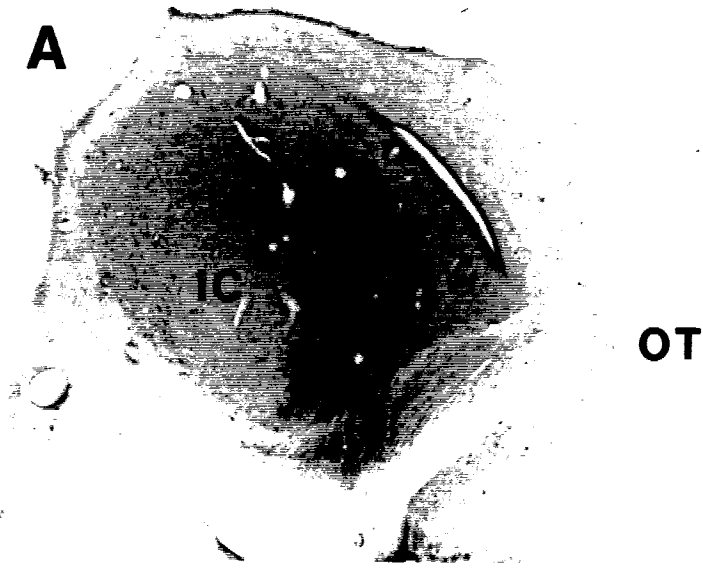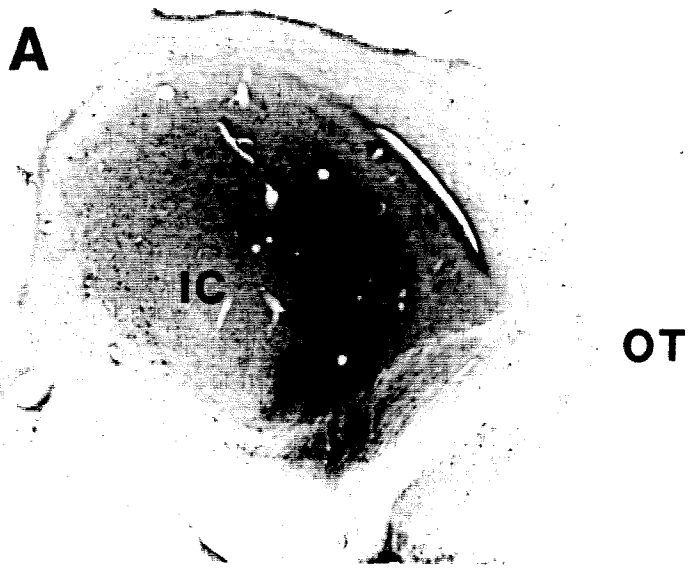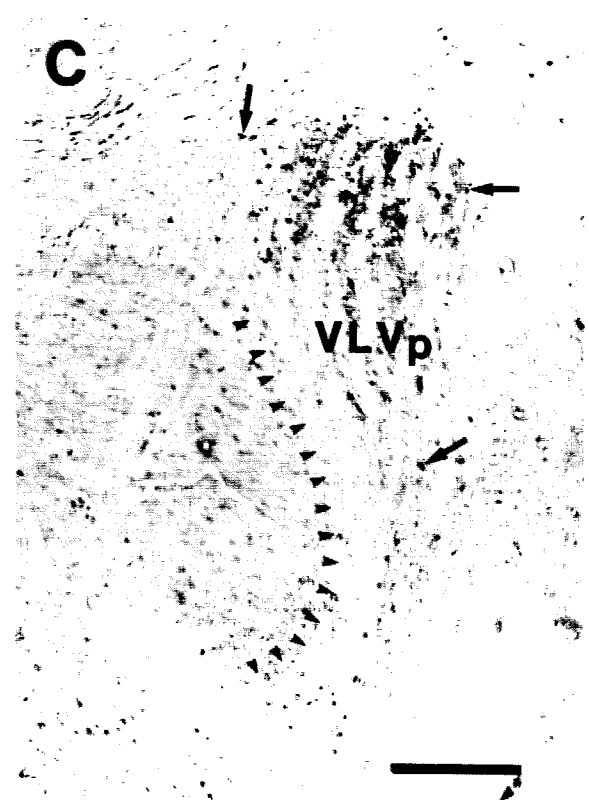
Figure 4.3: **Camera lucida reconstruction of the injection site of cholera toxin in owl 438.** For each section, the normalized antero-posterior distance from the anterior pole of IC is given (anterior pole of IC = 0.00; posterior pole of IC = 1.0). Scale bar = 1.0mm.

0.05

0.20   IC   OT

0.35

0.50   IC

0.65

0.80

0.95
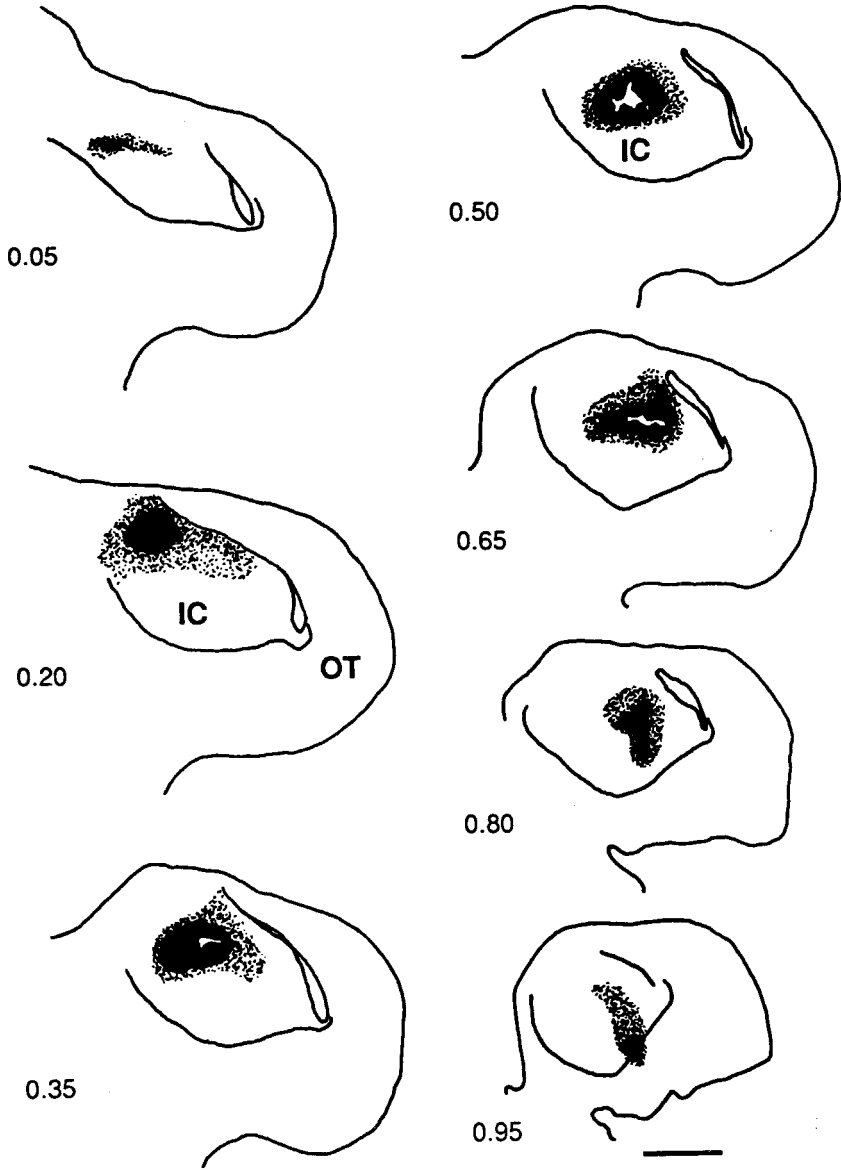
Figure 4.4: **Camera lucida reconstructions of retrograde label of cholera toxin for owl 438. A.** Retrogradely labelled cells (dots) were plotted individually in the contralateral VLVp and VLVa. These two nuclei fuse anteriorly. Numbers indicate normalized antero-posterior distance from the anterior pole of VLV. Scale bar: 1.0mm. **B.** Retrogradely labelled cells in the ipsilateral VLVp, VLVa, and LLv. No labelled cells were seen in the ipsilateral VLVa. Numbers indicate normalized antero-posterior distance from the anterior pole of VLV. Scale bar: 1.0 mm.

A.

0.08

IC

0.23

0.39   VLVp

0.54

0.69   VLVp

VLVa

0.85   VLVp

B.

0.15

0.31   VLVp

VLVa

0.46

LLv

0.62

0.77   VLVp

VLVa

LLv

0.92   VLVp
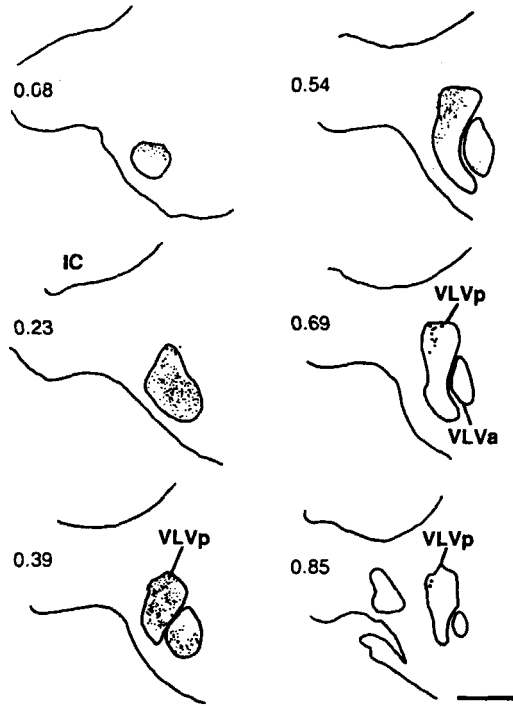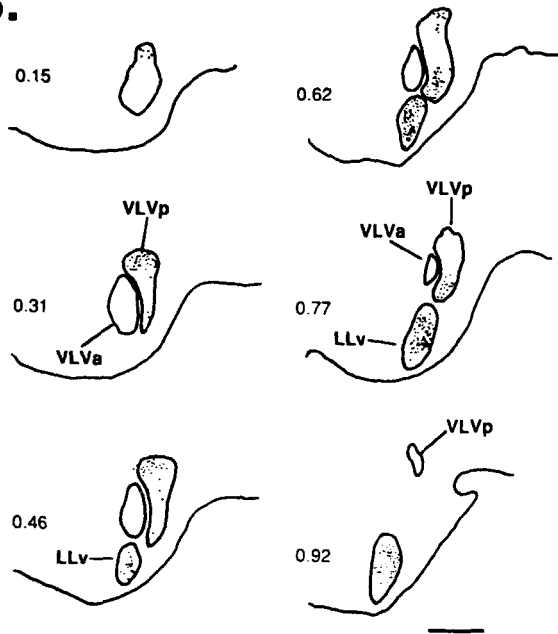
Figure 4.5: **The VLVp projects bilaterally to the inferior colliculus. A.** Anterograde label (arrowheads) is seen in the contralateral IC from an injection of PHA-L into VLVp. The pattern of label in IC suggests that the shell of ICc receives terminals from VLVp. Scale bar: 500 μm. **B.** Anterograde label (arrowheads) is seen similarly, although more faintly, in the ipsilateral IC. Scale bar: 500 μm. **C.** The injection site of PHA-L in the right VLVp is seen as the darkened region (curved arrow) and does not impinge upon VLVa (arrowheads). Scale bar: 500 μm. Owl 438.
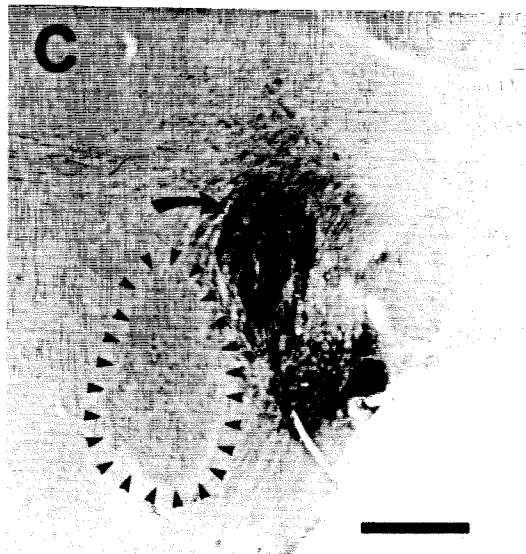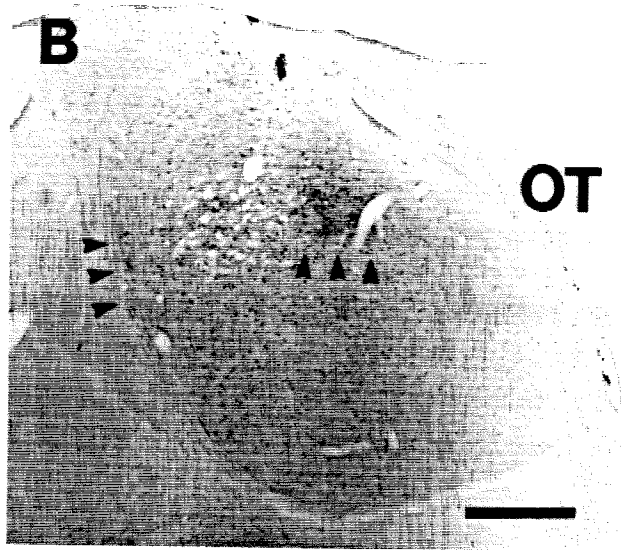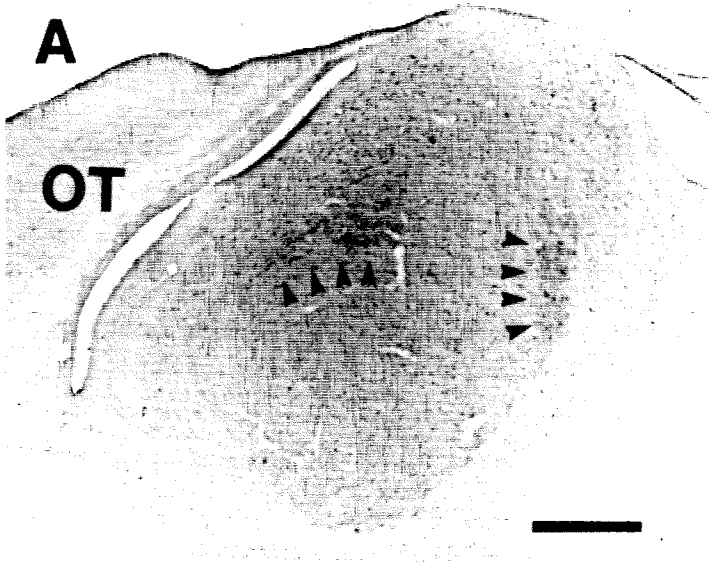
Figure 4.6: **Camera lucida reconstruction for PHA-L injection site and anterograde label for owl 438.** The right VLVp was injected; anterogradely labelled terminals are shown in stippling. Numbers indicate normalized distance from the anterior pole of IC; numbers in brackets indicate normalized distance from the anterior pole of VLV for VLVp and VLVa. Scale bar: 1.6mm.
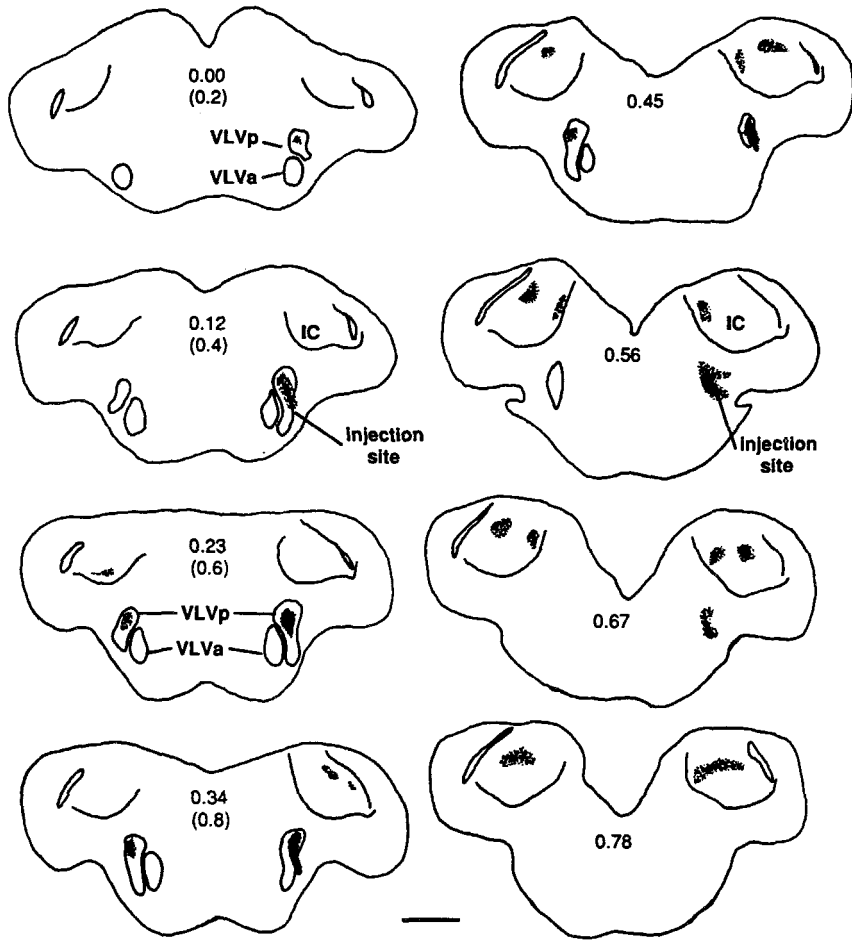
Figure 4.7: **Camera lucida reconstruction of retrogradely labelled cells in lemniscal nuclei from the PHA-L injection in owl 438.** Although weaker than the anterograde label, retrograde label could be seen in several cells (dots) in the contralateral VLVp. Numbers give normalized distance from the anterior pole of VLV. Scale bar: 1.0mm.
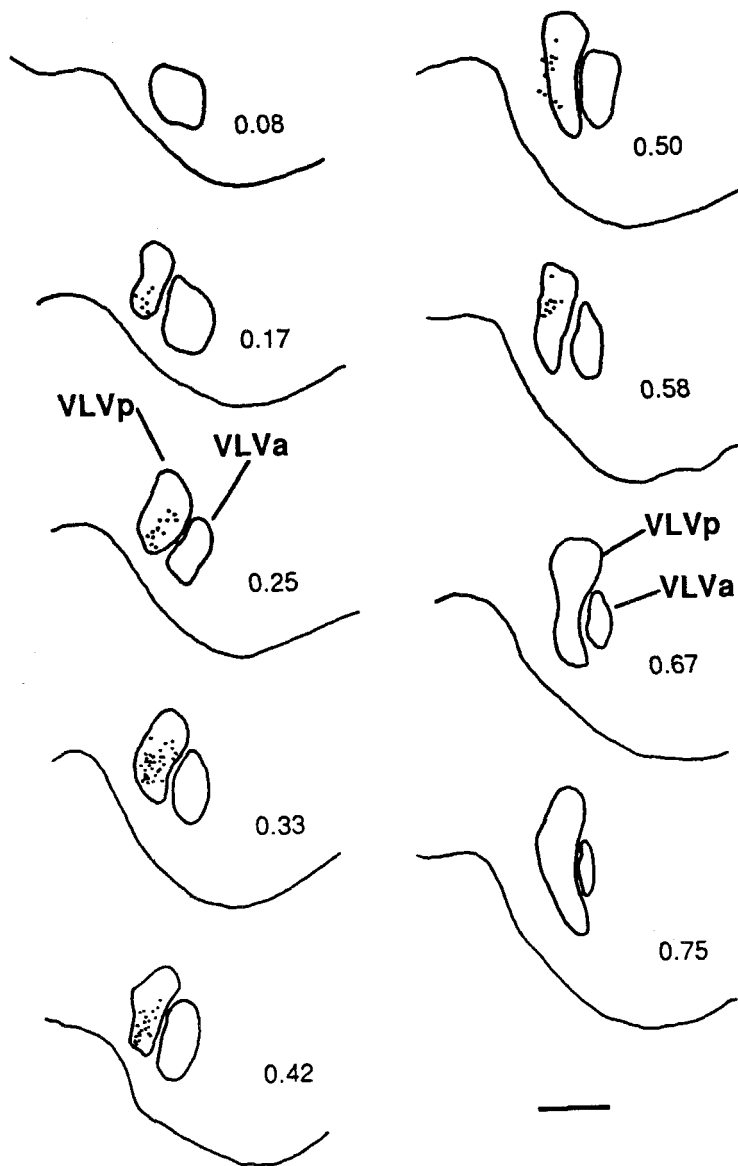
Figure 4.8: **The VLVp on each side are connected reciprocally.** From the PHA-L injection into the right VLVp in owl 438, anterograde label was seen in the dorsal region of the left VLVp (arrowheads), whereas retrogradely labelled cell bodies were seen in the ventral left VLVp (curved arrows). Scale bar: 250µm.
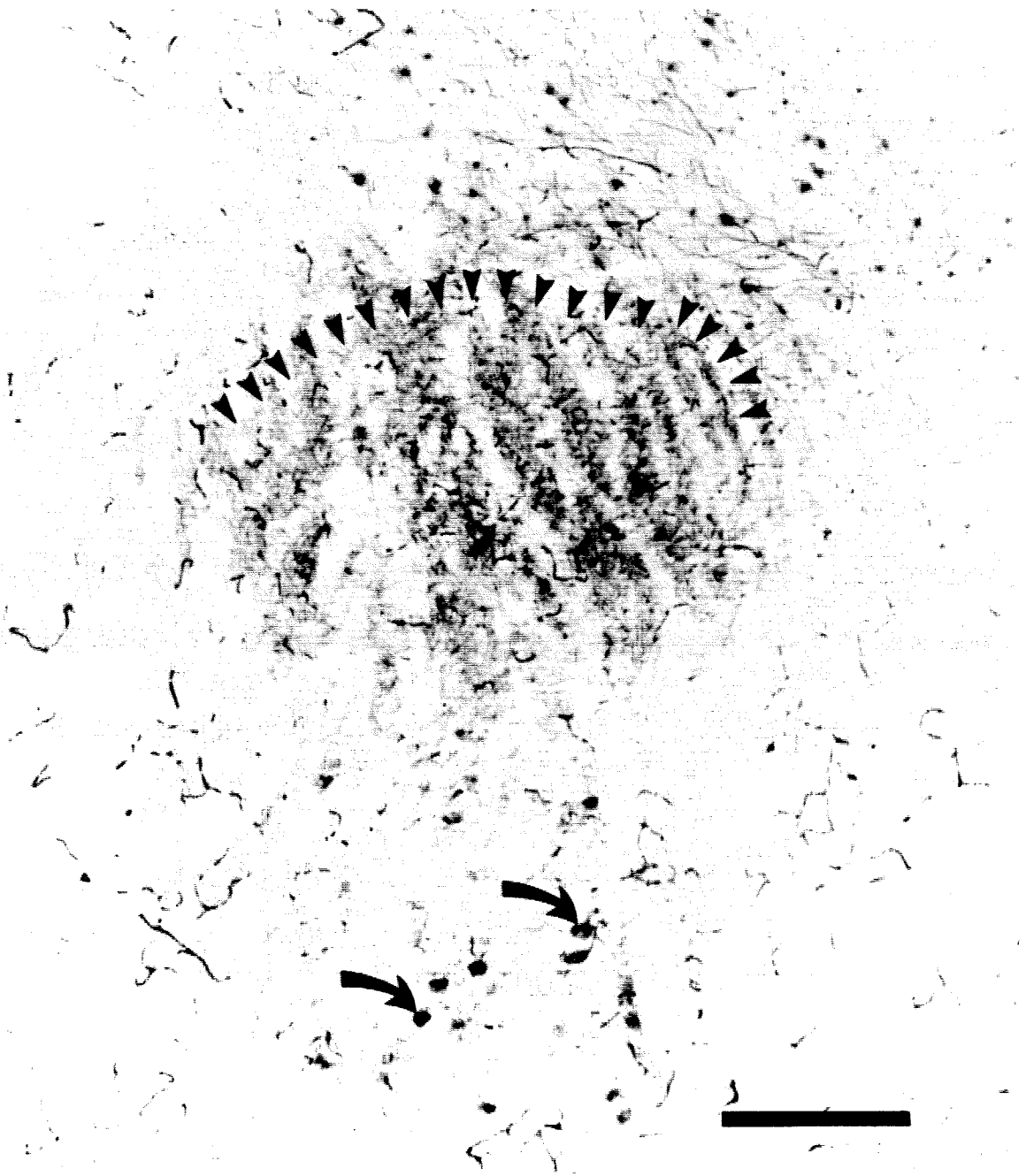
Figure 4.9: **VLVp projects bilaterally and topographically to IC.** Another example of an injection of PHA-L into the right VLVp (owl 451). **A.** PHA-L was injected at two sites in VLVp that contained neurons that responded to either very high, or to low frequencies. The injection sites cannot be distinguished histologically (large arrow; darkened region). Anterograde label was seen in the ipsilateral shell of ICc (small arrows). An injection of rhodamine-labelled beads is seen in the lateral shell (curved arrow) in this owl from another experiment. The section was also stained for acetylcholinesterase, resulting in other darkened structures in this black-and-white photomicrograph. **B.** Anterograde label is seen in the contralateral ICc in two distinct places (arrows), corresponding to the two regions of different frequency representation that were injected in VLVp. Frequency is mapped dorso-ventrally in IC. Scale bar: 750μm.
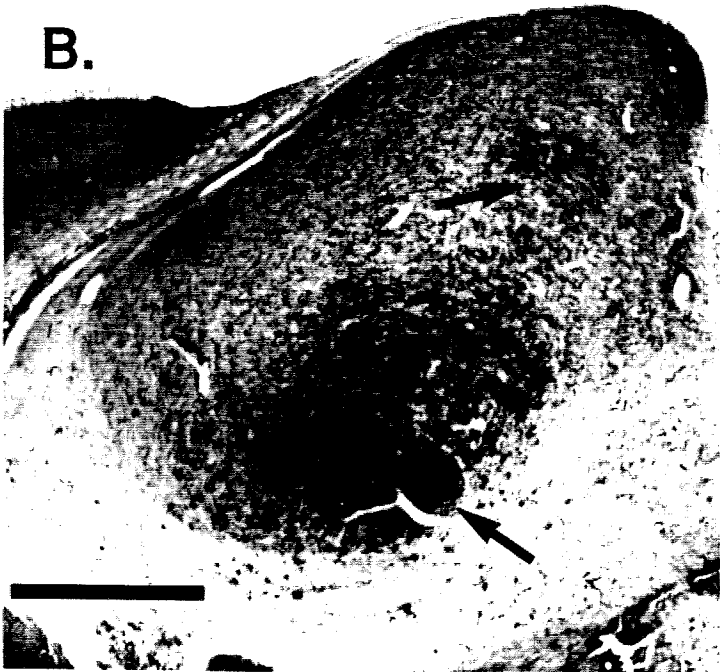
One owl received an injection of PHA-L into the lateral shell of ICc on one side. Anterogradely labelled fibers with terminals were seen as a prominent band that appeared to course along the iso-frequency dimension of the section laterally towards ICx (Fig. 10A, 11). Labelled terminals could be seen in ICx (Fig. 10B), confirming previous reports that the ICc provides input to ICx (Knudsen 1983). Retrogradely labelled cells were seen clearly in the contralateral VLV (Fig. 12). Since the injection site was at a very dorsal (low-frequency) position (Fig. 10A), the retrogradely labelled region of VLV was at the very anterior pole of the nucleus, where VLVa and VLVp are fused (Takahashi and Konishi 1988b), and where low frequencies are represented. This hodology again suggests that the projection from VLVp to IC preserves tonotopy. Since PHA-L was transported both anterogradely and retrogradely, this experiment shows that VLVp provides polysynaptic input to the map of auditory space in the ICx.

One owl received injections of fluorescently labelled latex beads into medial and lateral parts of the shell of ICc. Figure 13 shows the injections and a schematic of the retrogradely labelled cells that were scored in one section near the antero-posterior middle of VLVp. The data show that the lateral shell receives bilateral input from VLVp. The injection into the medial shell labelled only cells in the contralateral VLVp. No cells in VLVa were labelled. This experiment also addressed the question of where an individual cell in the VLVp projects. Figure 14 shows double-labelled cells in the contralateral VLVp. Thus, at least some cells in the VLVp project to both the medial and lateral parts of the contralateral shell.

From both the anterograde results with PHA-L (Figs.5,6), and the retrograde tracing with fluorescent beads (Fig. 13) it appears that medial and lateral parts of the shell receive equally strong projections from the contralateral VLVp, but that the ipsilateral projections are much stronger to the lateral shell than to the medial shell.

Figure 4.10: **The lateral shell of ICc projects to the ipsilateral ICx. A.** PHA-L was injected into the dorsal lateral shell of ICc under physiological guidance (straight arrow). A band of labelled fibers courses laterally to ICx. Curved arrow: same blood vessel as in B. Scale bar: 400μm. **B.** High-power photomicrograph of labelled terminals in the ICx (arrowheads). Scale bar: 50μm. Owl 415.
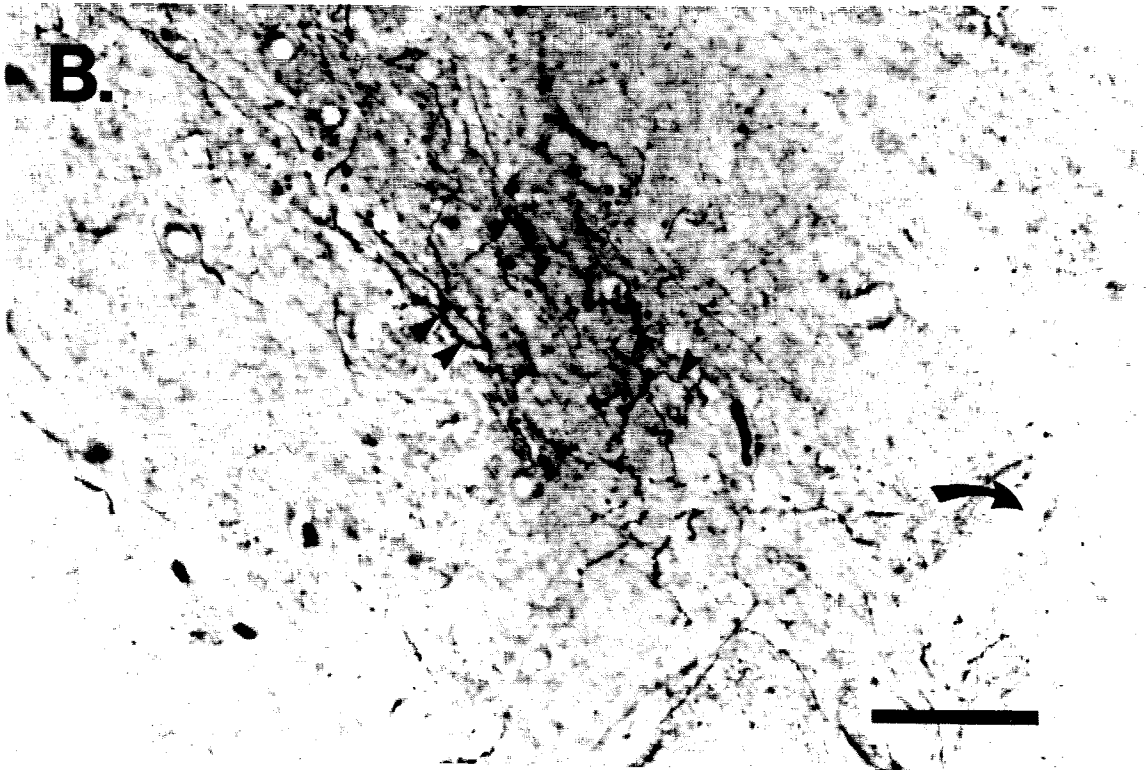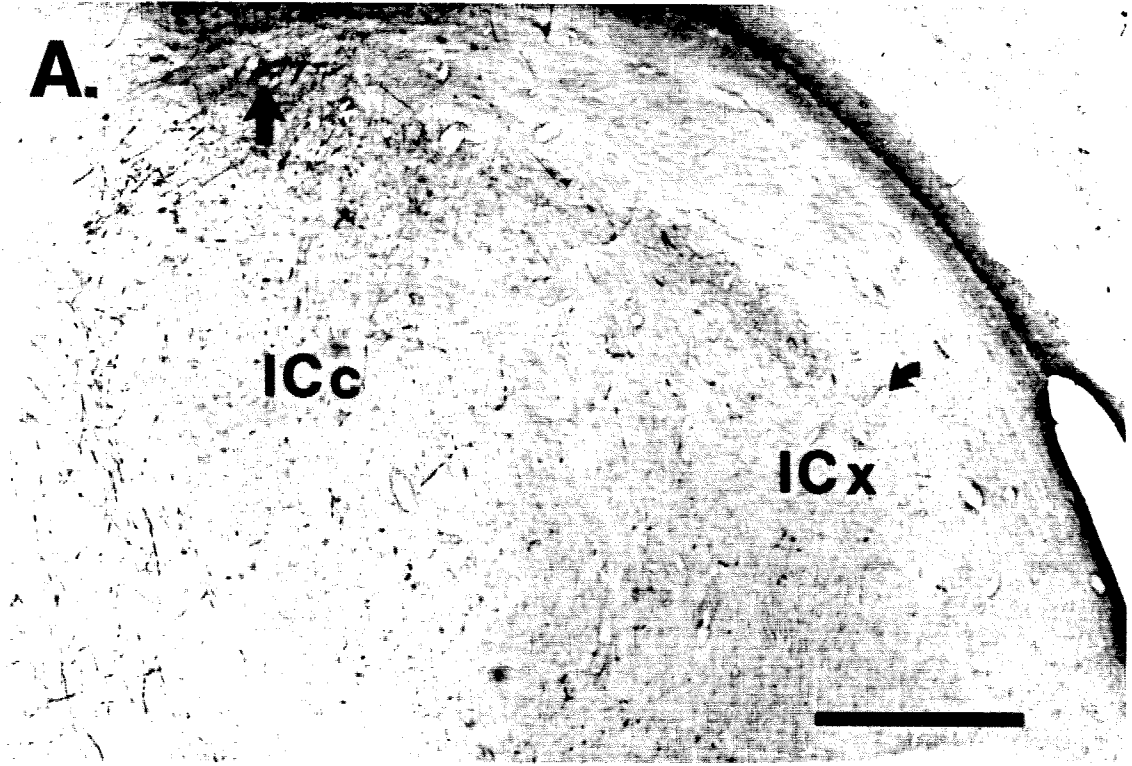
Figure 4.11: **Camera lucida reconstruction of injection site and anterograde label in IC from injection of PHA-L (owl 415).** Anterograde label is seen in the ICx from the injection in the lateral shell on the same side. Some retrogradely labelled fibers that course towards VLVp are also seen. Numbers give normalized distance from the anterior pole of IC. Scale bar: 1.0mm.

0.23

0.54

0.31

0.62

VLVa

0.38   IC

OT

0.69

VLVp

IC

0.46

0.90
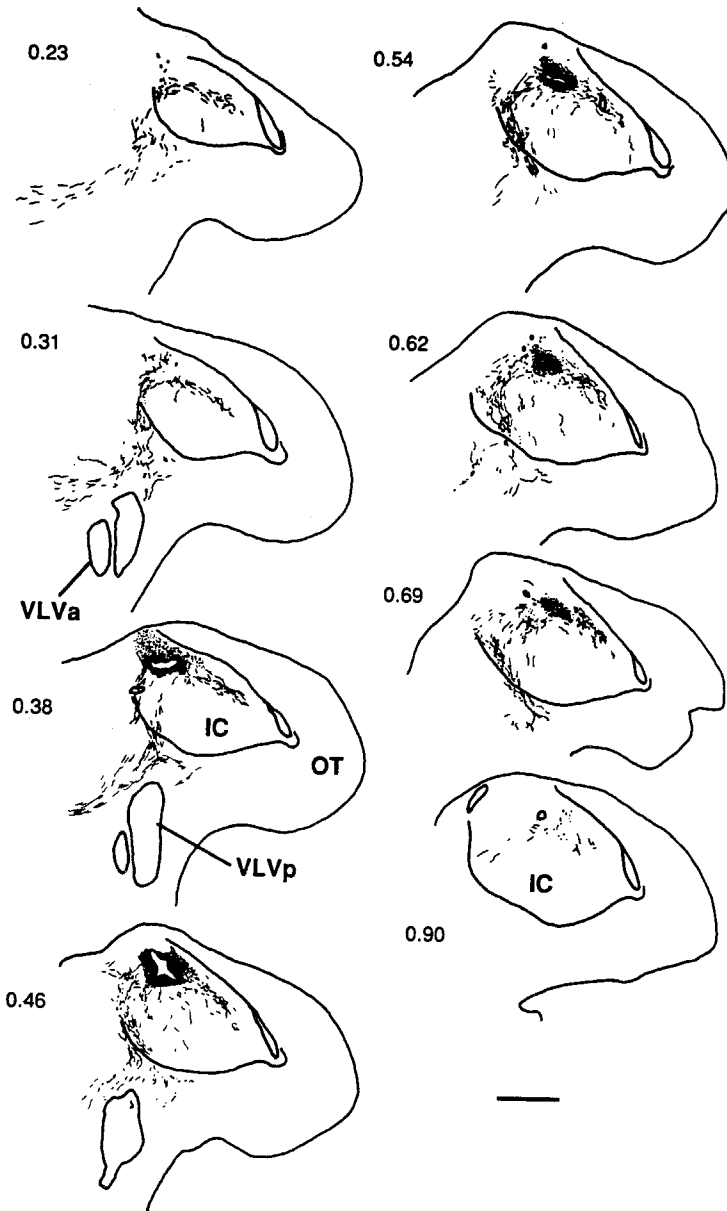
Figure 4.12: **The ICx receives polysynaptic input from the contralateral VLVp.** Retrograde label was seen in VLVp after the PHA-L injection in the lateral shell of ICc in owl 415. **A.** The very anterior pole of the contralateral VLV contains retrogradely labelled cells. The dorsal IC, where the injection site was, and the anterior VLV both map low frequencies, suggesting that the projection is topographic. Arrowhead: same blood vessel as in B. Scale bar: 1.0mm. **B.** High-power photomicrograph of VLV showing labelled cells (arrows). Scale bar: 150μm. **C.** Camera lucida reconstruction showing labelled cells in the VLVp and VLVa contralateral to the injection site. Label in the ipsilateral VLVp was not intense enough to unequivocally assign it significance. Numbers give normalized distance from the anterior pole of VLV. Scale bar: 1.0mm.

VLVp

VLVa

0.00

0.09

0.18

0.27

0.36

0.45
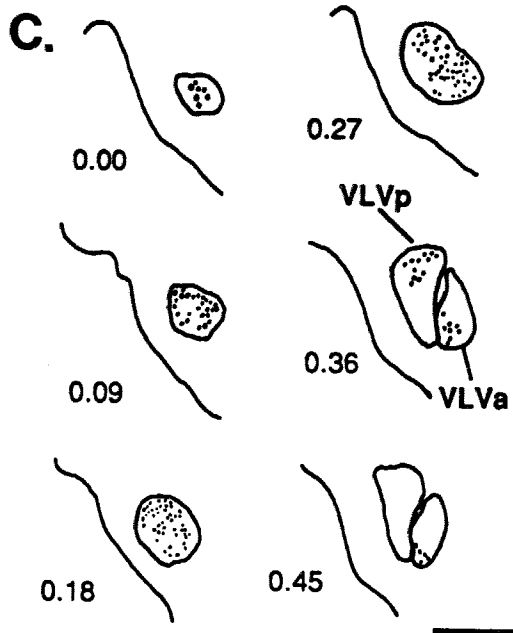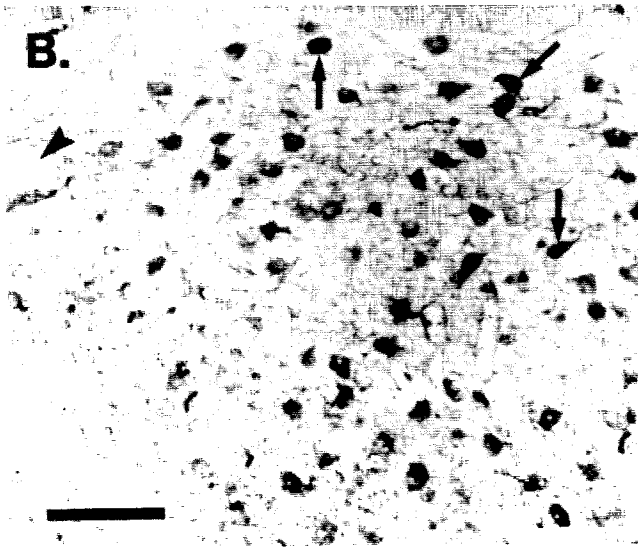
Figure 4.13: **The medial and lateral parts of the shell both receive inputs from VLVp.** **Top:** Fluorescein-labelled latex beads were injected into the medial shell (curved arrow) and rhodamine labelled beads were injected into the lateral shell on the same side (straight arrow). This epifluorescence photomicrograph shows the two injection sites in the right IC. **Bottom:** Retrogradely labelled cells were scored under epifluorescent illumination using a 60x objective with oil-immersion. Positions of cells were digitized using a motorized stage and custom software. The two sections through the antero-posterior middle of VLVp show retrogradely labelled cells in both the left (**left**) and right (**right**) VLVp. Solid dots denote rhodamine-labelled cells; open circles fluorescein-labelled cells. The left VLVp section has two cells that are double-labelled. Scale bar: 1.0mm. Owl 391.
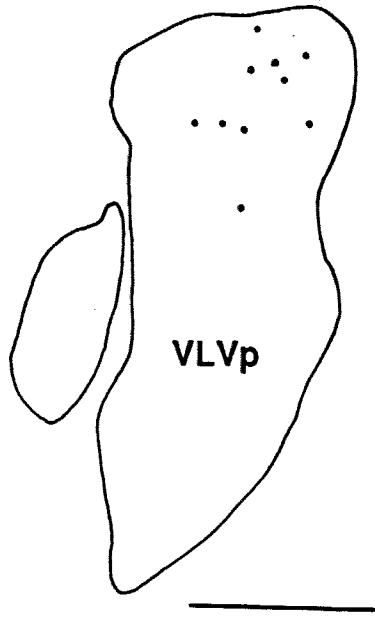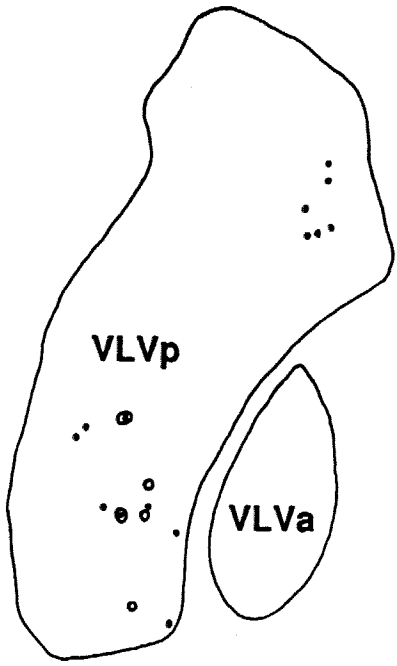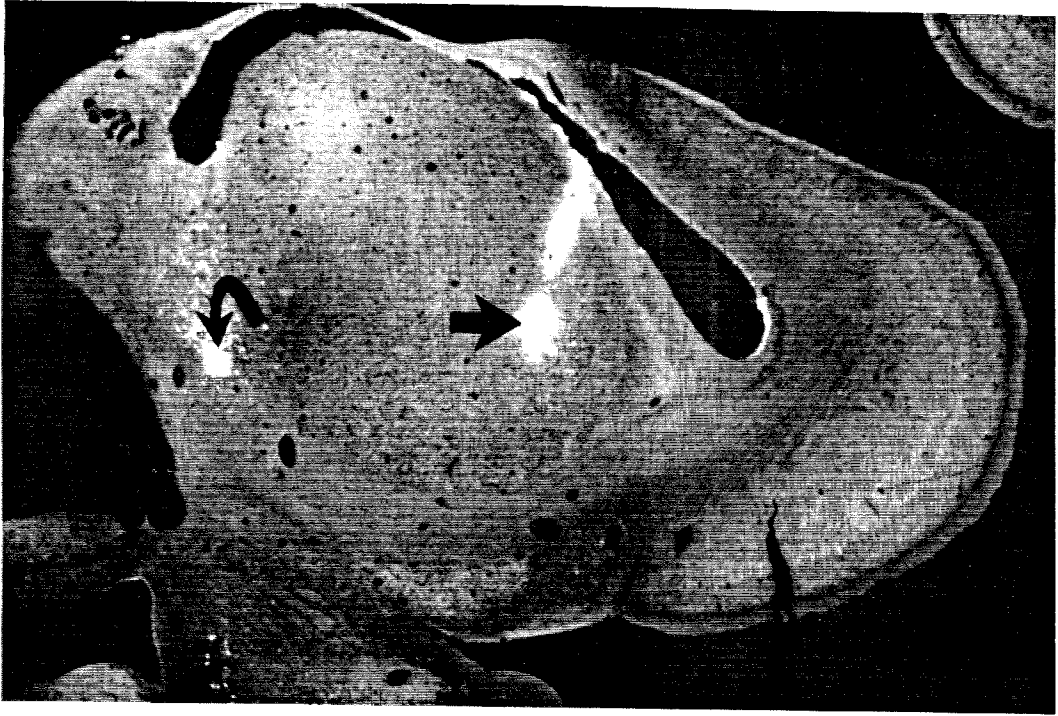
Figure 4.14: **Double-labelled cells in the contralateral VLVp.** Retrograde label of fluorescent beads was seen in cells of VLVp. Some cells in the contralateral VLVp (small arrows) were labelled both with rhodamine beads **(top)** and with fluorescein beads **(bottom)** in these photomicrographs taken under epifluorescence with filters specific to the fluorophore. Large arrowheads mark the same bloodvessel. Scale bar: 100μm. Owl 391.
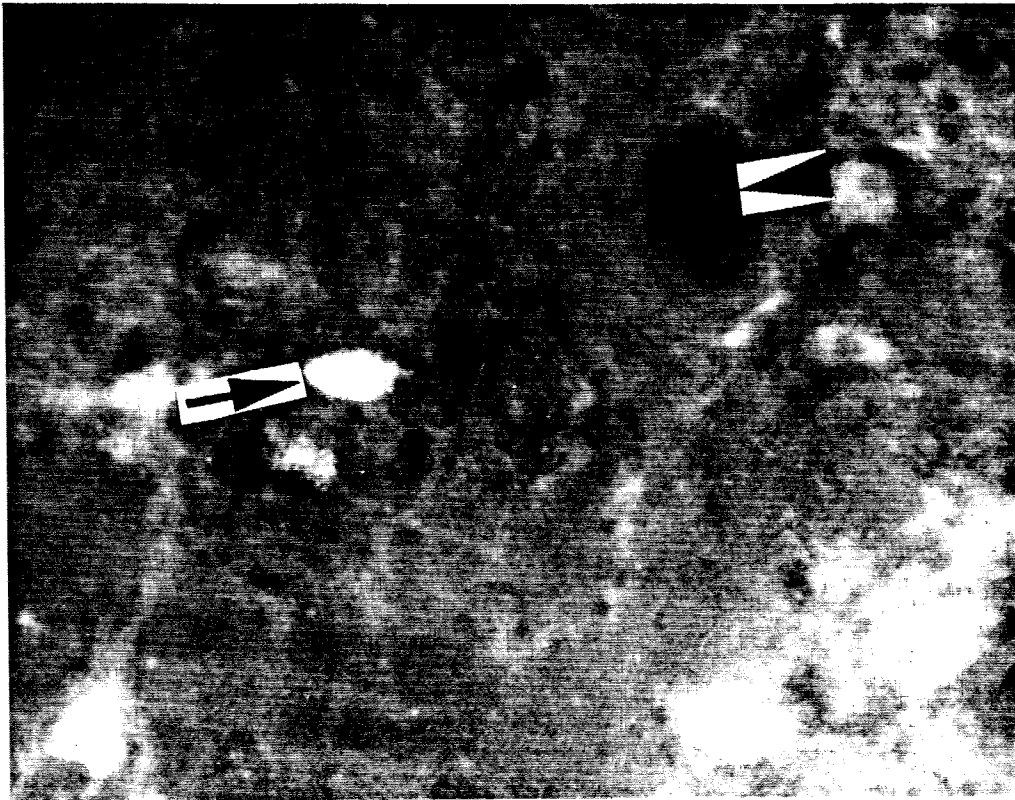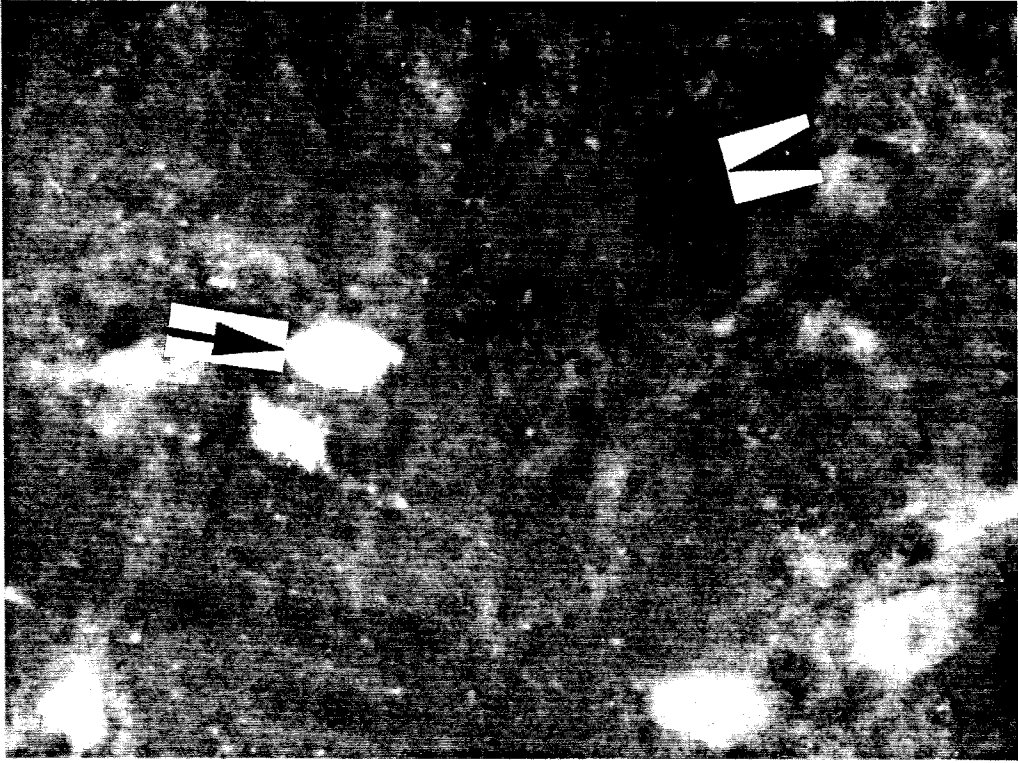
2. Responses in VLVp.

(i) VLVp Cells are Affected by Iontophoresis of GABA-A Receptor Agonists or Blockers.

Table 2 lists all owls used for the physiological studies reported here.

I first investigated the responses of cells in VLVp to iontophoretically applied GABA, and to the GABA-A receptor blocker bicuculline methiodide (BMI). Figure 15A shows an increased response to ILD as a function of time during and after ("post") BMI iontophoresis. Out of 32 neurons tested in 4 owls, 25 showed a reversible increase in firing rate with BMI application; the remaining cells showed no alteration in response properties. Iontophoresis of GABA either had no effect (5 cells) or decreased the firing rate of neurons (5 cells; 4 owls; Figure 15B). In all cases where these drugs had any effect at all, BMI increased the firing rate, while GABA decreased it. These effects were seen at all ILDs.

Controls were run by iontophoresing isotonic saline at currents and pH matched to that of the drugs that were iontophoresed; survey studies with other drugs (other inhibitory transmitters or their antagonists (data not shown): the muscarinic cholinergic antagonist atropine (10 cells), the transmitter glycine (2 cells), and its antagonist strychnine (17 cells), as well as the putative transmitter beta-alanine (7 cells)) served as controls as well. In none of these cases were any significant effects seen on VLVp cells.

These results indicate that at least some of the neurons in VLVp have GABA-A receptors, and that the magnitude of their evoked response can be increased by BMI, or decreased by GABA. This finding provided the basis for the experiments below in which these drugs were injected globally into VLVp in order to modulate the gain of the population response.

An additional drug used was the potent GABA-A agonist muscimol. Iontophoretic application of muscimol to VLVp cells resulted in a complete abolition of spontaneous and evoked firing (data not shown). Furthermore, this effect was irreversible on the time scale of an hour. To further test the efficacy of muscimol in blocking activity within VLVp, the following experiment was undertaken.

Table 42: **Owls used for Physiology.**

Owl #	Experiments
195	Iontophoresis in Lateral
203	Shell
299	
391	
203	Iontophoresis in VLVp:
386	BMI
715	
391	
715	
203	GABA
386	
391	
357	Muscimol
429	Inject muscimol VLVp; record contra VLVp
203	Inject VLVp; record contra
300	ICx: Lidocaine
398	
203	
357	BMI
424	GABA
357	Muscimol
195	Inject VLVp; record ipsi
299	ICx: Lidocaine
299	BMI
299	GABA
424	
424	Inject VLVp bilaterally;
451	record ICx
195	Inject Lateral Lemniscus;
250	record IC
300	

Figure 4.15: **Responses of cells in VLVp to BMI or GABA.** These surface graphs plot ILD versus response versus time. The **x-y plane** plots ILD in dB SPL (with negative values denoting ipsilaterally louder and positive values denoting contralaterally louder ILDs) versus minutes of drug iontophoresis. The **z-axis** measures mean spikes recorded from the cell per 100 msec of stimulus presentation of white noise. Each intersection in the surface grid represents a data point. Initial time values are minutes of continuous iontophoresis of drug; times marked "POST" measure time after cessation of iontophoresis. **A. Response to BMI. B. Response to GABA.**

A. 203/B03C

B. 203/B03
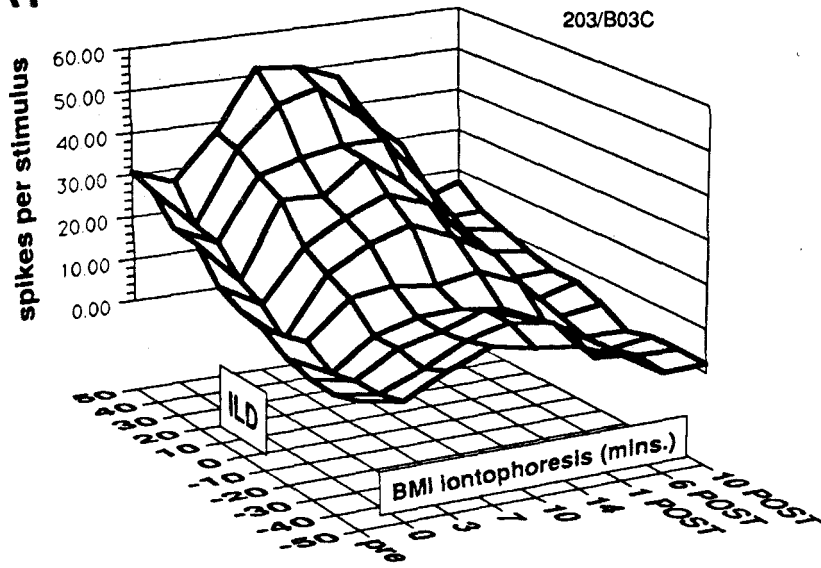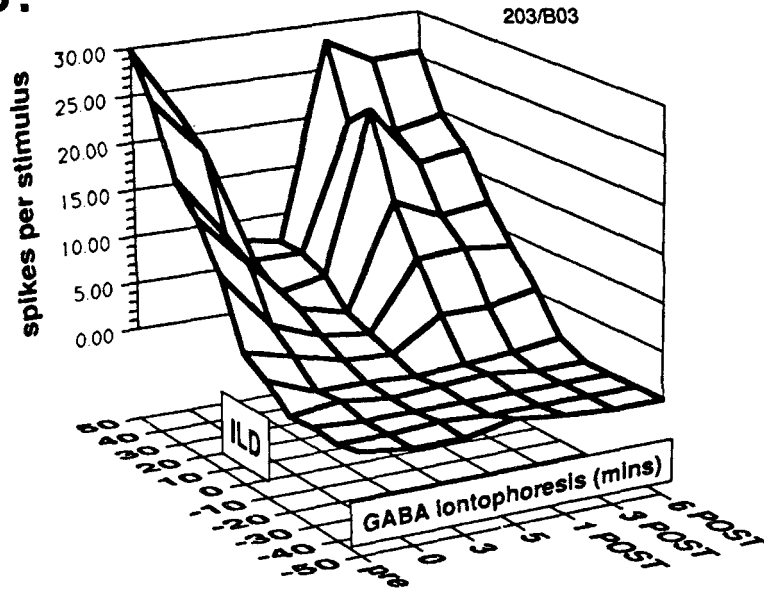
(ii) The Two VLVp Inhibit Each Other.

Muscimol was pressure injected into the VLVp on one side, while recording the response to interaural level differences in the VLVp on the opposite side. Figure 16 shows the response curve of a single neuron in the VLVp opposite to the injected side. Initially, the neuron is excited by sound at the contralateral ear, and inhibited strongly as sound level at the ipsilateral ear is increased; this response is typical of neurons in the dorsal regions of VLVp (Manley, Koeppl et al. 1988). Takahashi and Keller (1992) showed that the inhibition is due to the projection from the contralateral VLVp. After injecting muscimol into the opposite VLVp, the neuron is excited uniformly at all ILD, irrespective of the level of sounds at the ipsilateral ear (Fig. 16). This result shows that VLVp is effectively functionally silenced with muscimol injections, such that it cannot provide inhibition to the VLVp on the opposite side. The population response in the injected VLVp, recorded through the injection syringe, also showed that all activity was abolished in the nucleus when muscimol was injected. Again, these effects were irreversible on the time scale of an hour, probably due to the potency of muscimol at the GABA-A receptor.

When each of the 4 drugs (lidocaine, GABA, BMI or muscimol) were pressure injected into VLVp (see experiments below), the response of a population of neurons in VLVp near the injection site could be recorded through the syringe with which the drugs were injected. In all cases, the population responses thus recorded corroborated the iontophoretic results reported above.

Figure 4.16: **The VLVp provide reciprocal inhibition to each other.** Response of a neuron in VLVp to interaural level differences of a 5.0 kHz tone. The sound pressure at the contralateral ear was held constant at 40 dB while the ipsilateral sound level was increased from 0 to 60 dB SPL along the abscissa (corresponding to an ILD range from 40 to -20). The ordinate plots mean spikes per stimulus presentation before ("pre") and after ("post") injecting muscimol into the VLVp on the side opposite to the VLVp which was being recorded from. The muscimol injection caused an irreversible block of inhibition by sounds loud at the ipsilateral ear, but left the amount of excitation supplied by the contralateral ear unaffected.
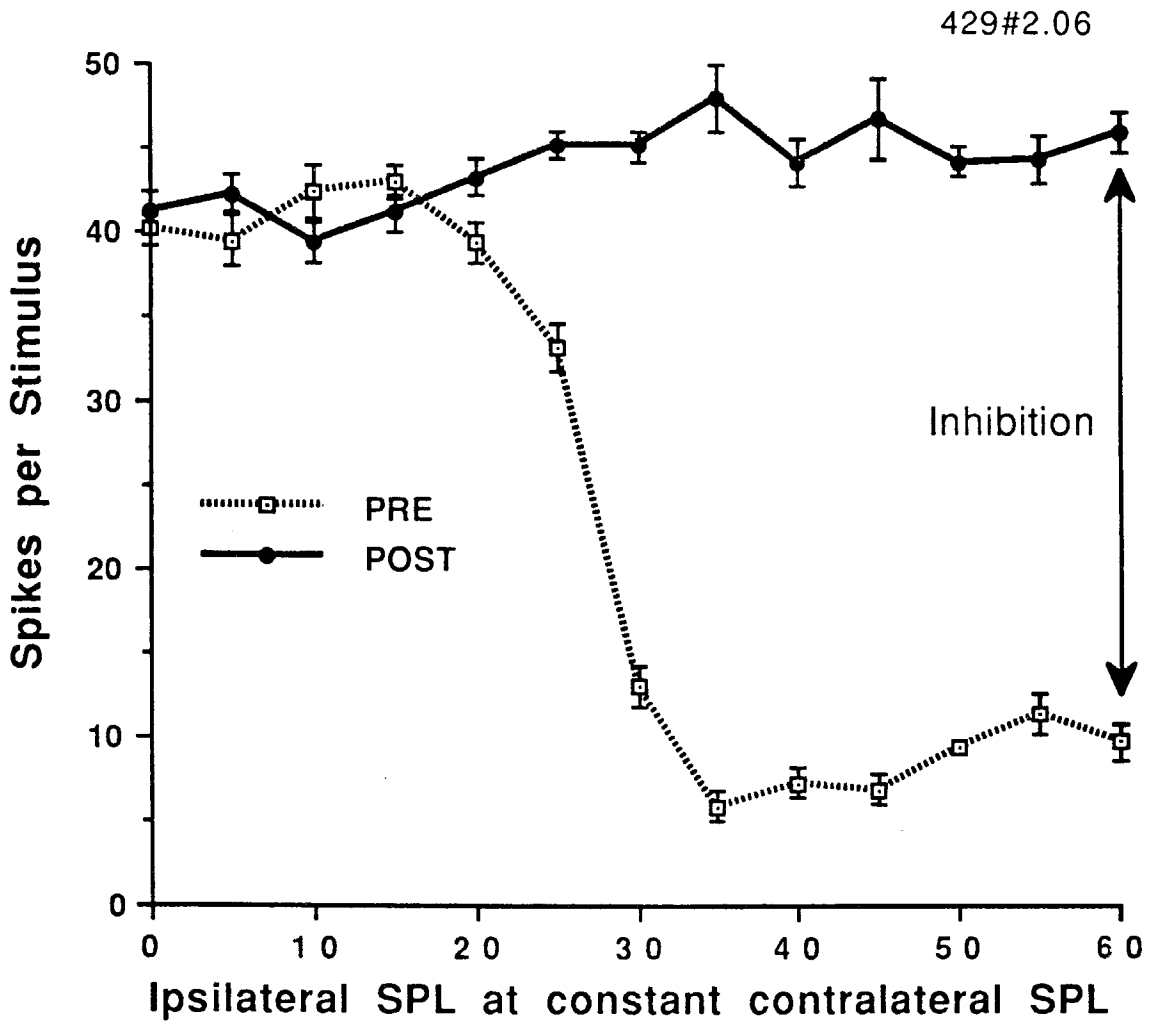
3. How Responses Tuned to ILD are Generated

(i) Physiology of Cells in the Inferior Colliculus

The responses of cells in the subdivisions of the inferior colliculus have been studied extensively by several investigators. I here concern myself only with the responses of cells in the lateral shell of ICc, and in the immediately adjacent ICx. Figure 17 shows typical responses to ILD in the lateral shell, and in the ICx. Cells in the ICx show sharp tuning to ILD, consistent with their role in representing spatially restricted sounds. Neurons in the lateral shell show more heterogeneous responses to ILD, ranging from monotonic responses, similar to those seen in the VLVp, to non-monotonic responses that resemble those of cells in the ICx.

What shapes the responses to ILD in the lateral shell? Answers to this question should shed light on the synthesis of responses in the ICx, to which the lateral shell provides auditory input. Figure 18 shows that some of the inhibition of response seen at certain ILD values in the lateral shell is GABA-ergic. All lateral shell cells are inhibited by sounds that are loud at the ipsilateral ear; some cells in the lateral shell are also strongly inhibited at contralaterally loud ILDs. Figure 18A shows that contralateral inhibition can be partially blocked by iontophoretic application of BMI. In some cells, inhibition at both extremes of ILD can be blocked with BMI iontophoresis (Fig.18B-D). Out of 22 cells recorded in the lateral shell of 4 owls, 20 cells showed a clear effect of block of inhibition by BMI iontophoresis. This suggests that lateral shell cells may receive a GABA-ergic input that provides strongest inhibition at one or both extremes of the ILD range.

Which structures might provide this inhibitory input to the lateral shell? Based on the hodology shown above, VLVp is a candidate for providing this

input.   I addressed this question by injecting drugs globally into VLVp while recording responses to ILD in the inferior colliculus.

Figure 4.17: **Typical responses to ILD of cells in the lateral shell and in the ICx.** Some cells in the lateral shell show monotonic ILD-response curves (**top**); they are excited by the contralateral ear and inhibited by the ipsilateral ear. Additionally, many cells in the lateral shell show response curves that are non-monotonic (**middle**) and approach the symmetrical shape of response functions typically seen in ICx (**bottom**). All lateral shell neurons are inhibited by sounds loud at the ipsilateral ear, but the degree to which they are inhibited at large contralateral ILDs varies. The stimulus was pseudo-random white noise in all three cases.

Lateral Shell

391/30za00

Spikes per Stimulus

ILD at ABI=35 dB

Lateral Shell

299/994f

Spikes per Stimulus

ILD at ABI=45dB

ICx

391/396B

Spikes per Stimulus

ILD at ABI=32
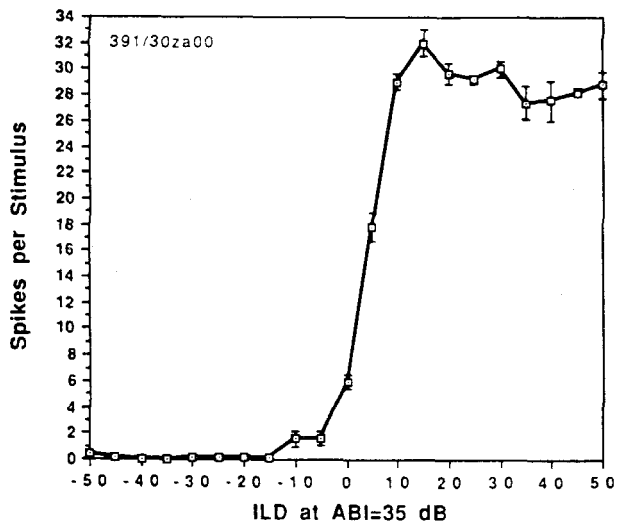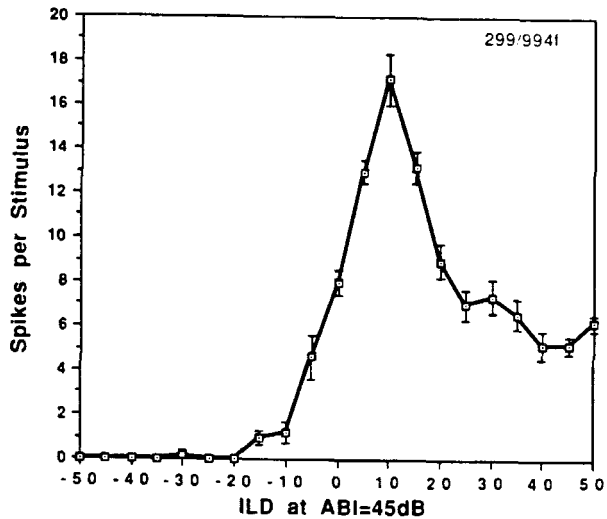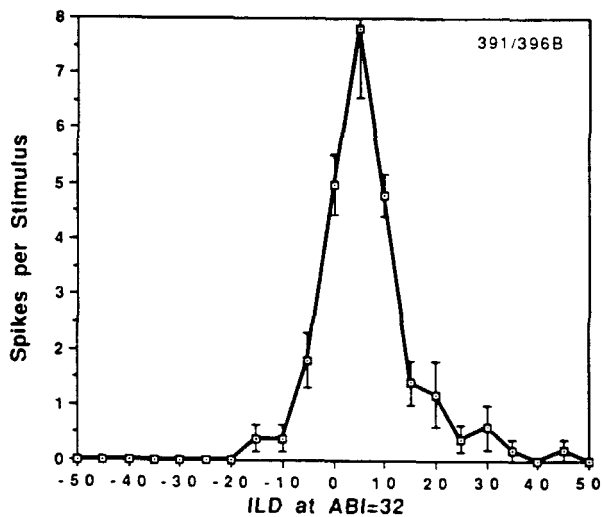
Figure 4.18: **Iontophoresis of BMI in the Lateral Shell. A:** BMI partially blocks the inhibition seen at large contralateral ILDs in this lateral shell cell. **B:** BMI blocks inhibition at both large contralateral and large ipsilateral ILDs in this cell. The ordinate plots the normalized spike count before ("pre") and after 10 minutes of continuous BMI iontophoresis ("BMI"). **C:** For the same neuron as in B., this surface plot shows how the ILD-response function varies with ABI (from 40 to 50 dB). The response of the neuron before BMI iontophoresis is shown. The surface was smoothed with a 4th-order spline for easier visualization. **D.** After 10 minutes of continuous BMI iontophoresis, this surface plot for the same cell shows that there are now significant monaural responses. At low ABI, the monaural response is greater than the binaural response. Stimulus was pseudorandom white noise in all cases.

A.

B.

C.

D.
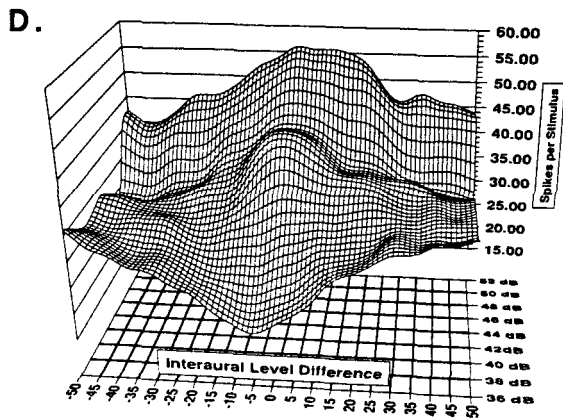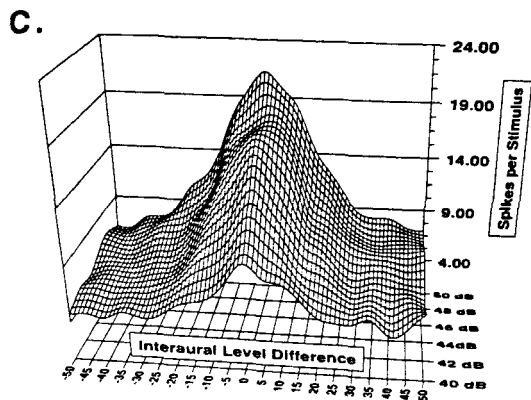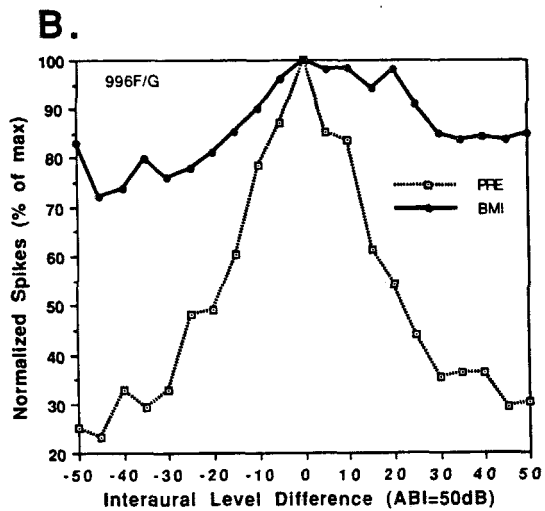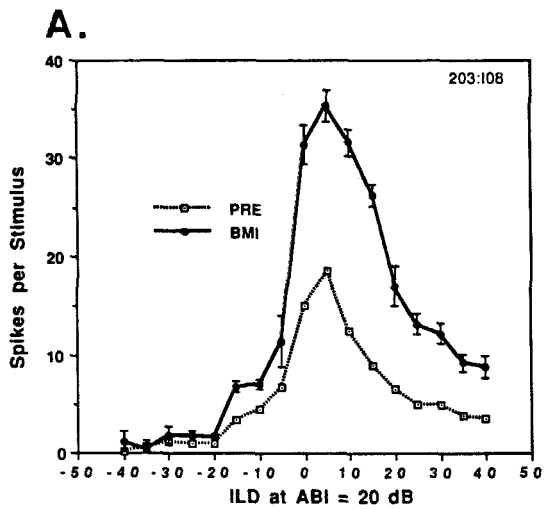
(ii) Manipulating Responses in one VLVp Affects Responses in ICx.

(a) The response in VLVp affects responses in the contralateral ICx.

The VLVp was injected with three separate drugs: lidocaine, GABA, or BMI. In each case, the drug was pressure injected into the nucleus while recording from a single cell in the contralateral ICx. Responses recorded through the injection syringe in VLVp showed a marked attenuation or abolition of activity in that nucleus with lidocaine and GABA, and an increase in activity with BMI. Figure 19A-B shows the responses to ILD of cells in the ICx before, and after injection of lidocaine or GABA into the contralateral VLVp. The effect obtained with GABA or lidocaine was similar. The time course of the effect was of shortest duration with GABA (about 10 minutes). The effect with lidocaine lasted about 15-20 minutes, in agreement with the time courses of other reports (Takahashi, Moiseff and Konishi 1984; Takahashi and Keller 1992). With both drugs, the change in response of the ICx cell was consistent with a decreased inhibition by sounds loud at the ipsilateral ear (Fig. 19A-B).

What happens when the response in VLVp is potentiated instead of attenuated? Figure 19C shows the result from a cell in ICx before, and after injecting BMI globally into the contralateral VLVp. Recordings through the syringe in VLVp confirmed that the population response in VLVp was increased at all ILDs. The effect on the response function of the ICx neuron was qualitatively the converse of that seen with the injections of the inhibitors of neural activity presented above. The inhibition by sounds loud at the contralateral ear was diminished this time. I also observed a variable increase in the inhibition by sounds loud at the ipsilateral ear. The ICx neuron's

selectivity for interaural time differences was also recorded during all these experiments, and remained unchanged (Fig.19).

The result with injection of BMI into the contralateral VLVp (Fig. 19C) can be explained by the connection between the two VLVp. Increasing the neural activity in one VLVp will increase the inhibition it provides to the VLVp on the other side. Thus, increasing the activity of the VLVp contralateral to the ICx recorded from would also decrease the activity in the VLVp ipsilateral to the ICx. If the ipsilateral VLVp, like the contralateral VLVp, provides polysynaptic inhibition to the ICx, the decrease of inhibition by sounds at the ear opposite the ICx could be explained.

Figure 4.19: **Manipulating activity in the contralateral VLVp affects responses in the ICx.** **A:** Lidocaine was injected into one VLVp and the response to ILD was recorded from a single neuron in the contralateral ICx. **Upper Left:** responses to ILD before ("PRE") and 7 minutes after injecting the lidocaine ("t=7mins.") are shown: there is an increase in the response at large ipsilateral ILD. **Upper Right:** the response recovers to the pre-injection case after 25 minutes. **Lower Left:** A surface plot showing the temporal evolution of this effect for the same experiment. The surface was smoothed with a cubic spline for better visualization. **Lower Right:** Response of the neuron to ITD during the experiment was unaffected. ITD was presented at the optimal ILD of the cell prior to injection. Stimulus was pseudorandom white noise in all cases. **B:** GABA was injected into VLVp and the response in the contralateral ICx was recorded. **Upper Left:** response of the ICx neuron before, and 1 minute after, the GABA injection: there is again an increase in the response at large ipsilateral ILD. **Upper Right:** The effect slowly recovers after 32 minutes. **Lower Left:** A surface plot for the same experiment, showing the detailed temporal evolution of the effect with GABA. The surface was smoothed with a 4th-order spline. **Lower Right:** Response to ITD at the neuron's optimal pre-injection ILD was unaffected. Stimulus was 4 kHz tone in all cases. **C:** BMI was injected into the contralateral VLVp while recording in the ICx. **Upper Left:** Response of a single neuron in ICx before, and 3 minutes after injecting BMI. There is an increase in the response to contralaterally loud ILD, the reverse of the effects seen with lidocaine and GABA. **Upper Right:** The effect recovers after 50 minutes. **Lower Left:** Surface plot of the same experiment showing temporal evolution; the surface was smoothed with a 4th-order spline. **Lower Right:** Response to ITD at the neuron's optimal pre-injection ILD was invariant. The stimulus was pseudorandom white noise in all cases.

A. Lidocaine
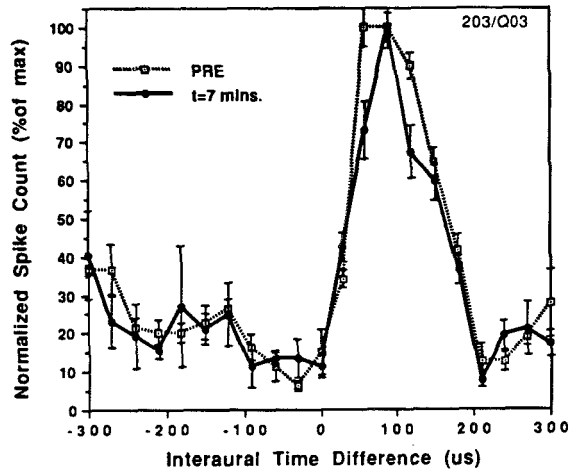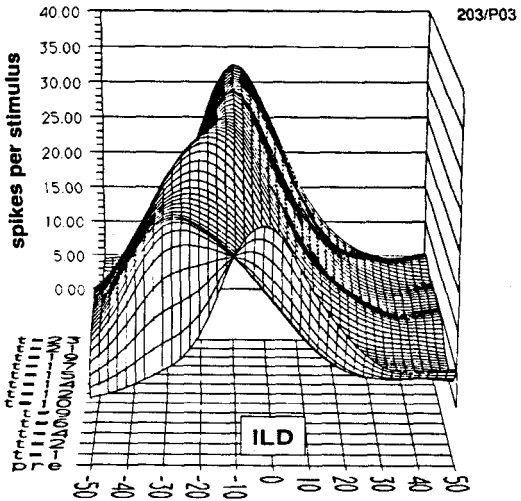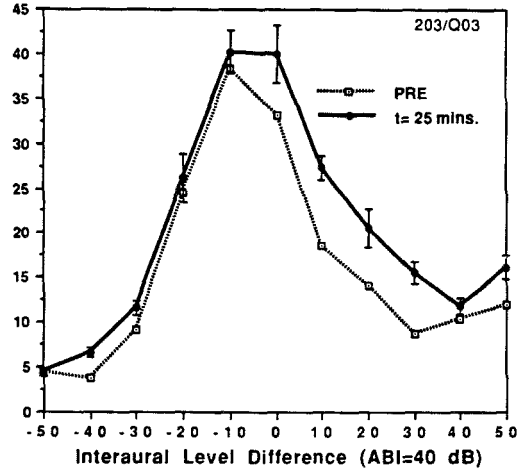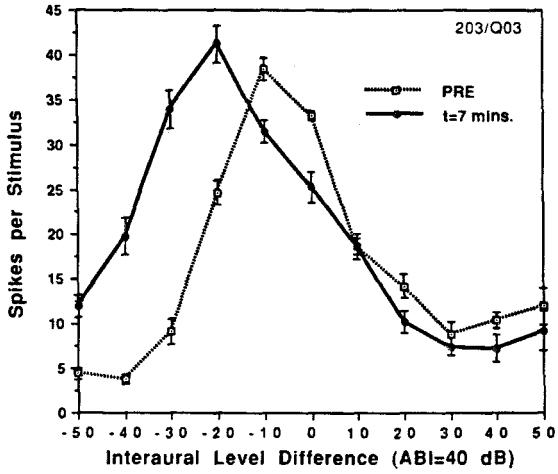
B. GABA
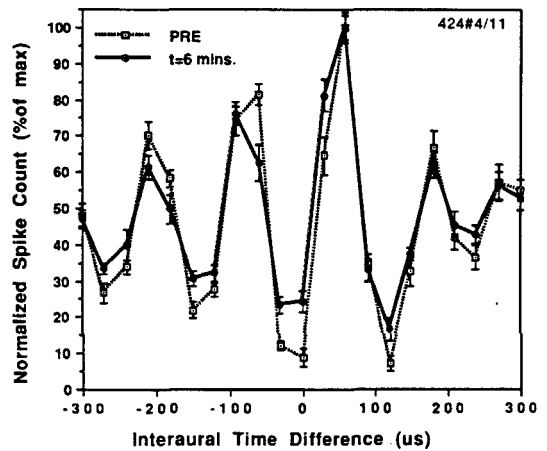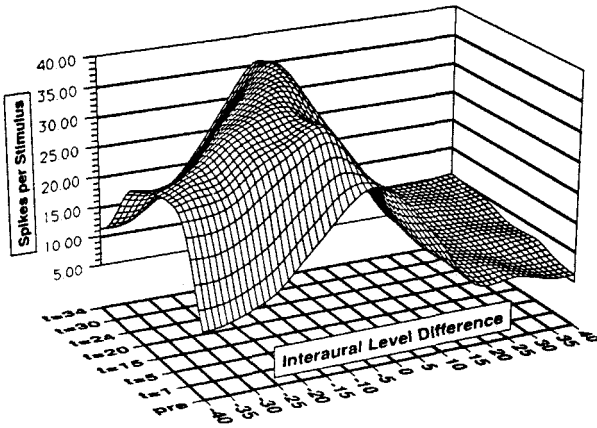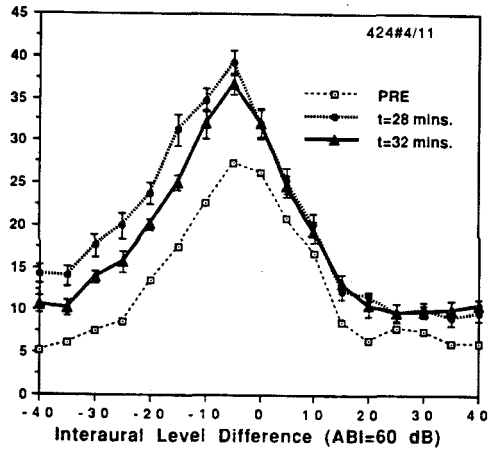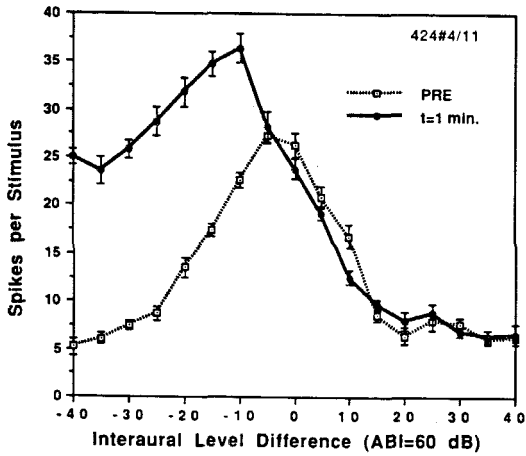
C. BMI
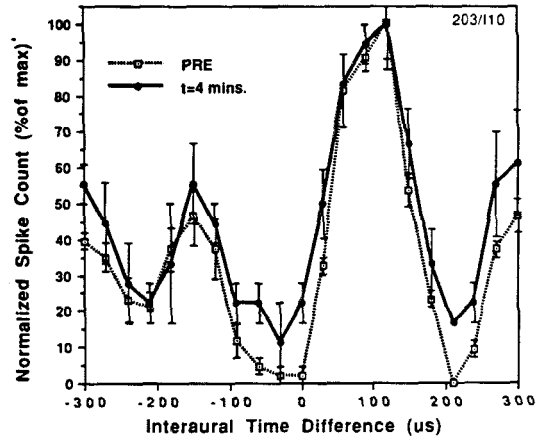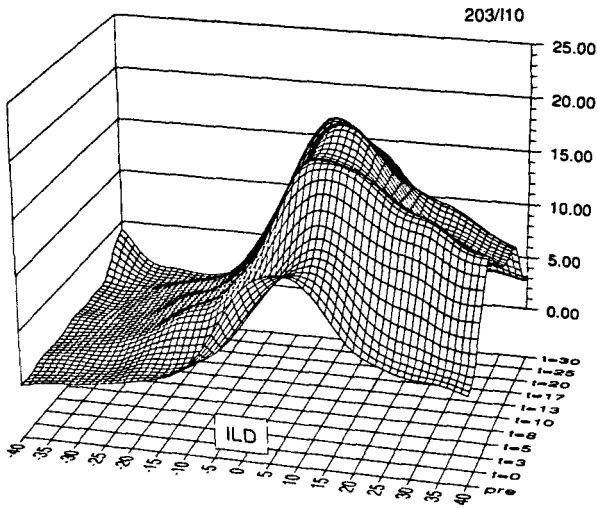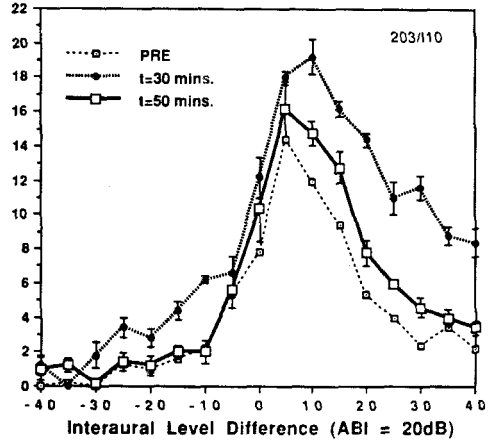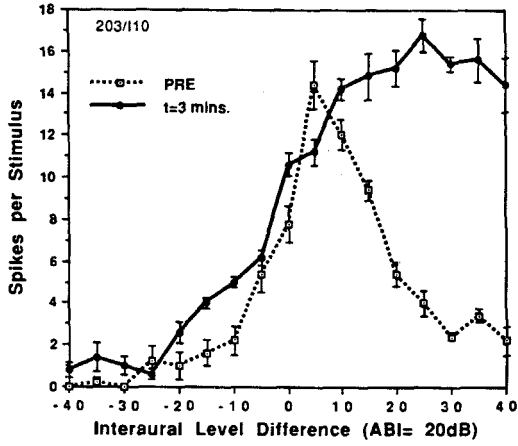
(b) VLVp can affect responses in the contralateral lateral shell.

A more specific test for the involvement of VLVp in providing inhibition to the inferior colliculus is shown in Figure 20A. BMI was injected into the contralateral VLVp to potentiate activity within that nucleus. The response of a single neuron in the lateral shell of ICc was recorded. The stimulus consisted in a constant level of sound at the contralateral ear, which excited this lateral shell neuron, while varying the sound level at the ipsilateral ear. This paradigm was the same as used previously in Figure 16, where the inhibition between the two VLVps was investigated. At loud ipsilateral sound levels, the lateral shell · neuron is inhibited below the tonic activity due to the contralateral sound (Fig. 20A, "pre"). Upon injection of BMI into the contralateral VLVp, this inhibition became much stronger (Fig. 20A). Increased activity in VLVp thus causes increased inhibition of lateral shell neurons.

An additional experiment to confirm that VLVp provides inhibition to the IC is shown in Fig.20B. The paradigm was the same as for Fig.20A: a constant level of excitation was provided to a lateral shell neuron by a fixed sound level in the contralateral ear. As the sound in the ipsilateral ear is made louder, it inhibits this tonic excitation ("pre"), and injection of muscimol into the contralateral VLVp nearly abolishes the inhibition. The effects were irreversible with muscimol. The results from this experiment are similar to those of Figure 16, where muscimol was injected into VLVp to decrease the inhibition it supplied to the VLVp on the opposite side. These experiments do not address the issue of whether the same cells in one VLVp may inhibit both the contralateral lateral shell and the contralateral VLVp.

Figure 4.20: **The contralateral VLVp provides inhibition to the lateral shell. A: Left:** Response of a lateral shell cell to a sound at constant contralateral level (30dB) while varying the ipsilateral level. The ipsilateral level is 50 dB at the origin, and decreases to 0 at the far right of the abscissa (this graphing keeps the way ILD is represented on the axis consistent with other data). Responses are shown before ("pre") and 8 minutes after injecting BMI into the contralateral VLVp. There is significantly more inhibition by ipsilateral sounds after BMI injection. **Right:** The effect recovers after 48 minutes. Stimulus was pseudorandom white noise. **B:** Response of a lateral shell cell to same stimulus as in A; this cell has a non-monotonic response to ILD. **Left:** After injecting muscimol, there is a small decrease in the strength of inhibition by ipsilateral sounds ("t=1"); the reverse of the effect seen above with BMI. **Right:** This effect increases and has not recovered at 71 minutes. Stimulus was pseudorandom white noise.

A. BMI
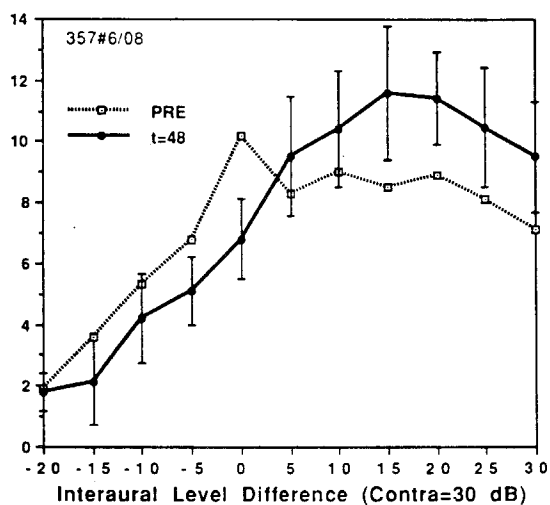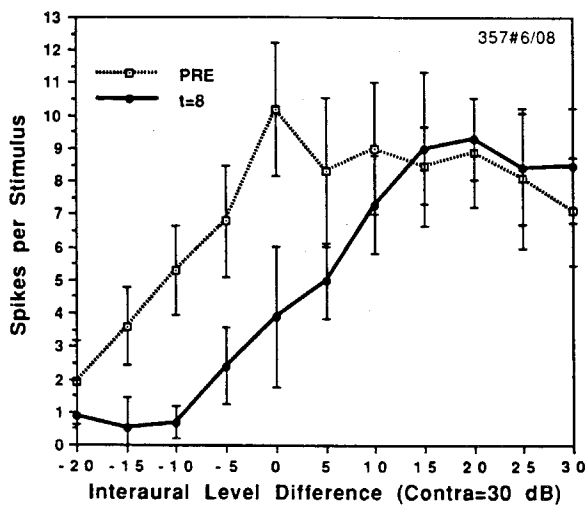
B. Muscimol
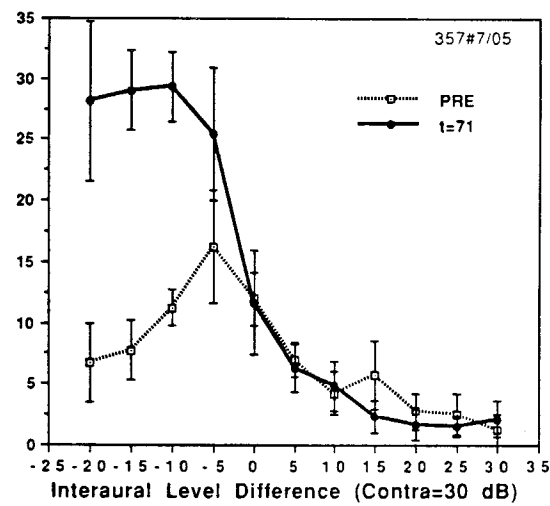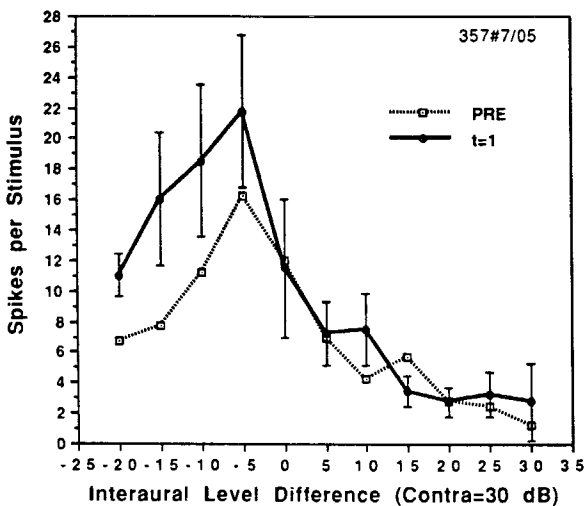
(c) The response in VLVp affects responses in the ipsilateral ICx.

All the above experiments of recording in the colliculus while injecting the contralateral VLVp may cause at least some of their effects via the ipsilateral VLVp, since the two VLVp are commissurally connected. A more direct test of the involvement of the ipsilateral VLVp is to inject it directly while recording from the ICx. Figure 21 shows the results of such an experiment. GABA was injected globally into the VLVp, and the response to ILD was recorded in the ICx on the same side. This injection attenuated the neuronal activity of cells in VLVp, as recorded through the injection syringe. In the ICx, a complete abolition of inhibition by contralaterally loud sounds was seen. The time course for these effects was similar to the results from injecting the contralateral VLVp. Lidocaine could not be used for the experiments at the ipsilateral VLVp, since it also blocks actional potentials in axonal fibers of passage. When lidocaine is injected into the ipsilateral VLVp, fibers of the lateral lemniscus providing input to the inferior colliculus on the same side are affected; the result is an abolition of all evoked neural activity in the ICx (see below, section 3 (v)).

(d) Summary: VLVp can affect cells in the colliculus on either side of the brain.

Results from experiments in which VLVp was injected and responses were recorded from ICx are summarized in Figure 22. When the contralateral VLVp was injected with GABA or lidocaine, I observed a decrease of inhibition at sounds ipsilaterally loud in the ICx. One measurement of such a decrease of inhibition is the shift of the center-of-mass of the region under the response curve of the ICx neuron. A decrease in inhibition by ipsilaterally loud sounds will shift the center-of-mass of the response to ipsilaterally loud ILDs. Figure 22 shows the average shifts in the center-of-mass of the response curve recorded

in the ICx upon injection of the contralateral VLVp with GABA (n=4), or lidocaine (n=11). Together with a decrease in inhibition by sounds loud at the ipsilateral ear, the center-of-mass can also be shifted in the same direction by an increase of inhibition by sounds loud at the contralateral ear. This latter effect was sometimes observed as well, although its magnitude depended on how completely inhibited the response at contralaterally loud ILD was in the first place.

The center-of-mass of the ILD-response curve in the ICx shifts in opposite directions with the injections of lidocaine or GABA on the one hand, and with BMI (n=7) on the other (Fig. 22). These shifts were statistically significantly different between Lidocaine and BMI (p=0.0005; Two-tailed Mann-Whitney U-test), and between BMI and GABA (p=0.0082).

The results from 7 experiments in which the ipsilateral VLVp was injected with GABA are also summarized in Figure 22; the response curve shift obtained when GABA was injected into the ipsilateral VLVp was in the opposite direction and differed significantly from the curve shift with GABA injected into the contralateral VLVp (p=0.0082; Mann-Whitney U-test).

To obtain additional histological evidence that VLVp was the nucleus injected in the above experiments, sections were examined for lesions that resulted from the injections. In general, injection experiments were repeated on the same animal until the effects were no longer completely reversible. In the case of lidocaine, this usually happened after 3-4 injections, and presumably was due to permanent lesions made by the fluid volume ejected. Figure 23 shows two cases in which VLVp was lesioned in this manner. The position of the lesion indicates that VLVp was the nucleus injected during the experiments.

The extent of the lesion encompasses most of the VLVp and does not impinge on the immediately adjacent nucleus VLVa.

Physiological evidence for the restricted region affected by the drug injections was also obtained during the experiments. Nucleus VLVa, which is adjacent to VLVp (Fig. 23), is involved in processing interaural time differences, and its neurons are sensitive to ITD, but not to ILD. Previous studies (Mazer and Adolphs 1991; pers. obs.) showed that injecting lidocaine, GABA or muscimol into VLVa affects ITD responses in the ICx. This observation was used as a control during the experiments reported here. When VLVp was injected with a drug, the response of the ICx neuron to both ILD and to ITD was recorded. In all cases reported here, only the response to ILD changed; the tuning to ITD remained invariant . In a few cases, when larger fluid volumes were injected into VLVp, an effect was seen on the ICx cell's response to ITD; all such experiments were not used for data because the site of action of the injected drug was presumably not restricted to VLVp.

Figure 4.21: **The ipsilateral VLVp provides inhibition to the lateral shell.** A single neuron in the lateral shell before ("pre") and at various times after injecting GABA into the ipsilateral VLVp. **A:** There is an immediate block of the inhibition by sounds at large contralateral ILD. **B and C:** This effect recovers over 27 minutes. **D:** Surface plot of the same experiment showing the temporal evolution of the effect; the surface was smoothed with a 4th-order spline. There was no change in the ITD tuning of the neuron after the injection (not shown). Stimulus was pseudorandom white noise.
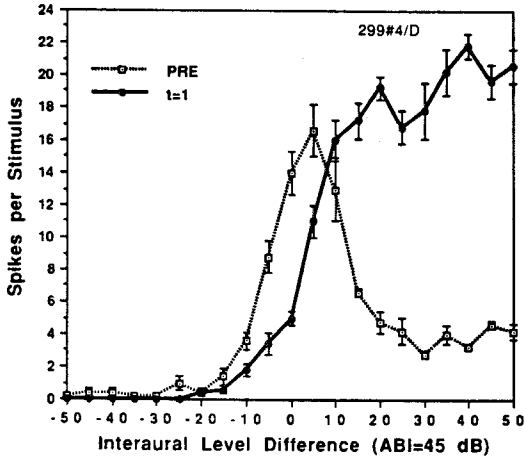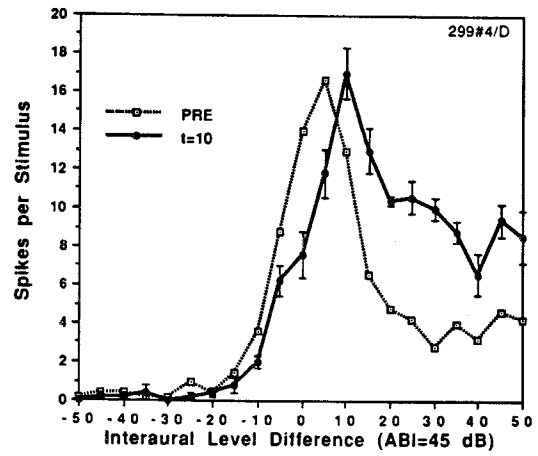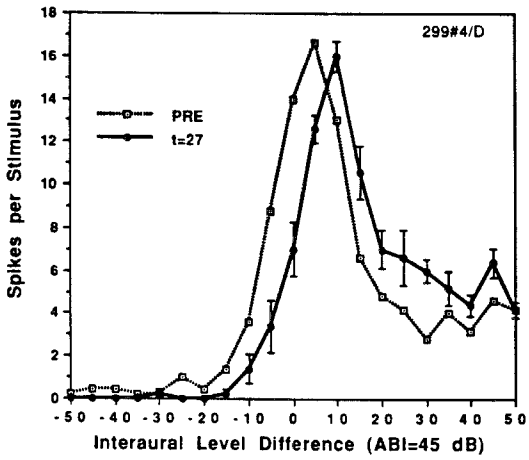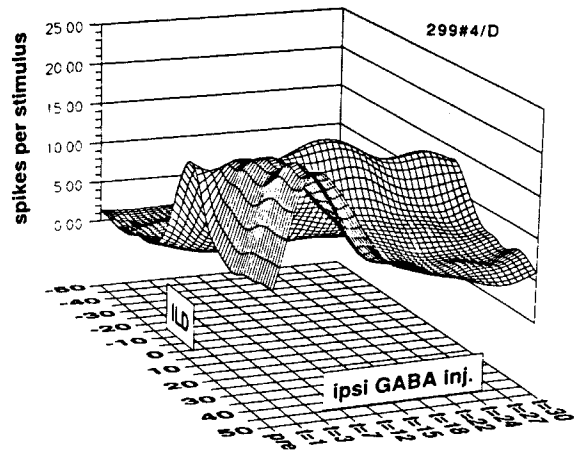
Figure 4.22: **Shift of the response curve in the ICx with drug injections into VLVp.** The maximal shift of the center-of-mass of the ILD-response function is plotted for all repeated experiments in which drugs were injected into VLVp and responses recorded in the ICx. The ordinate plots the maximal response-curve shift, computed for each experiment as the maximal difference between the first moment of the pre-response curve and response curves after injecting drugs into VLVp. The first moment of the response curve is calculated as $\Sigma(x_i y_i)/\Sigma(y_i)$, where x is the value of the ILD of the stimulus presented, and y is the response (mean spikes per stimulus presentation). This gives the value of the ILD of the center-of-mass of the area under the curve. The abscissa plots this response curve shift for experiments in which the contralateral VLVp was injected with lidocaine (open circles; n=11; mean=-11.3; s.e.m.=1.6), BMI (open triangles; n=7; mean=9.3; s.e.m.=2.3) or GABA (open squares; n=4; mean=-5.2; s.e.m.=1.9), and in which the ipsilateral VLVp was injected with GABA (closed triangles; n=7; mean=4.8; s.e.m.=2.3). A positive response curve shift means the response to contralaterally loud stimuli was potentiated and/or the response to ipsilaterally loud stimuli was attenuated. A negative response curve shift means the response to ipsilaterally loud stimuli was potentiated and/or the response to contralaterally loud stimuli was attenuated.
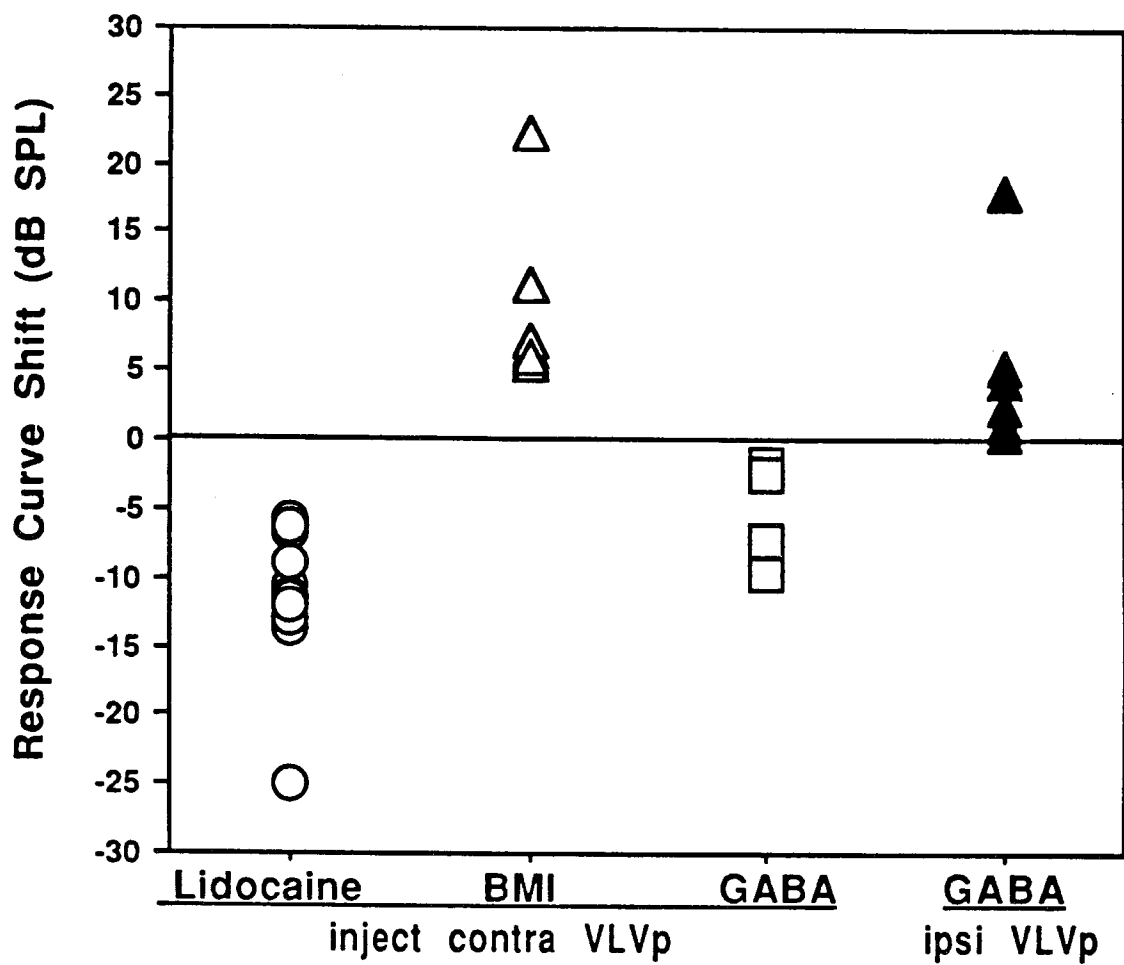
Figure 4.23: **Site of drug injections in VLVp.** Two examples are shown in which enough fluid was ejected into VLVp during the physiological experiments to result in a permanent lesion. In each case, the region lesioned (arrowheads) was confined to VLVp and did not impinge on the medially adjacent VLVa. The dorsal and ventral borders of VLVp are demarcated with straight arrows. **Left:** Left VLVp lesion in owl 299. **Right:** Right VLVp lesion in owl 424. Both sections were stained for acetylcholinesterase. Scale bar: 1.0mm.
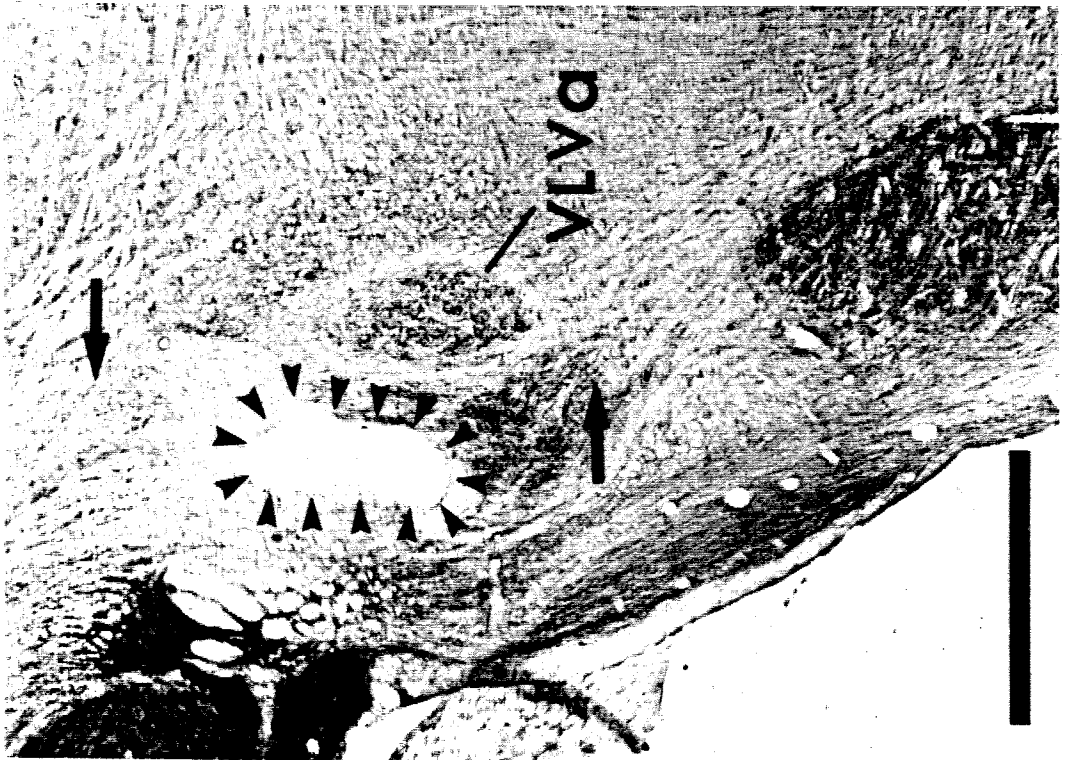
(iii) Bilateral Injection of VLVp Affects the ICx.

All the above experiments, in which one VLVp was injected with a drug, are in part confounded by the commissural connection between the VLVp on each side of the brain. Changing the response in one VLVp will also change the response in the VLVp on the opposite side (in the opposite direction). This makes an analysis of the effect of each VLVp on the responses in ICx difficult.

To attempt to eliminate this confounding factor, I carried out further experiments in which VLVp was bilaterally injected with GABA. Figure 24 shows the responses recorded from an ICx neuron before, after injecting one, and after injecting both VLVp. This experiment was also done by first injecting one VLVp several times until that nucleus was lesioned, and then injecting the other VLVp with GABA; the results from these experiments were the same. For an ICx neuron, inhibiting activity in the contralateral VLVp decreases the inhibition by sounds loud at the ipsilateral ear. Inhibiting the ipsilateral VLVp decreases the inhibition by sounds loud at the contralateral ear. Bilateral inhibition of VLVp leads to decreased inhibition at either extreme ipsilateral or contralateral ILD (Fig. 24). Out of 11 experiments in 3 owls in which VLVp was injected bilaterally, clear decreases in inhibition at both extremes of the ILD scale were seen in 6 cases.

(iv) VLVp Provides Inhibition to the Response of Neurons in ICx.

If VLVp provides polysynaptic input to the ICx that is functionally inhibitory, it should be possible to completely inhibit ICx neurons if the neuronal activity in VLVp is raised sufficiently. I tested this by injecting large quantities of BMI into VLVp. Multi-neuron recordings through the syringe in VLVp indicated strong neural activity even with no auditory stimulus to drive the cells. Under such conditions, VLVp might provide inhibitory input to the colliculus at all ILDs.

Figure 25 shows the results of experiments in which VLVp was injected with 0.5μl of BMI solution (more than twice the volume used in other experiments). In Figure 25A, the contralateral VLVp was injected; in Figure 25B, the ipsilateral nucleus was injected. In both cases responses to ILD of a single neuron in the ICx were recorded. In each case, the response was dramatically attenuated upon the injection of BMI into the VLVp. This effect was not always completely reversible on the time-scale of the experiment, probably due to the large amount of drug injected.

(iv) Some Lateral Shell Neurons Receive Direct GABAergic Inhibition.

All the above experiments show that VLVp provides inhibition to the ICx. The hodology shows that this inhibition must be polysynaptic. Since VLVp projects directly to the lateral shell, which in turn projects to the ICx, there are two theoretical possibilities: either VLVp inhibits lateral shell cells, which in turn provide excitatory input to the ICx, or VLVp excites lateral shell cells which are inhibitory to ICx cells. Since responses to ILD are rather similar in the lateral shell and in the ICx, the former possibility seems more likely.

Experiments were carried out to address this issue. I injected BMI into the contralateral VLVp while recording from a lateral shell cell. An effect was seen (increase in inhibition) on the response of the collicular cell, as reported above. Now, BMI was additionally applied iontophoretically to the lateral shell cell from which I was recording. Figure 26 shows that BMI iontophoresis onto the lateral shell neuron can block the inhibition induced by increasing the activity of the contralateral VLVp. The effect of the iontophoresis was reversible, as was, with a longer time-course, the effect of the VLVp injection. Two such experiments (in one owl), which gave identical results, suggest that there are neurons in the
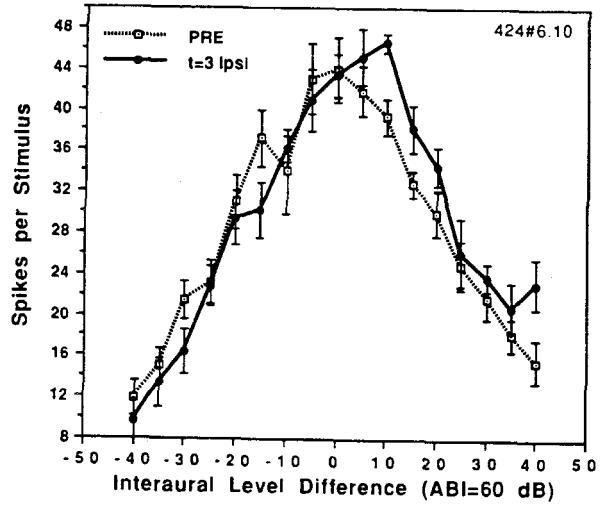
lateral shell of ICc that receive direct, GABAergic inhibition from the contralateral VLVp.

The identical paradigm was carried out with the ipsilateral VLVp. Figure 27 shows that injecting BMI into the ipsilateral VLVp causes an inhibition in the lateral shell. This inhibition can be partially blocked by iontophoretic application of BMI. In this experiment, however, the iontophoresis provided only a slight block of the induced inhibition.
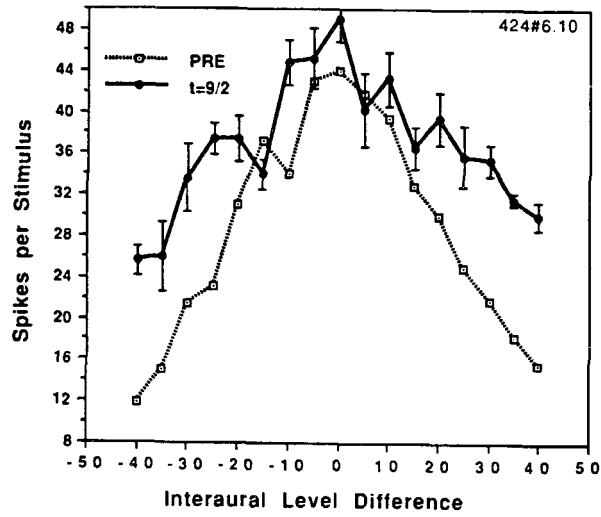
These results, together with the tracing studies reported, suggest that VLVp provides direct, bilateral, and probably GABA-ergic inhibition to the lateral shell of the inferior colliculus.

Figure 4.24: **Bilateral Injections of VLVp affect the ICx. A:** Response of ICx neuron before ("pre") and 3 minutes after injecting GABA into the ipsilateral VLVp. There is an attenuation of the inhibition at large contralateral ILD. **B:** Response 9 minutes after the ipsilateral VLVp injection, and 2 minutes after an additional injection of GABA into the contralateral VLVp. There is attenuation of inhibition at large contralateral and ipsilateral ILD. **C:** The effect shows partial recovery 30 minutes after the first injection. The response to ITD at the neuron's optimal pre-injection ILD was unaffected (not shown). Stimulus was pseudorandom white noise.

A.

424#6.10

PRE
t=3 Ipsi

Interaural Level Difference (ABI=60 dB)

B.

424#6.10

PRE
t=9/2

Interaural Level Difference

C.

424#6.10

PRE
t=30/23

Interaural Level Difference
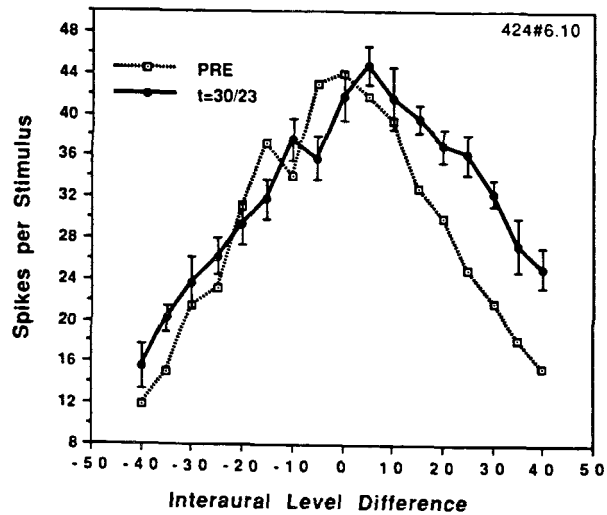
Figure 4.25: **Large injections of BMI into VLVp inhibit responses in ICx at all ILDs. A:** 0.5 µl BMI were injected into the VLVp while recording responses to ILD in the contralateral ICx. **Left:** Response of the ICx neuron before ("pre") and 1 minute after injecting the contralateral VLVp. There is a marked attenuation of the response at nearly all ILD values. **Right:** The effect recovers after 30 minutes. Stimulus was pseudorandom white noise. **B:** 0.5 µl BMI were injected into the VLVp while recording in the ipsilateral ICx. **Left:** There is a marked attenuation of the response in the ICx 10 minutes after the injection. **Right:** There is partial recovery of the effect after 90 minutes. Stimulus was pseudorandom white noise.
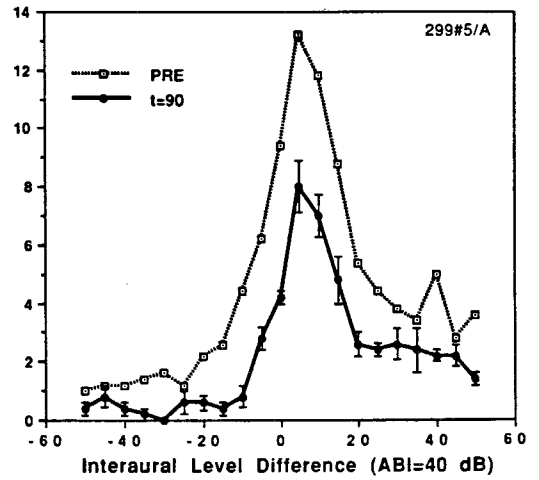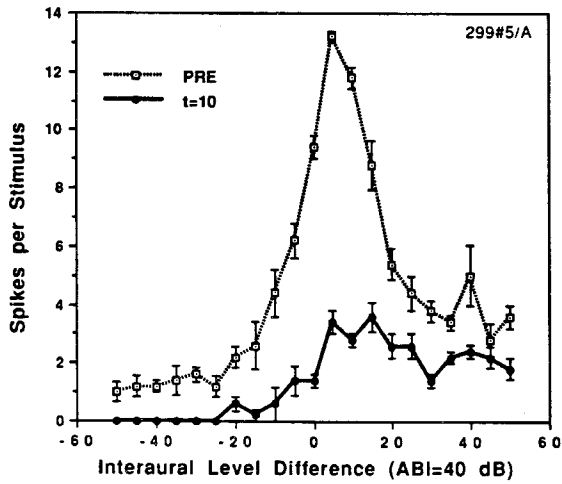
A. Contra VLVp
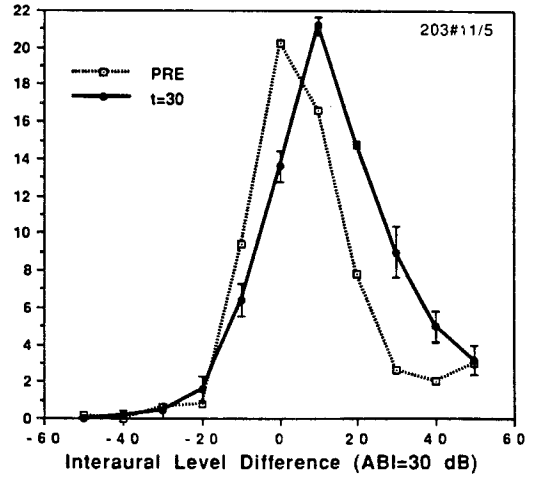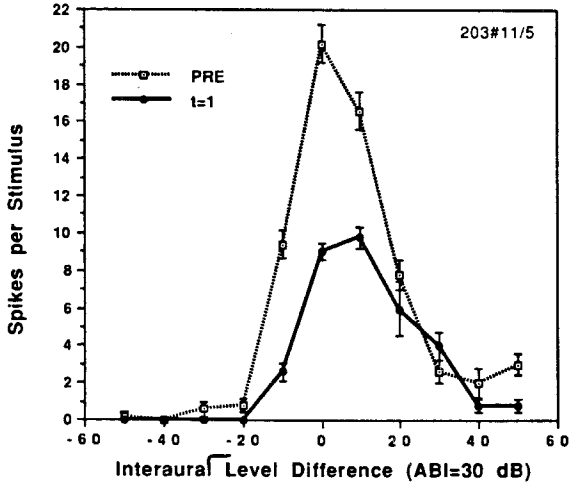
B. Ipsi VLVp

Figure 4.26: **The contralateral VLVp provides GABA-ergic inhibition to the lateral shell.** A 0.5 µl injection of BMI was made in VLVp while recording from a neuron in the contralateral lateral shell. After the response of the lateral shell neuron had been inhibited, BMI was applied iontophoretically to the lateral shell neuron to see if the inhibition could be locally blocked. **A.** Response of a neuron in the lateral shell to ILD before, and 2 minutes after injecting BMI into the contralateral VLVp. **B.** Response 7 minutes after the injection into VLVp, and 2 minutes after beginning continuous iontophoresis of BMI onto the lateral shell neuron ("t=7/2 ionto"). The inhibition due to the VLVp injection is attenuated. 12 minutes after the initial VLVp injection and 4 minutes after terminating the BMI iontophoresis ("t=12/4 post"), the effect of the iontophoresis has recovered, but the effect of the VLVp injection is still present and the cell is strongly inhibited again, as in A. **C.** After 1 hour, the response of the lateral shell neuron has recovered to the pre-injection case as the effect of the BMI injected into the VLVp has worn off. **D.** Surface plot of the same experiment with time along the x axis. The prominent peak in the middle shows the block of induced inhibition by BMI iontophoresis. The surface was smoothed with a 4th-order spline. Stimulus was pseudorandom white noise.
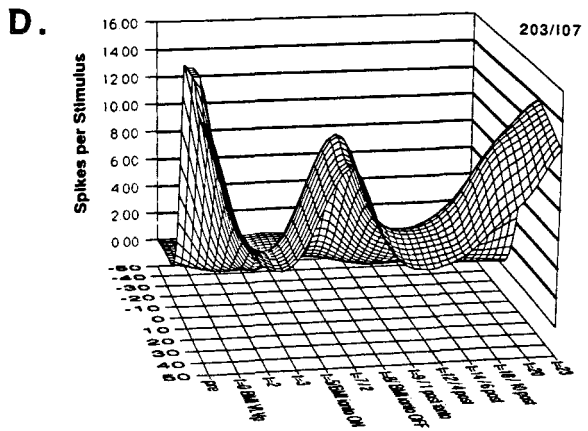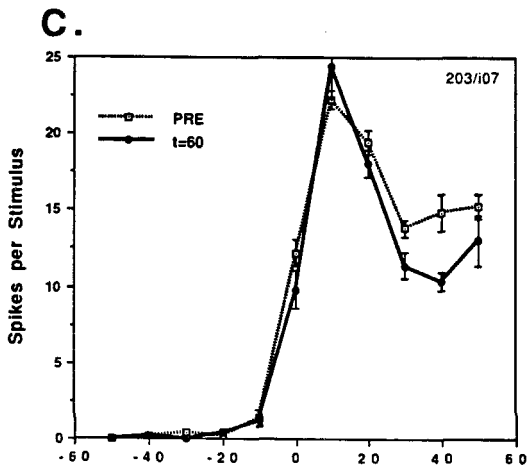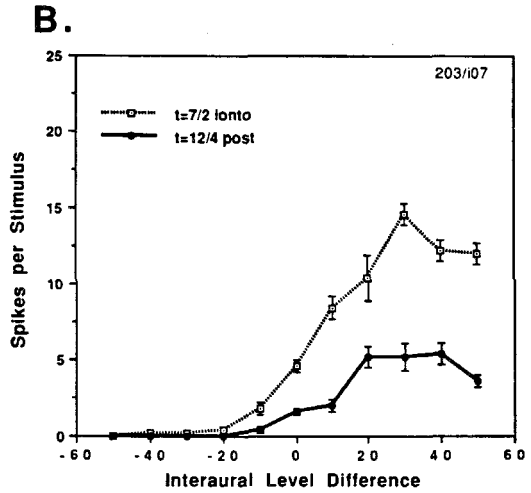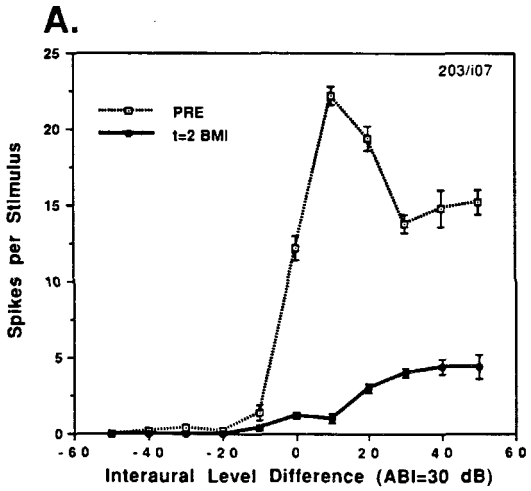
Figure 4.27: **The ipsilateral VLVp provides GABA-ergic inhibition to the Lateral Shell.** See Figure 26 for protocol. The ipsilateral VLVp was injected this time. **A.** Response of lateral shell neuron to ILD before ("pre") and 3 minutes after injection of BMI into the ipsilateral VLVp. The response is markedly inhibited. **B.** Response 5 minutes after the VLVp injection ("t=5"); and response 7 minutes after the VLVp injection plus 1 minute after beginning iontophoresis of BMI onto the lateral shell neuron ("t=7/1"). The BMI iontophoresis partially blocks the inhibition at some ILDs. **C.** Response after 6 minutes of continuous BMI iontophoresis and a total of 12 minutes after the VLVp injection ("t=12/6"); and at 30 minutes after the VLVp injection and 15 minutes after iontophoresis was stopped ("t=30"). After the iontophoresis was stopped, the cell recovered from the effects of locally applied BMI, and the strong inhibition due to the VLVp injection was unmasked again. **D.** After 80 minutes, the cell slowly recovers from the effect of the BMI that was injected into the ipsilateral VLVp. **E.** Surface plot of the same experiment, with time along the x-axis. The surface shows a cross-section through the data grid at ILD=15dB for better visualization of the maximal effect. The surface was smoothed with a 4th-order spline. Stimulus was pseudorandom white noise.

A.

B.

C.

D.

E.
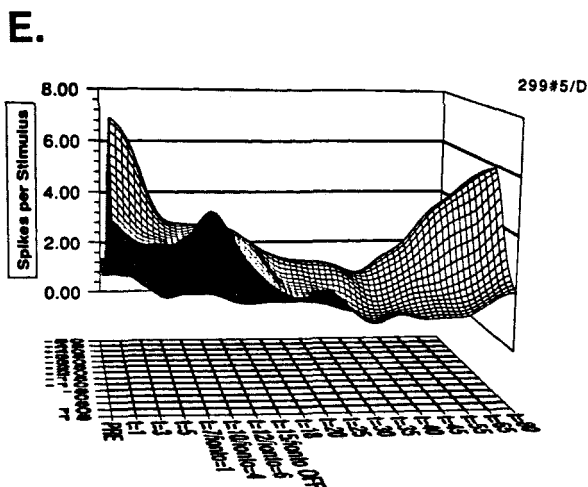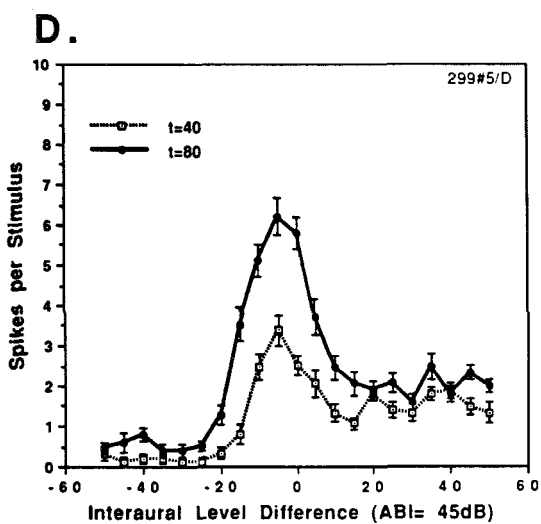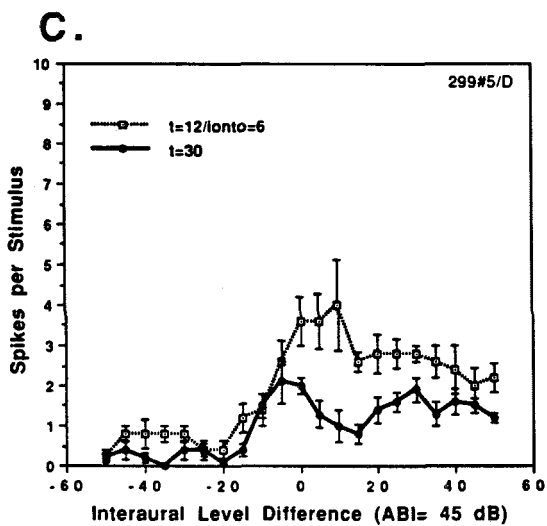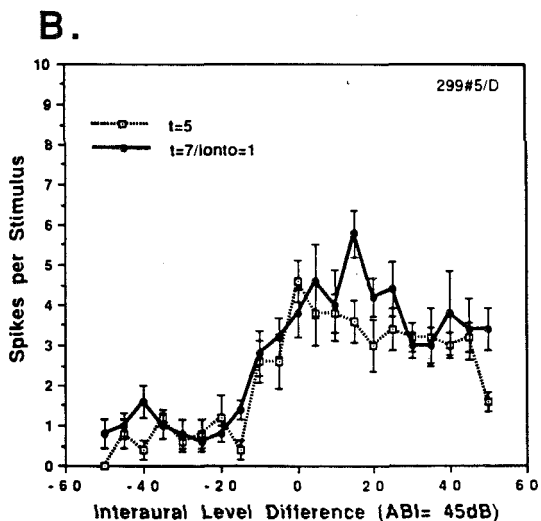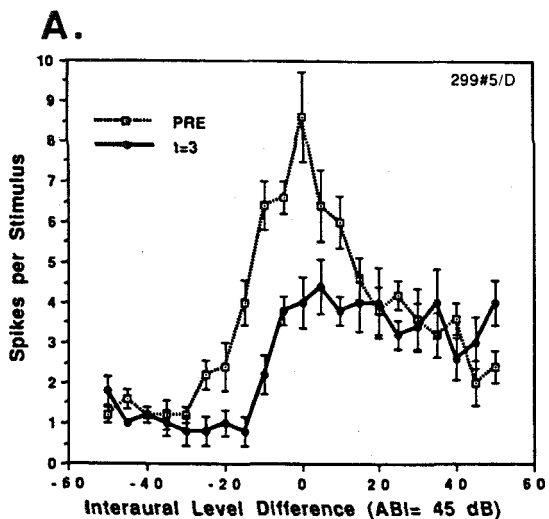
(v) Nuclei or Fibers of the Lateral Lemniscus may Provide the Excitation to the Lateral Shell and to ICx.

If VLVp provides bilateral inhibition to the inferior colliculus at extreme ILD values, where does the excitation at optimal ILD come from? Information about sound pressure level, and binaural level differences, is conveyed to the inferior colliculus in the lateral lemniscus by fibers originating from several nuclei. The local anesthetic lidocaine can be used to block action potentials in these fibers.

I injected 0.6 μl of lidocaine hydrochloride (5% aqueous, as for the above experiments) into the lateral lemniscus in 3 owls. Figure 28A shows the results from an injection of the left lateral lemniscus while recording from the right lateral shell of ICc. The responses of fibers recorded through the injection syringe were insensitive to ITD, excited by the left ear, and inhibited by the right ear ("0E"). These responses are best explained by fibers originating from the left nucleus angularis. Anesthetizing these fibers with lidocaine led to a nearly complete abolition of activity in the contralateral lateral shell (Fig.28A; 3 cells in 2 owls). Figure 28B shows that similar results obtain when lidocaine is injected into the ipsilateral lateral lemniscus (2 cells, 1 owl). Thus, at least some of the excitatory input to the lateral shell comes from lemniscal nuclei and/or the superior olivary nucleus and/or nucleus angularis, all of whose fibers course in the lateral lemniscus.

Since the lateral shell projects to ICx, a similar effect might occur for ICx cells. Figure 28C shows the response of a cell in ICx after injecting lidocaine into the contralateral lateral lemniscus. The response was reversibly blocked at all ILDs.

(vi) Inhibition From VLVp Renders Cells in ICx Binaurally Exclusive.

One model, which is presented in more detail in the discussion, is that ICx receives excitation, perhaps in part via polysynaptic inputs from the lateral lemniscus, that are not tuned to ILD. This excitation would lead to a response also for monaural stimuli, as is seen in all nuclei below the level of ICx. One of the defining features of cells in the ICx (and also of some cells in the lateral shell), however, is that they are binaurally exclusive. Cells in ICx are tuned to a particular ILD and will be inhibited by monaural stimuli. VLVp may provide such inhibition. Figure 29 summarizes the results from several experiments in which VLVp was injected and responses were recorded in ICx. The ordinate plots the change in the response to monaural stimuli recorded in the ICx when VLVp is injected. When activity in VLVp is reduced, there is a larger response in ICx to monaural stimuli. These data support the idea that VLVp provides inhibition at monaural stimuli, with the VLVp on each side inhibiting responses that are loud at one ear.

Figure 4.28: **Fibers and/or nuclei of the lateral lemniscus provide excitation to the lateral shell and to ICx.** A large (0.6 µl) injection of lidocaine hydrochloride was made into the lateral lemniscus lateral and ventral to VLVp while recording from a cell in the lateral shell. **A.** The contralateral lemniscus was injected and the response in the lateral shell was attenuated 3 minutes later (left). It recovered after 25 minutes (right). **B.** Injection of the ipsilateral lemniscus also resulted in a notable attenuation of the response in the lateral shell after 3 minutes ("t=3"; left), with recovery after 21 minutes (right). **C.** Response to ILD recorded from a neuron in ICx while injecting lidocaine into the contralateral lateral lemniscus. The surface plot (smoothed with a 4th-order spline) shows a nearly complete abolition of neural activity in ICx that recovers after 19 minutes. Stimulus was pseudorandom white noise in all cases.

A. Contra
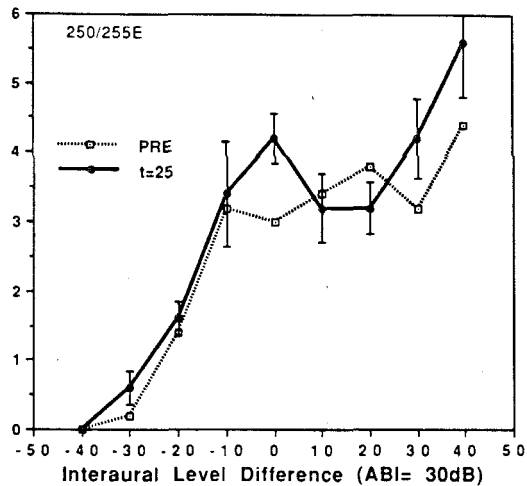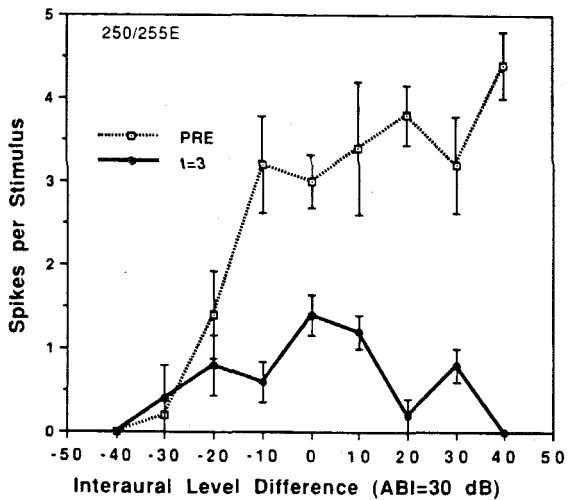
B. Ipsi
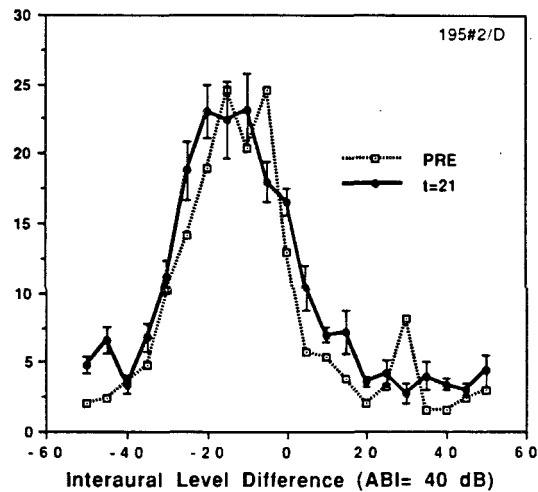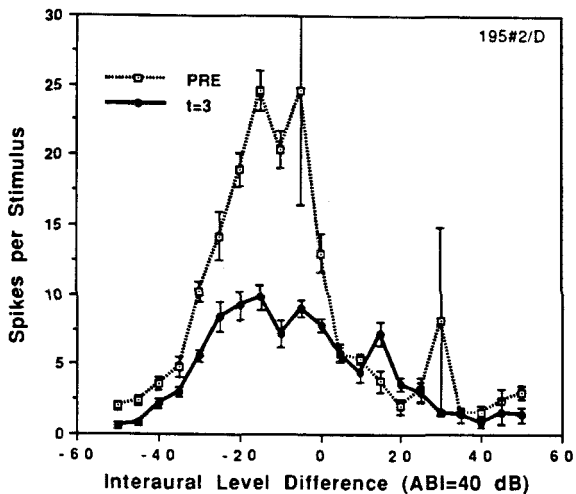
C.
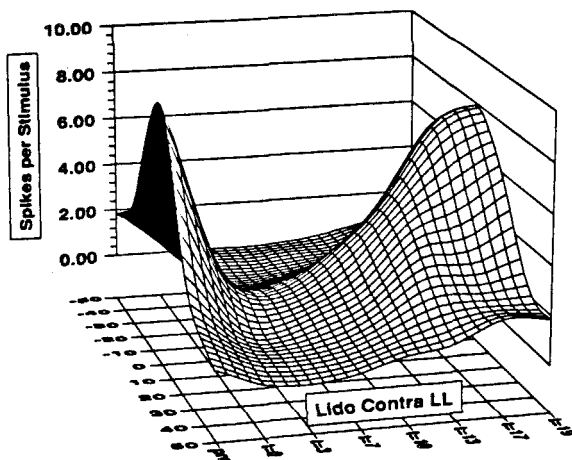
Figure 4.29: **Injections of drugs into VLVp alter responses to monaural stimuli in the ICx.** The mean changes in responses of ICx neurons to monaural ipsilateral (solid bars) or contralateral (hatched bars) stimuli and s.e.m. are plotted. This is a summary of experiments in which the contralateral VLVp was injected with lidocaine (n=11), BMI (n=6), or GABA (n=3), or in which the ipsilateral VLVp was injected with GABA (n=7). Monaural responses were normalized to the response of the ICx cell at its optimal ILD at an ABI equal to that of the monaural stimulation. Negative changes denote decreases in the monaural firing rate and generally underestimate the effect of induced inhibition since monaural firing rates of the ICx cells were near zero in most cases.
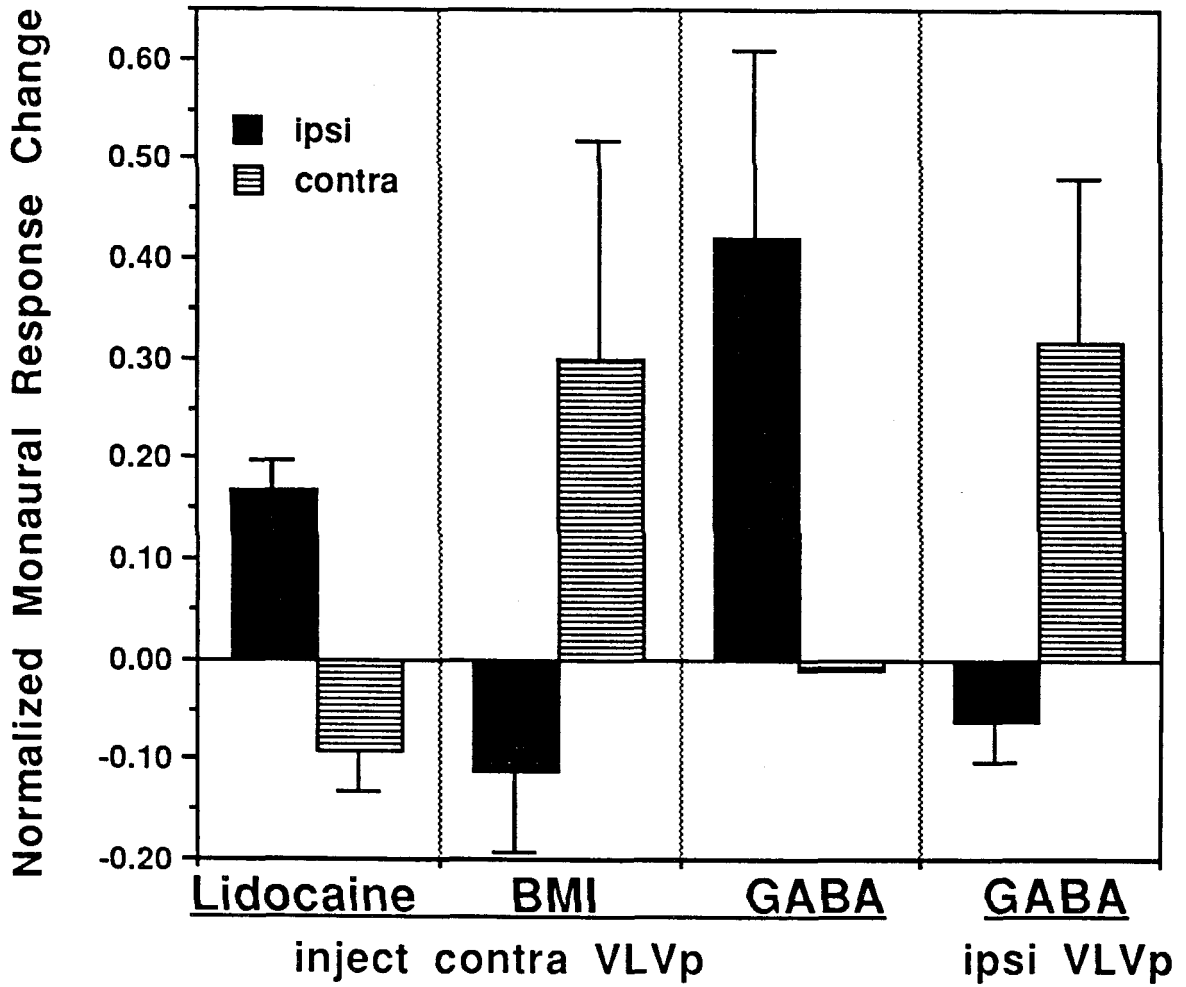
DISCUSSION

Connectivity and Physiology of VLVp

The strategy of the present paper was to select a level of processing in the owl's intensity pathway just below the top of the hierarchy. Given neurons tuned to ILD in the owl's auditory space map, how can a lower level of processing generate such response properties? Hodological investigations were the first step in answering this question: What is the circuitry?

Previous studies showed that VLVp projected contralaterally to the medial shell of ICc (Adolphs 1988) and preliminary studies suggested that VLVp might also project to the contralateral lateral shell of ICc (pers. obs.; T. Takahashi, pers. comm.). I found that VLVp projects bilaterally, and probably in a topography that preserves the mapping of frequency, onto the lateral shell of ICc. The projection to the ipsilateral lateral shell appears to come mostly from the dorsal portion of VLVp (Fig.2). Some cells in VLVp project collaterally to both the medial and lateral parts of the contralateral shell. In Figure 2, a few retrogradely labelled cells were seen in VLVa when the contralateral lateral shell was injected. Possibly, a very restricted ventral portion of VLVa projects contralaterally to the lateral shell of ICc. However, the extent of the injection site is also consistent with a projection from VLVa to the contralateral core of ICc, which may have had a small amount of tracer present due to spread from the injection of the adjacent lateral shell. Anterograde tracer injections into VLVa will be necessary to determine this connectivity. The medial shell also receives a strong projection from the contralateral VLVp, but an ipsilateral projection appears to be much weaker than in the case of the lateral shell (cf. Figures 5,6,13). This hodology is consistent with a physiological model that I present below.

The generation of responses to binaural stimuli has recently been explored in VLVp, which is the first binaural site in the ascending intensity pathway. Neurons in VLVp are inhibited by stimuli loud at the ipsilateral ear (Moiseff and Konishi 1983; Manley, Koeppl et al. 1988). Moreover, the strength of this inhibition changes in an orderly way across the nucleus: neurons in the top of (dorsal) VLVp are very strongly inhibited, while neurons near the bottom of the nucleus are nearly insensitive, or only very weakly inhibited, by ipsilateral stimuli. All neurons in VLVp are excited by sounds loud at the contralateral ear. The functional circuitry that leads to these response properties has recently been elucidated. Takahashi (1988b) showed that VLVp receives its excitatory input from the contralateral nucleus angularis. The inhibitory input comes from the contralateral VLVp (Figure 16), a result that has recently also been reported by Takahashi and Keller (1992), who used lidocaine instead of the muscimol used in my experiment. Because the polysynaptic inhibitory pathway from angularis to VLVp crosses the brain twice, it accounts for the inhibitory responses to ipsilateral sounds. Muscimol has the advantage over lidocaine that it affects only cells proximal to the injection site, and not possible fibers of passage. Lidocaine blocks both cellular activity and action potential propagation through axonal fibers.

Several models attempt to explain the gradient of inhibition in VLVp. Manley, Koeppl and colleagues (1988) proposed that the responses of VLVp neurons could be explained simply by subtracting the monaural inhibitory input from the monaural excitatory input, but this model was put forth before the source of the inhibition was known. The finding (Takahashi 1988; Takahashi and Keller 1992) that the two VLVp on each side of the brain inhibit each other, led Pearson and colleagues (Spence and Pearson 1989) to model the gradient of inhibition as due to a criss-cross reciprocal connectivity. In this model, the

ventral region of the VLVp on one side inhibits the dorsal region of the VLVp on the other side. The ventral VLVp in turn receives little or no inhibition from the other VLVp, accounting for the response properties seen. This overall scheme has received some recent preliminary support from detailed anatomical studies (Takahashi and Keller 1992; T. Takahashi pers. comm.). The present study (Figure 8) provides further evidence for this particular hodology. The injection of PHA-L was probably sufficient to label the entire dorso-ventral extent of one VLVp. Retrograde label in the ventral pole, and anterograde label in the dorsal pole of the opposite VLVp suggest that the ventral VLVp provides most of the efferent fibers, while the dorsal VLVp receives most of the inhibitory input. This circuitry between the two VLVp will need to be confirmed by tracing studies with more resolution.

It is unlikely that interneurons serve to provide inhibition in VLVp, since neurons with the necessary response properties have not been found in any study (Moiseff and Konishi 1983; Manley et al. 1988; Takahashi and Keller 1992; pers. obs.). This raises the possibility that neurons in VLVp that are themselves inhibited may in turn project to the contralateral VLVp to inhibit cells there. This reciprocity could result in responses with complex temporal structure (Takahashi and Keller 1992). However, it would appear that any such cell-to-cell reciprocity would be prevalent only near the middle of VLVp; the dorsal VLVp receives mostly afferents, and the ventral VLVp is mostly efferent in this circuit.

Carr, Fujita et al. (1989) reported that cells staining for glutamic acid decarboxylase (GAD) are more numerous in dorsal than in ventral VLVp. It is an intriguing possibility that this GAD-positive gradient relates to the physiological gradient of inhibition in VLVp, although the models outlined above would seem to require a higher density of inhibitory neurons ventrally in

VLVp rather than dorsally. The involvement of GABA in VLVp neurotransmission, however, is unclear. The results of the present study show that VLVp cells can be inhibited by GABA and that there is GABAergic inhibition of VLVp cells that can be blocked by BMI (Figure 15). This does not prove that the VLVps inhibit each other via a GABAergic projection. The finding that BMI appears to disinhibit cells in VLVp rather uniformly at all ILDs (Figure 15A) suggests that there may be a tonic GABAergic inhibition that is independent of ILD. My results only show that pharmacological agents acting at the GABA-A receptor can be used to alter the firing rate in VLVp.

However, the higher density of GAD-positive cells in the dorsal VLVp is consistent with my suggestion that the projection from VLVp to the inferior colliculus is GABA-ergic. Cells in the dorsal VLVp project to the colliculus, and in the case of the ipsilateral VLVp, it is predominantly the dorsal VLVp that supplies this projection (Fig. 2).

There are confounding issues concerning the effects of BMI in VLVp (Fig. 15A and injection experiments). One possibility is that the cells I found to respond to BMI were local interneurons, and a different population from the neurons of VLVp that project to the ICx. In this case, the iontophoretic results would not allow an interpretation of the results of global injection of BMI in subsequent experiments. Several points argue against such a complication. No heterogeneity in response properties in VLVp has been found (aside from the gradient of inhibition and the mapping of frequency). There is no evidence for functionally different subsets of neurons, with the exception of the gradient of inhibition. In particular, there are no neurons that are inhibited by contralateral sounds (as might be expected from inhibitory interneurons). I also recorded the multi-unit evoked response in VLVp through the syringe with which drugs were injected. BMI injection increased the global firing rate in VLVp as recorded

through the syringe, also making it unlikely that BMI had effects only on a small set of interneurons.

Synthesis of Responses in the Inferior Colliculus

I used pharmacological methods to functionally dissect the processing of ILD. The results show that VLVp provides inhibition to the inferior colliculus such that peaked responses to ILD are generated.

Inhibitory mechanisms that contribute to restricting auditory receptive fields in space have been suggested by previous findings. Knudsen and Konishi (1978) found evidence of an inhibitory surround to the receptive fields of neurons in ICx, as has also been found in the owl's optic tectum (Brainard, Knudsen et al. 1992). None of these studies address the locations of the neural sites where such inhibition might be implemented. I provide evidence that VLVp inhibits neurons in the lateral shell via a GABAergic projection (Figures 26,27). In view of the response properties of neurons of the lateral shell, and of their alterations when activity in VLVp is manipulated, it appears that such GABAergic inhibition must be direct. Were interneurons in the lateral shell involved, one would expect to find neurons in the lateral shell that can be excited by VLVp; there is no evidence for this. The inhibitory processes that sculpt sharply peaked responses to ILD appear to involve GABA, since blocking GABA-A receptors locally results in large responses at extremes of ILD (Figure 18). GABAergic inhibition has been reported at many levels of the pathway that processes ITD in the owl (Mori, Fujita et al. 1990; Fujita and Konishi 1991). It is not known if inhibitory transmitters other than GABA are additionally involved, nor what the nature of the excitatory transmitters in the ILD pathway is.

Figure 30 presents a model that explains the results reported in this paper. Cells in the lateral shell of the inferior colliculus, and in the ICx, receive

excitatory input from unknown and probably diverse sources. At least some of these excitatory projections appear to course in fibers of the lateral lemniscus on each side. This excitation results in a significant response at all ILDs. The role of VLVp is to provide inhibition, such that cells in ICx become binaurally exclusive and have sharply peaked response curves to ILD. The results of all experiments in which the activity within VLVp was manipulated can be interpreted within this scheme (Figure 31). Furthermore, the streams that process ITD and ILD appear to remain segregated even into the inferior colliculus: altering responses to ILD in the ICx had no effect on the tuning to ITD (Figure 19).

This model (Figs. 30, 31) could also account for the response properties of the medial shell. The tracer studies I report here suggest that the medial shell receives input primarily from the contralateral VLVp, and only a very weak connection from the ipsilateral VLVp. Inhibition of the medial shell by the contralateral VLVp would inhibit only one side of the ILD-response curve, resulting in a monotonic, sigmoid function. This is what one observes in the medial shell (Adolphs 1988; pers. obs.). Preliminary experiments with the same design as the ones of the present paper, but in which responses were recorded in the medial shell instead of the lateral shell or the ICx, support this picture (pers. obs.).

There are other models that have attempted to explain the synthesis of ILD tuning in the ICx. One of the most detailed is the model of Pearson and colleagues (Pearson, Spence et al. 1990). This model assumes a non-linear gradient of inhibition in VLVp and then takes the derivative of the response across the dorso-ventral axis of the nucleus' tissue to generate a non-monotonic curve as the ICx output. The gradient of inhibition in VLVp can be visualized as a sigmoid function; taking the derivative of a sigmoid function will

yield a peaked function with the peak located at the value of ILD at which the strength of inhibition in VLVp was changing most rapidly. Detailed simulations of this model, which have been presented elsewhere (Pearson, Spence et al. 1990), give results in close agreement with some of the data.

A simpler model, which does not make any requirements on the nature of the gradient of inhibition in VLVp (it could be linear) can also qualitatively account for my results (S. Volman, pers. comm.). This model simply takes the difference between the responses in the dorsal and ventral VLVp. Such an algebraic subtraction will yield a non-zero difference (a peak) at those ILDs at which the responses differ most. This difference dictates the position of the peak in the ICx. Both this model, and the one by Pearson and colleagues, have the virtue of providing a role for the gradient of inhibition found in VLVp. However, they do not explain the results I report here that ipsilateral and bilateral injections of VLVp affect responses in ICx. They would also predict that injecting dorsal or ventral regions of VLVp should have different effects, which is not borne out by experiments.

A different model that attempts to explain synthesis of a peak in the ICx convolves the response of one VLVp with that of the VLVp on the opposite side (T. Takahashi originated the idea, pers. comm.). Although multiplying the response functions of the two VLVp in this manner does generate a peaked function, this model runs counter to the results reported in the present study that VLVp provides inhibition to the colliculus.

The tuning of ICx neurons to ILD does not shift with average binaural intensity, nor with ITD. The latter invariance can be explained by the independence of the time- and intensity pathways. The invariance of ILD-tuning with ABI would appear to be a good design strategy, since the system should encode where a sound is coming from independent of how loud the sound is.

Neurons in VLVp, however, are generally sensitive to both ILD and ABI (Manley, Koeppl et al. 1988), suggesting that this invariance emerges only at the level of the inferior colliculus. The model I present here could qualitatively account for this observation. The response to ILD in one VLVp will shift with ABI symmetrically to the way the response shifts in the VLVp on the opposite side of the brain. Bilateral inhibition of collicular cells by VLVp will thus tend to cancel any shift of ILD with ABI by symmetry.

The model that I present in Figures 30 and 31 is the only model that accounts for the hodological and physiological results reported. However, none of the other models are conclusively ruled out. It is possible that several, different models may in combination be a correct description of the mechanisms that underlay peaked ILD-response functions. Further experiments on the microcircuitry of connections to and within the lateral shell, and of the transmitters used, are necessary to decide the status of all these models.

Figure 4.30: **Model of the contribution of VLVp to selectivity for ILD in the inferior colliculus.** Schematic of the bilateral projections from VLVp to the inferior colliculus (IC). It is proposed that the IC obtains inhibitory input (solid circles) from VLVp, and excitatory input (open triangle) from unknown sources projecting in the lateral lemniscus. The excitation need not be selective for ILD (dotted line on response-ILD graph for IC). Rather, selectivity for ILD is shaped by inputs from the VLVp to generate the bell-shaped curve (response versus ILD, top graph). It is known that the VLVp on each side are also connected reciprocally and inhibit each other, resulting in sigmoid response curves to ILD. This commissural connection also permits VLVp to influence responses in the colliculus via two routes: directly, and indirectly through the opposite VLVp. The abscissa on all graphs represent stimuli loud at the left ear on the left side, and stimuli loud at the right ear on the right.
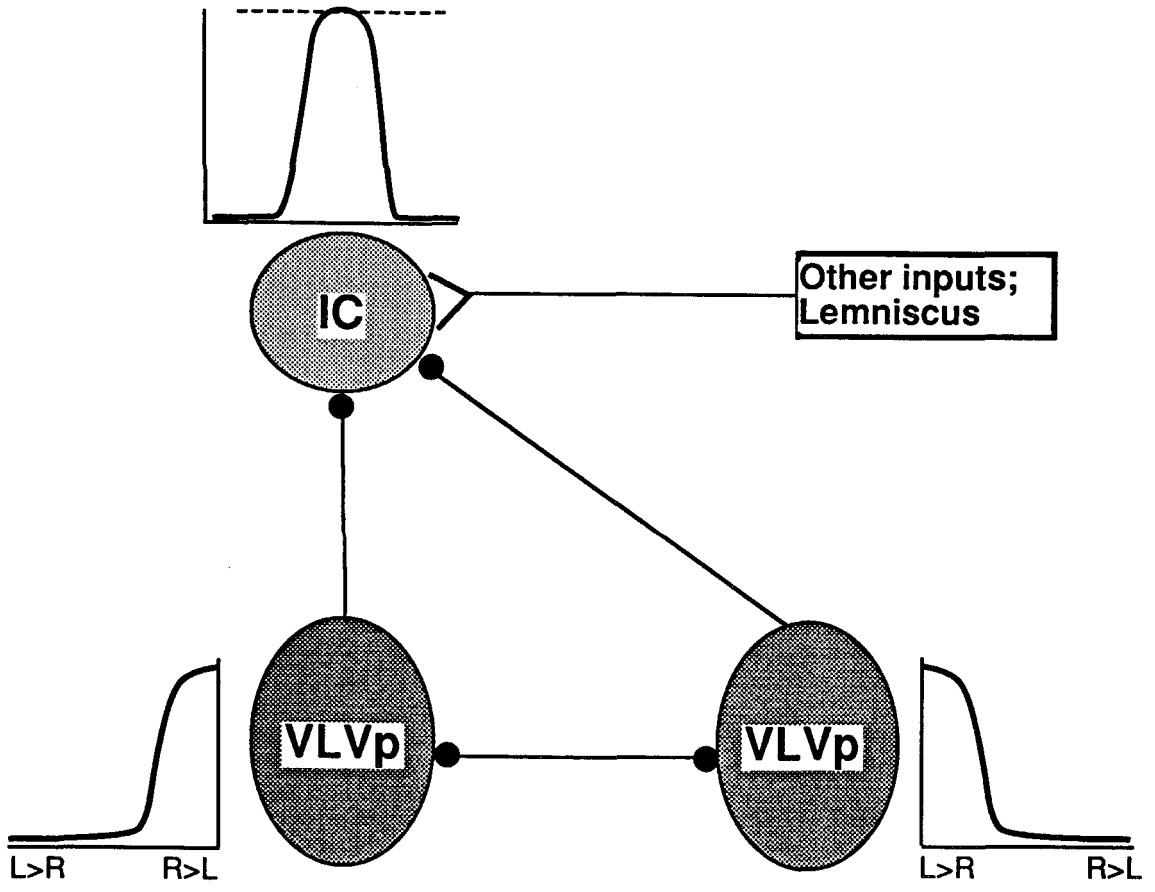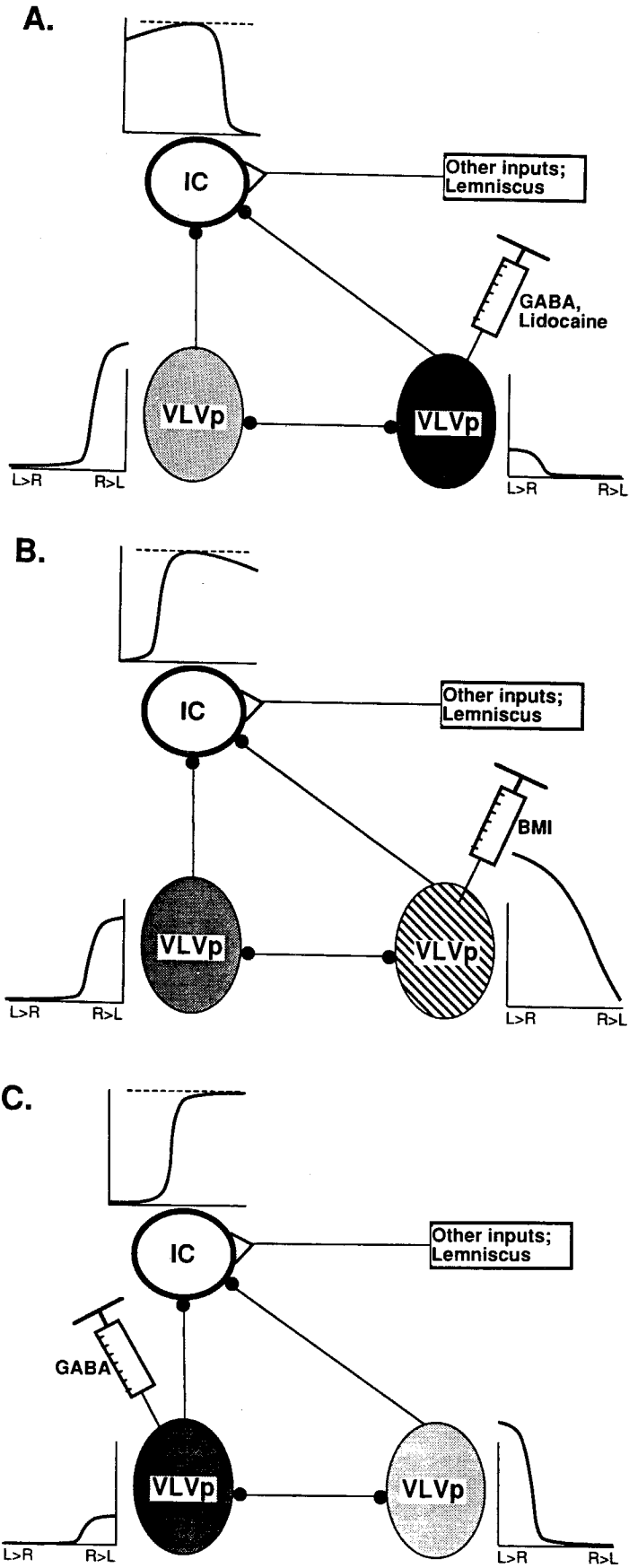
Figure 4.31: **The activity in VLVp affects the response to ILD in the colliculus. A.** The results from injecting the contralateral VLVp with lidocaine or with GABA can be explained with this scheme. Injecting the VLVp attenuates the response within the nucleus (and may variably increase the response in the other VLVp). Since the inhibition that the injected VLVp normally provides is now less, the ILD-selective neuron in the colliculus will respond at ipsilaterally-loud ILD that previously inhibited it. **B.** Similarly, injecting BMI into the VLVp increases the response within that nucleus (striped region), and may decrease the response in the other VLVp. The neurons in the colliculus show a change in response that is qualitatively the converse of that seen in B. **C.** Injecting GABA into the ipsilateral VLVp abolishes the inhibition at contralaterally-loud ILD in the colliculus, again the opposite from A. Darker shading denotes decreased neural activity in VLVp; hatching denotes increased neural activity.

A.

IC

Other inputs;
Lemniscus

GABA,
Lidocaine

VLVp

VLVp

L>R   R>L

L>R   R>L

B.

IC

Other inputs;
Lemniscus

BMI

VLVp

VLVp

L>R   R>L

L>R   R>L

C.

IC

Other inputs;
Lemniscus

GABA

VLVp

VLVp

L>R   R>L

L>R   R>L

Comparison with Mammals and with other Owl Species

The auditory brainstem of the barn owl shares several features in common with mammalian sensory systems. Neural maps of sensory space, parallel processing streams, and hierarchical architectures are found both in the owl (Konishi 1986; Konishi et al. 1988), and in such well studied mammalian sensory systems as the primate visual system (DeYoe and Van Essen 1988; Van Essen et al. 1990) and the biosonar system of the bat (Suga 1988, 1989). Aside from these gross processing similarities, however, it is difficult to find counterparts to the owl's auditory brainstem circuitry. The auditory brainstem of mammals is both anatomically and physiologically more complex.

It has been suggested that some of the auditory nuclei in the owl are analogous to nuclei found in mammals; in particular, it has been suggested that VLVp might be analogous to the dorsal nucleus of the lateral lemniscus (DNLL) in mammals, on the basis of response properties and hodology (Takahashi and Konishi 1988b; Takahashi and Keller 1992). Most neurons in DNLL respond to binaural stimuli (Aitkin, Anderson et al. 1970); nearly all are excited by sound loud at the contralateral ear. Some low-frequency neurons in the nucleus are sensitive to phase disparities, but nearly all neurons tuned to higher frequencies are excited by the contralateral ear and either unresponsive to or inhibited by the ipsilateral ear ("EO" or "EI"; Brugge, Anderson et al. 1970). These binaural response classes are also common in the inferior colliculus in mammals, and one proposal is that the DNLL provides inhibitory input both to the colliculus and to the opposite DNLL (Shneiderman, Oliver et al. 1988). Like VLVp, the DNLL on the two sides are connected via a reciprocal commissure. Unlike VLVp, however, the DNLL appear to be connected point-to-point, rather than in a criss-cross fashion (Shneiderman, Oliver et al. 1988). There is support

for the inhibitory role of DNLL from findings that many of its projection neurons appear to be GABAergic (Adams and Mugnaini 1984; Moore and Moore 1987).

Another mammalian nucleus with binaural responses sensitive to ILD is the lateral nucleus of the superior olive (LSO), which is excited by sounds loud at the ipsilateral ear and inhibited by sounds loud at the contralateral ear (Boudreau and Tsuchitani 1968), just the opposite of VLVp. Like VLVp, the LSO receives a direct excitatory projection and an indirect inhibitory projection from a cochlear nucleus (Glendenning, Hutson et al. 1985; Caspary and Finlayson 1989). This hodology supports models in which ILD-coding is derived by a comparison between the firing rates of neurons in cochlear nuclei on each side (Colburn and Moss 1981). The inhibition in the LSO appears to be glycinergic (Caspary 1990), although both GABA and glycine are found in the LSO (Helfert, Bonneau et al. 1989). Some studies have suggested a gradient of inhibitory input in the LSO across the medio-lateral dimension, such that medial (high-frequency) LSO neurons receive denser glycinergic inputs than do more lateral (lower frequency) regions (Glendenning 1985; Sanes, Geary et al. 1987). A recent report suggests, however, that the physiology may not exhibit any inhibitory gradient; low-frequency neurons in the LSO also exhibit binaural inhibition, and they are additionally sensitive to phase (Finlayson and Caspary 1991).

Both the DNLL and the LSO project to the central nucleus of the inferior colliculus in mammals. The LSO projects bilaterally to the inferior colliculus (Moore 1988; Glendenning and Masterton 1983; Brunso-Bechtold, Thompson et al. 1981) and appears to provide both glycinergic inhibition and non-NMDA receptor-mediated excitation (Saint Marie, Ostapoff et al. 1989). The DNLL also projects bilaterally to the ICc (Roth, Aitkin et al. 1978; Shneiderman, Oliver et al.

1988), and may use GABA as an inhibitory transmitter (Adams and Mugnaini 1984).

Cells in the inferior colliculus of mammals exhibit a heterogeneity of response properties. Although some studies have reported a very discontinuous representation of ILD in the mammalian colliculus (Caird and Klinke 1987), there are now several studies that show ILD may be represented in an organized fashion in the inferior colliculi of bats (Wenstrup, Ross et al. 1986), and, perhaps more coarsely, of cats (Schreiner and Langner 1988; Irvine and Gago 1990). Psychophysical and acoustic studies suggest that mammals use ILDs to localize high-frequency sources in azimuth (Stevens and Newman 1936; Casseday and Neff 1973; Martin and Webster 1987), and possibly also to localize spectrally complex sounds in two dimensions (Martin and Webster 1989). In mammals, it appears that the ILD-tuning of cells can also be a function of average binaural intensity (Semple and Kitzes 1987; Irvine and Gago 1990), and of ITD (Yin, Hirsch et al. 1985; Caird and Klinke 1987; Pollak 1988). The barn owl has the advantage that the cue of ILD has a particularly clear neuroethological relevance in the localization of sound sources along one coordinate of auditory space (Moiseff 1989a,b). Unlike in mammals, ILD is processed by a pathway that is completely separate from processing of ITD.

It is interesting to compare the barn owl to other owl species. Although no other owl has been studied as intensively as the barn owl, there is physiological data on the great horned (Volman and Konishi 1989), long eared (Volman and Konishi 1990), and saw whet owl (Wise, Frost et al. 1988). The use of ILD as a cue for elevation relies on a vertical asymmetry in the directional sensitivities of the ears at high frequencies (Payne 1971; Coles and Guppy 1988; Moiseff 1989b). Ear asymmetry appears to have arisen independently in the evolution

of several species of owl (Norberg 1977; Volman 1990). Volman and Konishi (1989) examined the tuning properties of neurons in the inferior colliculus of the great horned owl, which has symmetrical ears, and found that in this owl auditory spatial receptive fields are not limited in elevation. In view of the utility of ears with vertical asymmetry, it is surprising that no animals other than certain species of owl appear to have evolved this feature, suggesting that perhaps aspects of the owl's auditory system are pre-adapted to take advantage of the occurrence of peripheral asymmetries (Volman 1990). Support for this hypothesis comes from the finding that cells in the great horned owl's ICx, while having auditory spatial receptive fields that are unlimited in elevation, show tuning to ILD (although this is not as sharp as in the barn owl). Perhaps owls with symmetrical ears possess much of the same brainstem circuitry as the barn owl, but their peripheral specializations do not support the use of ILD as a cue for elevation.

What purpose might tuning for ILD serve in a species such as the great-horned owl? Two possibilities that Volman and Konishi (1989) point out are that ILD serves as a cue for horizontal sound source position that is supplementary to ITD, or that the binaurally exclusive response properties seen in the ICx serve to provide a window within which other responses are permitted. For example, inhibition of monaural responses could serve to filter out spontaneous activity that is afferent from lower, monaurally responsive nuclei. Since the computation of ITD can only be made for binaural stimuli, such filtering could serve to increase the signal-to-noise of coding for ITD in the ICx. Such a function is also not ruled out in the barn owl, although in this species it is clear that ILD codes for aspects of sound source location as well. Future behavioral studies could address some of these issues. The results and models I have presented would predict that blocking activity in VLVp would lead

to errors predominantly in localizing sounds in elevation, since tuning to ILD represents vertical source position. If binaural exclusiveness serves other purposes, such an experiment might reveal a broader impairment in localizing sounds anywhere in space.

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