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Author

Sahley, Tony L.

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INVESTIGATIONS INTO THE POSSIBLE ROLE OF ENDOGENOUS COCHLEAR OPIOIDS ON LATERAL EFFERENT OLIVOCOCHLEAR MODULATION OF AUDITORY NERVE ACTIVITY IN THE CHINCHILLA

by

Tony L. Sahley

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INVESTIGATIONS INTO THE POSSIBLE ROLE OF ENDOGENOUS COCHLEAR OPIOIDS ON LATERAL EFFERENT OLIVOCOCHLEAR MODULATION OF AUDITORY NERVE ACTIVITY IN THE CHINCHILLA

Tony L. Sahley
Division Of Speech and Hearing Sciences
Department Of Otolaryngology
University Of California
San Francisco, California 94143

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Dedicated to my wife Jill, whose love, patience and support made this work possible.

Investigations Into The Possible Role Of Endogenous Cochlear Opioids On Lateral Efferent Olivocochlear Modulation Of Auditory Nerve Activity In The Chinchilla.

Tony L. Sahley

Abstract: Axons of olivocochlear cell bodies in the pontine lateral superior olivary region belonging to the "lateral efferent system" synapse directly upon the peripheral processes of spiral ganglion neurons that innervate inner hair cells in the cochlear organ of Corti. Medial periolivary nuclei send "medial efferent system" projections directly to the bases and circumnuclear regions of outer hair cells of the organ of Corti. The lateral efferent system termination on Type I ganglion cell afferent fibers suggests that these centrifugal projections modulate the sensitivities and spontaneous discharges of primary auditory system inputs. Both anatomical and developmental evidence suggest that lateral efferent synapses are inhibitory. Furthermore, products of the proenkephalin and prodynorphin opioid peptide families are found within lateral efferent terminals. Enkephalins primarily activate δ-opioid receptors, and their central nervous system effects are usually inhibitory. Dynorphins are K-opioid receptor agonists, and their effects are inhibitory as well.

In this study, the effects of intravenously administered opioid agonists on click-evoked, round window-recorded N1 and N2 components of auditory nerve compound

action potential and cochlear microphonic (mass receptor potential) responses were investigated in the chinchilla. Parenteral administration of μ-opioid receptor ligands like naloxone and fentanyl failed to alter these cochlear-generated neural and receptor potential responses. The racemic benzomorphan (±)-pentazocine (16mg/kg) and its levorotatory isomer (-)-pentazocine (8mg/kg) caused significant positive amplitude changes in CAP N1 and N2 response components relative to baseline values at near-threshold stimulus intensities. Following pentazocine, absolute response thresholds were improved by 5 to 7dB SPL. CAP response latencies and CM amplitudes were unaffected. By contrast, the non-opioid, dextrorotatory isomer (+)-pentazocine (8mg/kg) was without effect on all dependent measures, and its infusion did not affect neural response thresholds. These neural effects of (-)-pentazocine may have been partially blocked by the potent K-receptor antagonist nor-binaltorphimine, applied to the cochlear round window.

These data support the conclusion that K-opioid receptors are involved in the mediation of opioid efferent effects in the inner ear. Functional implications of these findings are discussed.

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group. As indicated in the text, the grand mean for a treatment group is defined as the mean obtained over the 0-90 minute baseline periods. Drug injection was initiated just after the 90 minute benchmark.

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Figure 4: (A) CAP evoked response in a representative chinchilla recorded before (lower trace) and after (upper trace) an iv injection of 16mg/kg (±)-pentazocine. These responses were evoked by near-treshold (in this animal 30dB SPL) stimulation, and displayed negative-up. (B): Effects in the same animal of 16mg/kg (±)-pentazocine on the amplitudes of the far-field auditory brainstem response waves I-IV, recorded at near-threshold. The lower ABR was recorded during baseline. The middle and upper ABRs were recorded 30 and 60 minutes respectively, post iv infusion of (±)-pentazocine. All wave potentials are displayed negative up.

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CHAPTER 1

The Efferent Olivocochlear System

General Overview: The olivocochlear (OC) system consists of axons arising from neurons within the brainstem superior olivary region, and supplies bilateral efferent innervation to the cochlea. These descending OC efferent fibers can be separated into two anatomically and presumably functionally distinct divisions, designated as the "lateral" and "medial" efferent systems (e.g. Liberman and Brown, 1985; 1986; Spangler and Warr, 1991; Warr, 1975; 1978; 1980, 1988; Warr, Guinan and White, 1986). Lateral efferent neurons project preferentially to the radial Type I afferent dendrites (peripheral processes) within the inner hair cell (IHC) region, bilaterally, but with predominantly uncrossed fibers. Medial OC neurons project preferentially to basal and circumnuclear regions of the outer hair cells (OHCs), also bilaterally, but with predominantly crossed fibers (Spangler and Warr, 1991; Warr, 1978; 1980; 1988; Warr et al., 1986).

The anatomy notwithstanding, virtually nothing is known regarding the function of lateral efferent OC neurons. Several functional roles have been postulated for the medial efferent OC neurons, however (Cody and Johnstone, 1982b; Dewson, 1967; Nieder and Nieder 1970a; 1970b; Liberman, 1988a; Tolbert, Morest and Yurgelun-Todd, 1982). Considerable physiological evidence indicates that the

medial efferent system may compensate for the compressive effects of noise (e.g. Costalupes, Young and Gibson, 1984; Gibson, Young and Costalupes, 1985; Winslow, 1988; Winslow and Sachs, 1984; 1985; 1987) by reducing the noise-driven response rates of single auditory fibers (Dewson, 1967; Nieder and Nieder 1970a; 1970b). This then, restores the range of discharge rates available for encoding intensity changes in a stimulus. Indeed, medial efferent activation produces a four-fold increase in Type I auditory fiber rate-level response (dynamic) range, allowing for the encoding of intensity changes in noise backgrounds at relatively lower signal/noise ratios (Winslow, 1988; Winslow and Sachs, 1984; 1985; 1987).

Acetylcholine (ACh) is an important neurotransmitter for both the lateral and medial divisions of the descending efferent OC system (e.g. Altschuler and Fex, 1986; Bledsoe, 1986; Bledsoe, Bobbin and Puel, 1988; Eybalin and Altschuler, 1990; Eybalin and Pujol, 1987), and cholinergic receptors have been detected on mammalian OHCs (Plinkert and Zenner, 1989; Zenner, Reuter, Plinkert, Zimmermann and Gitter, 1989). Recent evidence indicates that these receptors may be of the muscarinic M_3 variety (Guiramand, Mayat, Bartolami, Lenoir, Rumigny, Pujol and Recasens, 1990a; Guiramand, Mayat, Bartolami, Lenoir, Pujol and Recasens, 1990b), though there is also evidence in support of nicotinic OHC receptors (Canlon, Cartaud and Changeux,

1990; Plinkert and Zenner, 1991; Plinkert, Zenner and Heilbronn, 1991).

There is substantial evidence that products of proenkephalin and prodynorphin synthesis occur endogenously within brainstem efferent OC cell bodies, and within efferent fiber bundles and terminals innervating the cochlea (Altschuler and Fex, 1986; Altschuler, Reeks, Fex and Hoffman, 1988; Eybalin and Altschuler, 1990; Fex and Altschuler, 1981; 1985; 1986; Eybalin and Altschuler, 1990). Indeed, proenkephalin and prodynorphin gene products coexist within the same lateral efferent cell bodies (Abou-Madi, Pontarotti, Tramu, Cupo and Eybalin, 1987; Altschuler et al., 1988). There is additional evidence of multiple opioid receptor interaction within the cochlea (Eybalin, Pujol and Bockaert, 1987). Such evidence supports the co-localization and/or coexistence of multiple opioid receptors within the terminals of lateral efferent fibers and/or postsynaptic target sites within the cochlea. Taken together with the evidence that products from both opioid peptide families also coexist with choline acetyltransferase (ChAT) within the same lateral efferent perikarya (Abou-Madi et al., 1987; Altschuler, Fex, Parakkal and Eckenstein, 1984a), a potentially complex neuromodulatory role within the cochlea is suggested for endogenous neuroactive opioid substances and ACh. What follows is a condensed review of the anatomy, physiology, and neurochemistry of the mammalian lateral and medial efferent systems.

Superior Olivary Complex Anatomy

The superior olivary complex (SOC) consists of a number of small nuclear masses located within the pontine tegmentum. In the cat, about twelve distinct nuclei have been identified (e.g. Warr, 1972). The three principal nuclei associated with the ascending auditory pathway are the lateral superior olive (LSO), the medial or accessory superior olive (MSO), and the medial nucleus of the trapezoid body (MNTB) (Stotler, 1953). Both the MSO and LSO receive second or third order auditory input from lower brainstem regions (see: Helfert, Snead and Altschuler, 1991) and both structures are well developed in the guinea pig, dog, monkey and cat (Harrison and Howe, 1974a). In the cat as in most species where it is well developed, the thin crescent-shaped MSO consists of large radially oriented bipolar and multipolar neurons (Harrison and Feldman, 1970; Scheibel and Scheibel, 1974; Stotler, 1953).

The LSO is occupied by spindle-shaped fusiform neurons and by a smaller number of round or star-shaped cells arranged in irregular layers (Boudreau and Tsuchitani, 1970; Cant, 1984; Goldberg and Brown, 1968; Harrison and Feldman, 1970; Scheibel and Scheibel, 1974; Tsuchitani, 1978). The response properties of LSO units are known and have been investigated (e.g. Tsuchitani, 1977). The layers of cells within the LSO create a convex-concave "S" configuration (Boudreau and Tsuchitani, 1970; Cant, 1984; Scheibel and Scheibel, 1974). The concavities created by the S-shaped

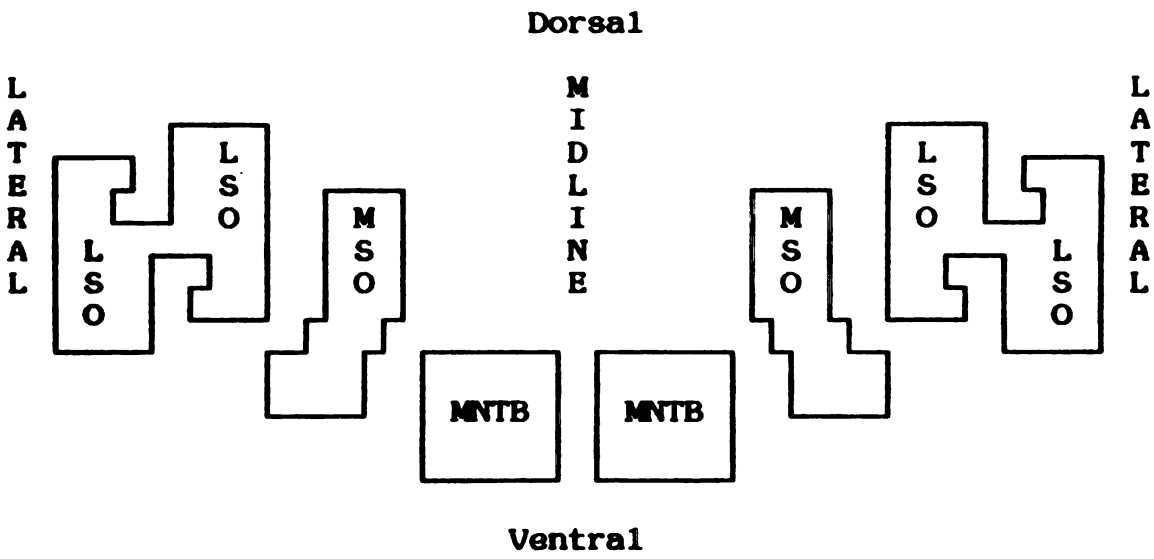
cellular arrangement form a ventromedially directed medial hilus, and a dorsomedially directed dorsolateral hilus (Stotler, 1953). The dorsolateral hilus in particular is a region into which many afferent fibers enter, and from which many lateral efferent fibers exit (Elverland, 1978; Osen, Mugnaini, Dahl and Christiansen, 1984; Scheibel and Scheibel, 1974; Spangler, Warr and Henkel, 1985). The MNTB, the third major division of the superior olivary complex, is discussed below as one source of the descending medial efferent OC system of fibers. The response properties of MNTB units are known and have been investigated (Boudreau and Tsuchitani, 1968; 1970; Brugge and Geisler, 1978; Caird and Klinke, 1983; Goldberg and Brown, 1968; 1969; Guinan et al., 1972b; Tsuchitani, 1977). The response properties of the MSO are also known and have been investigated (Cant and Casseday, 1986; Goldberg and Brown, 1968; 1969; Guinan et al., 1972b; Warr, 1966). The primary nuclei of the SOC are schematically depicted below in Illustration 1.

Efferent Olivocochlear (OC) System Anatomy

Introduction: The descending OC system consists of cell bodies and axons that provide centrifugal (efferent) innervation to the cochlea. The early work of Rasmussen (1942; 1946; 1953; 1955) established what has since been confirmed as a projection of axons originating bilaterally in the region of the superior olivary nuclei of the brainstem. The descending fibers travel via crossed or un-

Illustration 1

Highly schematized frontal view illustrating the major auditory nuclei of the brainstem superior olivary complex.



crossed midline brainstem pathways and terminate at two separate and distinct target regions within the organ of Corti. The early observations made by Rasmussen have been extended by others in a variety of preparations employing various histologic methods such as lesion/degeneration or ACh histochemistry combined with light or electron microscopy. Those species investigated to date have included mice (Nakai, 1972; Osen et al., 1984) chinchillas (Iurato, Smith, Eldredge, Henderson, Carr, Ueno, Cameron and Richter, 1978; Osen et al., 1984) guinea pigs (Hilding and Wersall, 1962; Kimura and Wersall, 1962; Smith, 1975; Wright, 1975; Wright and Preston, 1973; 1975), rats (Osen et al., 1984) rabbits (Borg, 1973) cats (Arnesen and Osen, 1984; Liberman, 1980b; Morest, 1968; Osen et al., 1984; Osen and Roth, 1969; Spoendlin, 1969; 1972; Spoendlin and Gacek, 1963) monkeys (Bodian, 1983; Ishii, Murakami and Gacek, 1967) and humans (Gacek, 1961; Nadol, 1983a; 1983b). The use of horseradish peroxidase (HRP) axonal transport labeling methods (LaVail and LaVail, 1972; 1974) for tracing neural pathways has confirmed earlier observations, and has led to an even clearer understanding of the origin and termination of descending efferent OC neurons.

Substantial evidence has accumulated supporting the existence of two separate descending efferent OC fiber systems. There are "lateral" and "medial" efferent systems of descending fibers. The two systems differ in a number of morphologically and presumably functionally distinct ways

(Spoendlin, 1988; Warr, 1988). The lateral and medial efferent olivary cell bodies have also been positively identified by retrograde transport investigations using HRP in the cat (Adams, 1983; Spangler and Henkel, 1982; Warr, 1975; 1978; 1980; Warr, White and Nyffeler, 1982; Warr et al., 1986), rat (White, 1984; White and Warr, 1983), guinea pig (Strutz, 1981; Strutz and Bielenberg, 1984; Strutz and Spatz, 1980; Thompson, Cortez and Igarashi, 1984), chinchilla (Bianchi and Salvi, 1990) and primate (Strominger, Silver, Truscott and Goldstein, 1981; Thompson et al., 1984). With the added results from investigations employing anterograde axonal transport of tritiated amino acids (Brown, 1985a; 1985b; 1987b; Guinan et al., 1983; 1984; Liberman, 1982; Liberman and Brown, 1985; 1986; Robertson, 1984; Warr, 1978; 1980; Warr and Guinan, 1979; Warr et al., 1986), and fluorescent tracers (Robertson, Cole and Harvey, 1987), it is now well established that the medial group of descending efferent OC axons project to the basal and circumnuclear regions of the cochlear outer hair cells. The lateral efferent fiber division, on the other hand, projects to dendrites of Type I ganglion cells, innervating the inner hair cells (IHCs) in the mammalian cochlea (e.g. Warr, 1988).

Organization Of Efferent OC Nuclei: A conglomerate of smaller cell groups identified as either "periolivary nuclei", or as "nuclei of the trapezoid body" surround the larger principal SOC nuclei (e.g. Aitkin, Irvine and

Webster, 1984; Boudreau and Tsuchitani, 1970; Caird and Klinke, 1983; Elverland, 1978; Goldberg and Brown, 1968; Harrison and Feldman, 1970; Harrison and Howe, 1974b; Morest, 1968; 1983; Stotler, 1953; Tsuchitani, 1977; 1978; Warr, 1969; 1972; 1975; 1978; 1980; 1982). *These small-cell clusters are the locus of origin of the two morphologically distinct efferent axon bundles which differ with respect to: a) the size of their perikarya of origin (Adams, 1983; Osen et al., 1984; Spoendlin, 1988; Strutz and Bielenberg, 1984; Warr, 1972; 1975; 1978; 1980; Warr and Guinan, 1979; White, 1983; White and Warr, 1983); b) their specific loci of origin (Osen et al., 1984; Strutz and Spatz, 1980; Warr, 1972; 1975; 1978; 1980; Warr and Guinan, 1979); c) their postsynaptic targets within the cochlea (Guinan et al., 1983; Warr, 1978; 1980; Warr and Guinan, 1979); d) their preferred side of projection (Guinan et al., 1983; 1984; Warr, 1978; 1980); and (e) their pattern of development (Ginzberg and Morest, 1983; 1984; Pujol, 1985; Pujol, Carlier and Devigne, 1978; 1979; Pujol, Carlier and Lenoir, 1980; Whitehead, 1986).

The Lateral And Medial Efferent Systems

Lateral Efferent System: The lateral group of efferent OC nuclei lie in close proximity to (i.e. on the margins), or directly within the LSO as in rodent species such as rats, mice, guinea pigs, and chinchillas (Altschuler, Parakkal and Fex, 1983; Bianchi and Salvi, 1990; Osen et

al., 1984; Osen and Roth, 1969; Robertson et al., 1987; White and Warr, 1983). In the cat and in some (i.e. Strutz, 1981; Strutz and Bielenberg, 1984; Strutz and Spatz, 1980), but not all reports in the guinea pig (i.e. Altschuler et al., 1983; Bianchi and Salvi, 1990; Robertson et al., 1987), lateral efferent cell bodies are predominantly concentrated within: the dorsolateral hilus (H), within the dorsal and dorsolateral periolivary nuclei (DPO and DLPO), and within the lateral nucleus of the trapezoid body (LNTB) (see: Osen et al., 1984; Osen and Roth, 1969; Spangler and Warr, 1991; Warr, 1975; 1978; 1980; Warr et al., 1986)*. The lateral efferent OC nuclei together with the principal auditory nuclei of the SOC are schematically depicted below in Illustration 2.

The location of the LSO hilus was discussed earlier. As depicted in Illustration 2, the DPO nucleus is located dorsal and slightly medial to the LSO, while the DLPO nucleus is located dorsal and just lateral to the S-segment. These two small nuclei are separated by fibers entering and leaving the dorsal hilus. In the cat, the LNTB is bounded ventromedially by the VNTB, and consists of a dense plexus of cell bodies, dendrites and afferent collaterals that lie in close proximity to the ventral border of the LSO. The boundaries between the LNTB and the non-olivocochlear ven-

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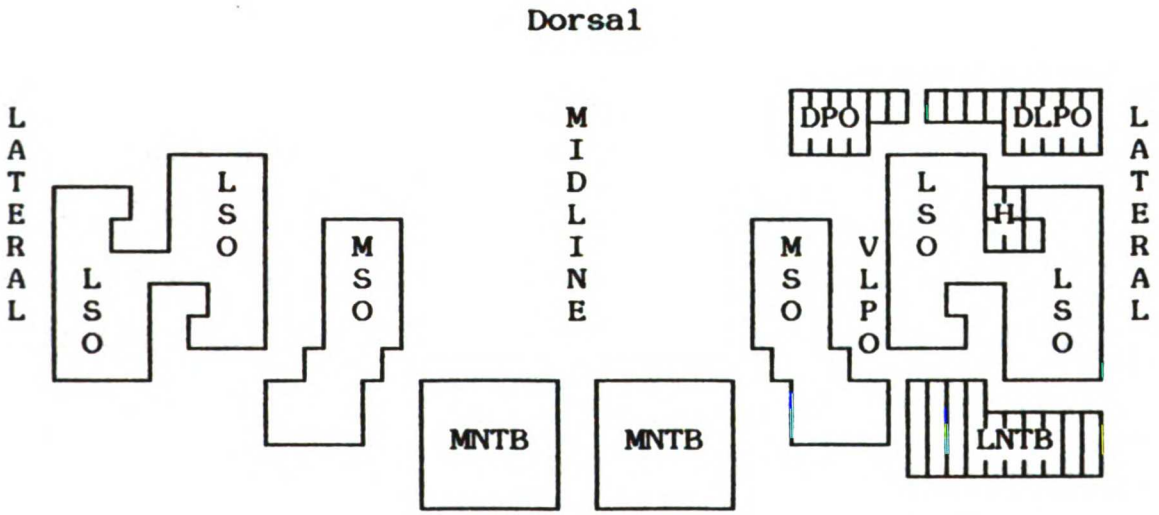
* The dorsolateral periolivary nucleus (DLPO) has also been referred to as the anterolateral periolivary nucleus. The lateral nucleus of the trapezoid body (LNTB) is sometimes referred to as the posterior periolivary nucleus, or as the lateral preolivary nucleus.

Illustration 2

Highly schematized frontal view illustrating the distribution of lateral efferent olivocochlear nuclei (vertically hatched) of the brainstem superior olivary complex. The lateral efferent nuclei DPO, DLPO, Hilus (H), and LNTB are shown together with the primary olivary nuclei (LSO, MSO and MNTB) on the right. Only the principal olivary nuclei are shown on the left.



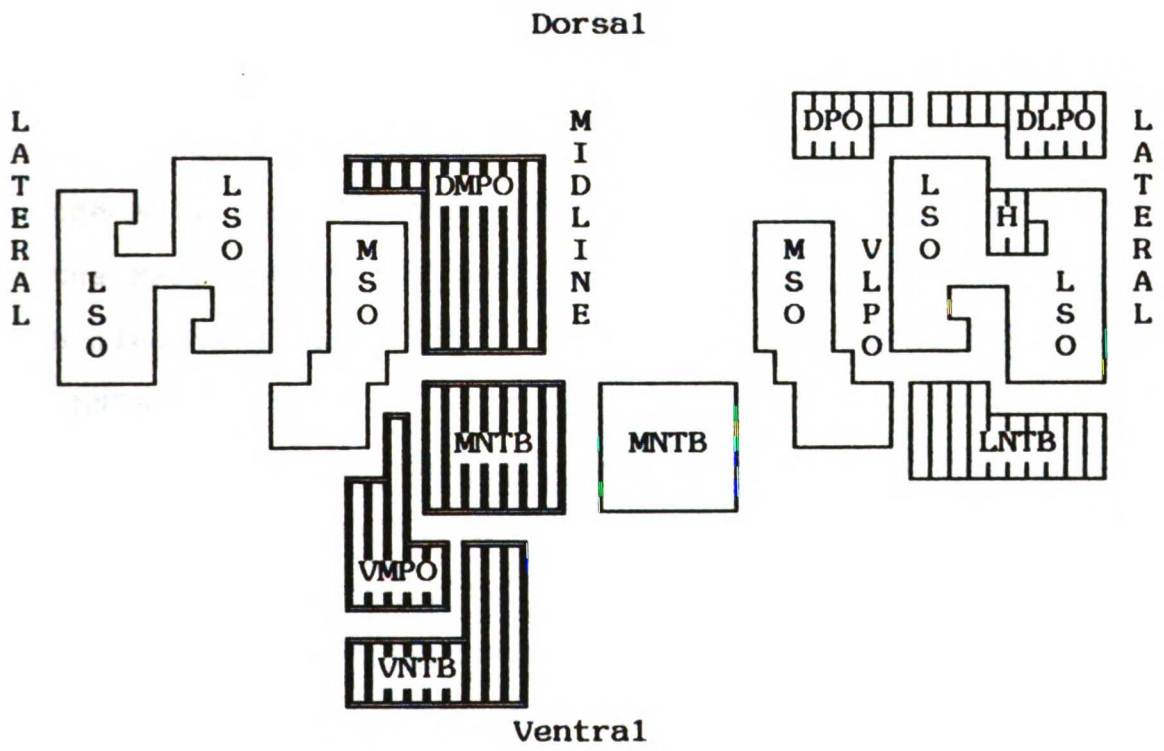
trolateral periolivary nuclei (VLPO) are poorly defined (e.g. Adams, 1983; Boudreau and Tsuchitani, 1970; Elverland, 1978; Goldberg and Brown, 1968; Guinan et al., 1972b; Harrison and Howe, 1974b; Osen et al., 1984; Tsuchitani, 1977; Warr, 1975; 1978; Warr et al., 1986).

Medial Efferent System: Medial efferent OC perikarya are generally larger than lateral efferent cell bodies and are multipolar or radiate in shape (Adams, 1983; Morest, 1968; Osen et al., 1984; Osen and Roth, 1969; Warr, 1972; 1975; 1978; 1980; White, 1984; White and Warr, 1983). In the cat, guinea pig and chinchilla, medial OC fibers originate from the cell bodies located: a) within the dorsomedial and ventromedial periolivary nuclei of the MSO (DMPO and VMPO); and b) from within the medial and ventral nuclei (MNTB and VNTB, respectively) of the trapezoid body (Osen et al., 1984; Osen and Roth, 1969; Strutz, 1981; Strutz and Spatz, 1980; Thompson et al., 1984; Warr, 1972; 1975; 1978; 1980; Warr et al., 1986). Both the medial and the lateral efferent OC nuclei together with the primary auditory nuclei of the SOC are schematically shown in Illustration 3.

The MNTB is a major component of the ascending auditory pathway, having major projections to the LSO (e.g. Gacek, 1972; Morest, 1968; Stotler, 1953). The MNTB therefore plays a dual role in the auditory system since it also contributes descending efferent fiber bundles as part of the medial efferent OC system (e.g. Warr, 1975; 1968). The MNTB lies ventromedial to the MSO, within and slightly dorsal to the

Illustration 3

Highly schematized frontal view illustrating the medial efferent olivocochlear nuclei MNTB, DMPO, VMPO, and VNTB (double vertical hatched) together with the principal nuclei (LSO, MSO and MNTB) of the brainstem superior olivary complex depicted at the left. As in Illustration 2, the lateral efferent nuclei DPO, DLPO, Hilus (H) and LNTB (single vertical hatched) are shown together with the principal olivary nuclei at the right.



fiber bundles of the trapezoid body (Goldberg and Brown, 1968; Harrison and Howe, 1974b; Morest, 1968; Osen et al., 1984; Stotler, 1953). Within the MNTB, the large scattered stellate cells (also referred to as multipolar or radiate cells) sampling a wide range of CFs (Morest, 1968; Osen et al., 1984; Stotler, 1953; Tsuchitani, 1978; Warr, 1982) are important since they project many descending fibers into the medial efferent OC system (e.g. Warr, 1975; 1988).

The DMPO ("retro-olivary nucleus" of Rasmussen, 1946) is a major contributing source of the crossed medial OC system (Warr, 1975). It is located just lateral to the abducens nerve rootlets dorsal to the MNTB and dorsomedial to the MSO. The VMPO nucleus is positioned directly ventral to the MSO, lateral to the MNTB and dorsal to the VNTB. The VNTB lies ventral to the MSO and VMPO and ventrolateral to the MNTB (e.g. Guinan et al., 1972b; Harrison and Feldman, 1970; Harrison and Howe, 1974b; Morest, 1968; Warr, 1975; Warr et al., 1986). In the rat and mouse, medial OC perikarya are confined to the more rostral region of the VNTB (Osen et al., 1984; White, 1984; White and Warr, 1983). In primates, the overall distribution of medial OC neurons is similar to the distribution observed in cats (Strominger et al., 1981; Thompson, et al., 1984). The locations of the medial efferent nuclei (MNTB, DMPO, VMPO, and VNTB) are schematically depicted in the above Illustration 3.

Terminal Distribution Of Descending Olivocochlear (OC) Fibers

General Fiber Pathways: The course of descending efferent OC fibers originally described by Rasmussen (1946; 1953) has been confirmed and is well documented (e.g. Arneson and Osen, 1984; Godfrey, Park and Ross, 1984; Osen et al., 1984; Warr et al., 1986; White and Warr, 1983). In general, OC fibers pass dorsally from their cell bodies through the reticular formation to the floor of the fourth ventricle. Together with the crossed vestibular efferents, OC axons form a compact bundle just ventral to the anterior end of the facial nerve genu. In the cat, the region where efferent fibers pass is about 8 to 12mm rostro-lateral to the obex (Galambos, 1956). At that location, fibers destined to travel ipsilaterally veer laterally, coursing through or around the descending facial nerve root or its genu (knee). Those fibers destined to cross the midline toward the contralateral cochlea, turn medialward to join with uncrossed OC and vestibular fibers. Lateral to the genu near the dorsal border of the descending root of the trigeminal nerve, axons from the ipsilateral vestibular efferents join with crossed and uncrossed OC fibers, and together with the crossed vestibular efferents course beneath the vestibular nuclei to the dorsal aspect of the trigeminal tract (Iurato, 1974). Continuing laterally as a well-defined bundle within the vestibular nerve root, efferent fibers pass the rostral pole of the ventral cochlear nucleus (VCN) and send

collateral projections into the cochlear nuclear complex before exiting from the brainstem with the vestibular nerve (Osen and Roth, 1969).

Efferent fibers travel with the vestibular nerve as it exits from the brainstem, and emerge from the inferior surface of the distal part of the saccular ganglion. They then enter the cochlea between the basal and second turn at the external margin of Rosenthal's canal and the spiral ganglion, via the vestibulocochlear anastomosis of Oort in the fundus of the internal auditory meatus (Arnesen and Osen, 1984; Brown, 1987b; Iurato, 1974). Traveling with myelinated (1.5 to 6.0μm diameter) and unmyelinated (0.1 to 1.0μm in diameter) fibers of the auditory nerve, efferent axons pass radially among the spiral ganglion cell bodies within Rosenthal's canal, turn, and proceed apicalward in the cat as 0.7 to 1.5μm fibers, and in the guinea pig as 0.2 to 2.0μm fibers of the (IGSB) intraganglionic spiral bundle (Arnesen and Osen, 1978; 1984; Brown, 1985b; 1987b; Gacek, 1972; Liberman and Brown, 1986; Robertson, 1984; Spoendlin, 1969; 1972; 1978; 1979). In the cat, efferent fibers traveling to the basal end of the cochlea may pass from the anastomosis of Oort to the organ of Corti without becoming part of the IGSB (Nomura and Schuknecht, 1965).

Turning outward from the IGSB, medial efferent fibers lose their myelin (Brown, 1985a; Liberman and Brown, 1986), and both medial and lateral efferent fibers travel collectively with auditory afferent dendrites within and to

the very edge of the osseous spiral lamina. Unlike the efferents, the radial Type I auditory afferents remain myelinated up to this location (Spoendlin, 1967; 1978; 1988). Efferent fibers enter the organ of Corti via the habenula perforata (foramin nervosa) alongside the exiting auditory afferent fibers (Lim, 1986; Smith, 1967; Spoendlin, 1967; 1978). Ten to twenty thin, unmyelinated fibers pass through each habenular opening (Spoendlin, 1967), and each fiber is surrounded by extensions of a single Schwann cell situated near the ventral surface of each aperture. Schwann cells bind the separate fibers, and serve to maintain a 200^{A} gap between each (Liberman, 1980b; Spoendlin, 1978). Within the organ of Corti, all fibers remain unmyelinated, although many increase in thickness distal to the habenular region (Liberman, 1980b; Spoendlin, 1978).

Medial Efferent Innervation Of The Cochlea: Myelinated axons (0.5 to 2.8μm) arise from the generally larger medial efferent cell bodies (Arnesen and Osen, 1984; Osen et al., 1984). Much of what is known regarding the anatomy of the descending medial and lateral efferent fiber distributions to the cochlea, has come from investigations in the cat. Efferent innervation to the OHCs of each cochlea in the cat is supplied by about 450 to 535 neurons arising from each medial OC region (Arneson and Osen, 1984; Warr et al., 1982; 1986). Approximately 72 to 74% of those fibers travel to the contralateral cochlea. The remaining 26 to 28% course

ipsilaterally (Guinan et al., 1983; 1984; Warr, 1975). These above-cited percentages have been generally consistent across different studies in the cat (Warr, 1978; 1980; Warr and Guinan, 1979; Warr et al., 1982; 1986). In the rat, about 240 medial OC neurons innervate each cochlea, 64% of which are crossed (White and Warr, 1983). In the mouse, only about 164 medial efferent neurons innervate each cochlea, and 123 of those (or 75%) are crossed (Campbell and Henson, 1988). In the guinea pig, about 395 medial efferent neurons innervate each cochlea, and about 296 (75%) of those are crossed (Robertson et al., 1987).

The Upper Tunnel-Crossing Radial Efferents: Large medial efferent fibers lose their myelin at the IGSB (Brown, 1985a; Liberman and Brown, 1986) and pass through the habenula with fiber diameters (in the cat) measuring 0.3 to 0.6μm. These unmyelinated fibers proceed to travel to the tunnel of Corti reaching the OHCs via direct or indirect routes (Ginzberg and Morest, 1983; 1984; Iurato et al., 1978; Liberman, 1980b; Robertson, 1984; Smith, 1975; Wright, 1975; Wright and Preston, 1973; 1975). Increasing in diameter (to 0.6 to 1.6μm in the cat; 0.2 to 1.3μm in the guinea pig), 5 to 10μm from the habenular opening (Liberman, 1980b; Spoendlin, 1978), some of the larger efferent fibers destined for the OHCs spiral apically or basally within the ISB for distances of 50 to 200μm (about 2 to 8 IHCs) or more (Brown, 1987b; Smith, 1975; Wright, 1975) before angling 90°, and branching into as many as 10 upper tunnel-crossing radial (TR) fibers

(Ginzberg and Morest, 1984; Guinan et al., 1983; Liberman, 1980b; Liberman and Brown, 1985; 1986; Smith, 1975; Wright and Preston, 1975). In the guinea pig, the larger diameter medial efferent fibers in the apical cochlear turns frequently travel for long distances (e.g. 100 to 600μm) within the ISB or TSB prior to branching as upper TR fibers (Brown, 1987b). More basally located TSB-traveling fibers customarily project apically and basally for much shorter distances in this species before branching as upper TR fibers (Smith, 1975; Wright and Preston, 1975).

Many fibers exiting from the habenula also take a more direct radial course to the tunnel of Corti without branching (Brown, 1987b; Ginzberg and Morest, 1983; Iurato et al., 1978; Robertson, 1984; Wright, 1975). These radially traveling efferent fibers, destined for the OHCs, eventually pass between the inner pillar cells, run across and between the spiral traveling fibers of the TSB, and traverse the tunnel as upper TR fibers (Bodian, 1983; Brown, 1987b; Engstrom and Ades, 1972; Ginzberg and Morest, 1983; 1984; Iurato et al., 1978; Spoendlin, 1967; 1969; 1972; Spoendlin and Gacek, 1963; Wright, 1975; Wright and Preston, 1973; 1975). Possible synaptic connections between the fine (IHC) spiral and larger (OHC) radial efferents have been observed in the vicinity of the TSB in the cat (Liberman, 1980b) and guinea pig (Brown, 1987b).

Course Of The Upper Tunnel Radials: Upper TR fibers cross the tunnel of Corti midway between its floor and roof

(Ginzberg and Morest, 1983; 1984; Spoendlin, 1967; 1969; Spoendlin and Gacek, 1963). In comparison to the thin (0.5-0.7μm), floor-crossing outer spiral OHC Type II afferents (Ginzberg and Morest, 1984; Spoendlin, 1969; 1972; 1988) entering the tunnel roughly 5 to 10μm lower (Liberman, 1980b), some upper crossing TR fiber diameters in the cat may reach 2 to 4μm in the basal cochlear turn (Ginzberg and Morest, 1983). Also in the cat and guinea pig, some efferent fibers begin their crossing near the tunnel floor where they appear to contact outer spiral afferent fibers before rising to exit the tunnel at the mid-level of the outer pillar cells (Brown, 1987b; Ginzberg and Morest, 1984). Indeed, approximately 5 to 15% of radial efferents contact the Type II outer spiral bundle afferents when first entering the region of the OHCs (Bodian, 1983; Bodian and Gucer, 1980; Ginzberg and Morest, 1984; Spoendlin, 1966; 1967; 1969; 1972; Spoendlin and Gacek, 1963).

Medial Efferent Innervation Of OHCs: Exiting from the tunnel, radial efferents pass through the intercellular gaps created by the outer pillar cells, pass through the innermost space of Nuel, and into the upper cytoplasmic region of Deiters cells at the OHC base (Engstrom and Ades, 1972; Spoendlin, 1969). In the cat, most efferent fibers entering the OHC region take a predominantly radial orientation independent of their distance from the round window (Liberman and Brown, 1986; Spoendlin, 1966). Most radial efferent fibers (about 1μm in diameter) make contact

with OHCs as they course through the tiny gaps created by each cell, in each of the three rows of Deiters' cells, and most of these radial efferents synapse with the OHCs at their base (e.g. Lim, 1986; Pujol and Lenoir, 1986; Smith and Rasmussen, 1965; Spoendlin, 1966; 1988). A smaller number terminate higher, at the circumnuclear region of the OHC (Altschuler and Fex, 1986; Engstrom and Ades, 1972; Iurato, 1974; Liberman and Brown, 1986; Spoendlin, 1969; Spoendlin and Gacek, 1963; Wright and Preston, 1973).

In general, medial efferent fiber diameters are thicker, and terminal endings are larger and more numerous in the basal cochlear turns (Ades and Engstrom, 1975; Engstrom and Ades, 1972; Ginzberg and Morest, 1983; Liberman and Brown, 1986). In general, medial OC nuclei send very few projections to the apical 20-25% of either cochlea, an area representing frequencies below 1kHz (Guinan et al., 1984; Liberman, 1982; Warr et al., 1986). In both cat and guinea pig, radially traveling efferent fibers often terminate exclusively on one or two first-row OHCs (Brown, 1987b; Liberman and Brown, 1986), while many main fiber trunks arborize, sending spiral collateral projections into the second and third OHC rows. Each of these collaterals may innervate from 6 to 12 OHCs (e.g. Spoendlin, 1988) within a restricted radius of approximately 2 to 7 hair cells.

Efferent-OHC Terminals: Medial efferent terminals are much more abundant than the dendrites of Type II afferents, and are always provided postsynaptically with a reticular

network of subsurface\subsynaptic cisternae within the OHCs (Ades and Engstrom, 1975; Engstrom and Ades, 1972; Lim, 1986; Lim, Hanamure and Ohashi, 1989; Nadol, 1983b; Pujol et al., 1980; Smith and Rasmussen, 1965). The subsurface cisternae consist of stacks of lamellar endoplasmic reticulum situated just beneath the subplasma and cell membranes of each OHC (Lim, 1986; Lim et al., 1989; Saito, 1983). A second, more basally located reticular complex of subsynaptic cisternae can be found superimposed upon, and connected by ducts to the laminated subsurface cisternae that characterize the more apically oriented, non-synaptic membrane regions of the OHC (Ades and Engstrom, 1975; Ginzberg and Morest, 1984; Lim, 1986; Lim et al., 1989; Pujol and Lenoir, 1986; Saito, 1983).

Lateral Efferent Innervation Of The Cochlea: The discussion now returns to the course of the lateral efferent fibers. Lateral efferent OC perikarya give rise to descending, unmyelinated axon fibers approximately 0.3 to 0.7μm in diameter (Arnesen and Osen, 1984; Osen et al., 1984). In the cat, each lateral olivary region contributes roughly 750 to 870 efferent fibers (Arneson and Osen, 1984; Warr et al., 1982; 1986). Approximately 89 to 91% of those fibers are destined for the ipsilateral cochlear IHC region. The remaining 9 to 11% project to regions beneath the contralateral IHCs (Guinan et al., 1983; 1984; Spoendlin, 1988; Warr, 1975). In the rat, and mouse an average of 240

and 311 lateral OC neurons innervate each cochlea, respectively (Campbell and Henson, 1988; White and Warr, 1983). While the uncrossed:crossed lateral efferent fiber ratio in the cat is about 5:1, lateral OC fibers in the rat and mouse are exclusively uncrossed (Campbell and Henson, 1988; Warr, 1975; 1978; 1980; Warr et al., 1982; 1986). In the guinea pig, all but approximately a dozen out of the much larger number (839 to 977) of lateral efferent fibers are uncrossed (Robertson et al., 1987; Strutz and Bielenberg, 1984).

Inner Spiral Bundle (ISB): The fine-diameter, unmyelinated lateral OC (spiral) efferent fibers entering the organ of Corti exhibit diameters of 0.1 to 0.6μm in the cat, and 0.17 to 0.9μm in the guinea pig. Upon entry, most (84%) travel about 150 to 200μm (60 to 300μm in the guinea pig) to their IHC target areas, and branch with collaterals traveling unidirectionally in an apical or basalward direction beneath the IHCs, as a densely packed and tangled inner spiral bundle (Ades and Engstrom, 1975; Brown, 1985b; 1987b; Engstrom and Ades, 1972; Ginzberg and Morest, 1983; 1984; Iurato et al., 1978; Liberman, 1980b; Lim, 1986; Spoendlin, 1978; 1988; Wright, 1975). In the cat, the inner spiral bundle (ISB) appears to distribute more fibers basally (Spoendlin, 1979), and often appears as several distinct bundles of fine spiral efferents. Also in the cat, a small number of fine diameter ISB efferent fibers frequently branch and spiral bidirectionally for distances of 500 to

600μm, or the span of about 70-80 IHCs (Ginzberg and Morest, 1983; 1984). In the guinea pig, a small number (16%) of thin bidirectional lateral efferents can take a spiral course within the ISB for distances of >1mm (Brown, 1987b; Smith, 1975; Wright and Preston, 1973). In both the cat and guinea pig, the fine spiral fibers may bifurcate to very thin diameters, often passing radially between the inner pillar cells (Liberman, 1980b). Traveling from the ISB into the tunnel of Corti, these fine diameter efferents often resume their spiral course for extended distances, forming the fascicles (bundles) of the tunnel spiral bundle (TSB) (Brown, 1987b; Ginzberg and Morest, 1983; 1984; Iurato et al., 1978; Lim, 1986; Smith, 1975; Wright, 1975; Wright and Preston, 1973; 1975).

Tunnel Spiral Bundle (TSB): The closely spaced efferent fiber fascicles entering the TSB from the ISB typically form recurring loops, encircling one or two inner pillar cells before returning to the ISB (Brown, 1987b; Ginzberg and Morest, 1984; Iurato et al., 1978; Wright and Preston, 1973; 1975; Smith, 1975). The TSB also consists of radial fiber bundles that persistently loop apically and/or basally for distances of 30 to 50 IHCs in all turns of the cochlea (Wright and Preston, 1973). The fine diameter efferent fibers of the TSB travel within, but never cross, the tunnel of Corti, and run adjacent to the inner tunnel wall surface formed by the tunnel side of inner pillar cell bodies (Brown, 1987b; Ginzberg and Morest, 1984; Liberman, 1980b;

Lim, 1986; Wright, 1975; Wright and Preston, 1973; 1975). Fiber fascicles of the TSB course 21 to 23μm above the tunnel floor (Liberman, 1980b), loop around the inner pillar cells, and end exclusively within the IHC region together with fibers of the ISB (Brown, 1987b; Ginzberg and Morest, 1983; Wright, 1975; Wright and Preston, 1975).

Summary: Lateral efferent cell bodies in the brain stem of the cat send bilateral terminal fiber projections exclusively to the cochlear Type I dendrites, located near the IHC base and the fibers are predominantly ipsilateral (uncrossed). Medial efferent neurons give rise to bilateral, and predominantly crossed fiber projections traveling to the cochlea, that preferentially terminate upon the OHCs (e.g. Warr, 1988). Indeed, the distribution pattern of descending efferent input to the cochlea, mirrors the pattern of ascending projections from the cochlea to the lateral and medial efferent nuclei within the brainstem (see: Spangler and Warr, 1991). That is to say, ascending auditory input is predominantly ipsilateral to the lateral efferent cells of origin, while medial brainstem nuclei receive bilateral, but predominantly contralateral auditory input (see also: Adams and Warr, 1976; Aitkin et al., 1984; Boudreau and Tsuchitani, 1968; 1970; Brugge and Geisler, 1978; Cant and Casseday, 1986; Cant and Morest, 1984; Goldberg and Brown, 1968; Guinan et al., 1972b;Harrison and Feldman, 1970; Harrison and Irving, 1966; Kane, 1977; Morest, 1968; Spangler, Warr and Henkel, 1984; Spangler et al., 1985;

TABLE 1

Summary Table of anatomical characteristics and fiber distributions for the separate descending medial and lateral efferent OC systems in the cat. Data are taken from various sources (i.e. Guinan et al., 1983; 1984; Warr, 1978; 1980; Warr and Guinan, 1979; Warr et al., 1982; 1986).

CHARACTERISTIC	LATERAL	MEDIAL
fiber diameter	0.3 to 0.7μM	0.5 to 2.8μM
myelinated ?	no	yes
number of descending fibers	750 to 870	450 to 535
% ipsilateral	89 to 91%	26 to 28%
% contralateral	9 to 11%	72 to 74%
% contribution to a given cochlea:	58%	42%
%ipsilateral:	50 to 54%	10 to 13%
%contralateral:	4 to 8%	29 to 32%
cochlear target	Type I Auditory Fibers	Base & Circum-nuclear OHC regions
cochleotopic projection		
ipsilateral:	uniform & relatively precise	relatively diffuse mid-range
contralateral:	converging cochlear input	relatively diffuse skewed basally

Stotler, 1953; Tolbert et al., 1982; Tsuchitani, 1977; 1978; 1983; Thompson and Thompson, 1987; Tolbert et al., 1982; Warr, 1966; 1969; 1972; 1982).

There is, therefore, an approximate 2.5:1 ratio of crossed to uncrossed medial fibers in the cat, a 3:1 ratio in guinea pigs, a 2:1 ratio in rats, and a larger 4:1 ratio in primates. The uncrossed/crossed ratio of lateral efferent fibers is 5:1 in cats and is 3:2 in primates. Taken together with the virtually absolute uncrossed to crossed lateral efferent ratios in rodents, it is apparent that the lateral efferent neurons which are mostly uncrossed, comprise the largest group of cochlear efferents in all species analyzed thus far (Bianchi and Salvi, 1990; Campbell and Henson, 1988; Robertson et al., 1987; Strominger et al., 1981; Strutz and Bielenberg, 1984; Thompson, et al., 1984; Warr, 1975; 1978; 1980; 1982; Warr et al., 1979; 1982; 1986; White and Warr, 1983). For the purpose of illustration, some of the anatomical and physiological properties of the lateral and medial efferent systems in the cat are summarized above in Table 1.

Lateral Efferent Innervation Of Type I Afferents

Overview: Beneath the IHCs, both efferent and afferent neurons form a densely packed network of unmyelinated, spiral and radially-oriented fibers (Ginzberg and Morest, 1983; 1984; Iurato, 1974; Liberman, 1980a; 1980b; 1982; Liberman and Oliver, 1984; Spoendlin, 1978; 1979). Traveling through this network of fibers are the Type I radial (IHC)

afferents, the Type II outer spiral (OHC) afferents, the large (radial) medial efferents destined for the OHCs, and the finer diameter, spiral efferents of the ISB and TSB that terminate primarily within the IHC region. In the cat and guinea pig, each fine-diameter lateral spiral efferent fiber, and its collaterals, exhibit numerous 'en-passant' swellings, making terminal axo-dendritic contacts with at least two radial (Type I) afferent dendrites (Brown, 1987b; Liberman, 1980b). The lateral efferent terminal endings, 0.8 to 1.5μm in diameter, are very rarely observed making direct contact with IHCs in adult species (e.g. Liberman, 1980b; Nadol, 1983a). Indeed, the disappearance of direct IHC synapses is one of the few changes occurring at the IHC level during mammalian synaptogenesis (e.g. Ginzberg and Morest, 1984; Pujol, 1985; Pujol et al., 1978; 1979; 1980; Pujol and Lenoir, 1986; Whitehead, 1986). Synaptic contacts made by these spiral lateral efferents, occurs at postsynaptic regions on the Type I dendrites identified by frequent constrictions and postsynaptic densities (Ginzberg and Morest, 1984; Liberman, 1980b; Pujol et al., 1980; Pujol and Lenoir, 1986).

A brief categorical description of the primary Type I radial afferent fibers is prerequisite to a summary of lateral efferent patterns of axodendritic innervation, and to the possible understanding of lateral efferent function. Additional mention will be made regarding the poorly understood auditory Type II spiral afferents also known to

innervate the OHCs (e.g. Brown, 1987a; Santi, 1988; Schwartz, 1986; Spoendlin, 1972; 1978; 1979; 1988). Below, therefore is a short description of the afferent auditory fibers which innervate the cochlea, integrated with a categorical description of the lateral efferent innervation of primary Type I afferents.

Segregation Of Axodendritic Contacts: Type I auditory cell bodies (within the spiral ganglia) are relatively large (12-20μm), and are surrounded by a compact myelin sheath. They give rise to fibers with 2 to 3μm diameters, peripheral processes with 1μm diameters, and are numerous in mammalian species (e.g. Schwartz, 1986; Spoendlin, 1988). Indeed, they comprise at least 95% of the total number of afferent fibers (e.g. Santi, 1988; Schwartz, 1986; Spoendlin, 1972; 1978; 1988). Therefore, Type I fibers constitute approximately 95% of the auditory afferent fibers totaling: 36,000 in humans, 50,000 to 60,000 in cats, and 15,000 to 20,000 in rats (see: Schwartz, 1986; Spoendlin, 1972; 1978; 1988). Type I fibers usually contact single IHCs, with one synapse per fiber, and each IHC is contacted by about 20 Type I fibers (Liberman and Oliver, 1984; Schwartz, 1986; Spoendlin, 1972; 1978; 1988).

Type I Fiber Segregation: Within a given CF region of the organ of Corti, three functional groups of radial afferent fibers have been distinguished both in cats and chinchillas. Existing evidence further indicates that each IHC is contacted by about 20 Type I afferents, belonging to

all three functional categories (Liberman and Oliver, 1984). The Type I fiber segregation is based upon fiber diameter, mitochondrial density, IHC synaptic complexity, synaptic location, and spontaneous discharge rate (Evans and Palmer, 1980; Harrison, 1988; Javel, 1986; Liberman, 1978; 1980a; 1980b; 1982; 1988b; Liberman and Oliver, 1984; Nadol, 1983a; Salvi, Henderson, Hamernik and Ahroon, 1983). In general, spontaneous discharge rate (SDR) can range from near 0, to 120 spikes/second. A Type I radial afferent exhibiting relatively higher rates of spontaneous discharge will also tend to exhibit a relatively lower threshold, a relatively higher maximum discharge rate, and a relatively narrower dynamic range (within 20 to 50dB SL, seldom exceeding 40 to 50dB SL) in response to an auditory stimulus (Evans and Palmer, 1980; Harrison, 1988; Javel, 1986; Liberman, 1978; 1988b; Salvi et al., 1983; Shofner and Sachs, 1986).

Lateral Efferent Innervation Of Pillar-Oriented Fibers: Most (about 60%) Type I radial afferents have relatively large diameter axons (0.3 to 1.3μm), high mitochondria densities, small synaptic endings, and relatively simple IHC synaptic junctions. These units, which contact the lateral (inner pillar) side of the IHCs, exhibit low thresholds (0 to 10dB SPL) and high (>18 to $\approx$200/spikes/sec.) SDRs, distributed around a mean of 50 to 60/sec. with only a few exhibiting SDRs in excess of 100/sec. These higher SDR-Type I fibers seem to comprise a homogeneous group with respect to threshold, such that a fiber exhibiting a SDR of 100

spikes/sec is no more or less sensitive than a frequency-matched unit exhibiting SDRs of 20 spikes/sec (Liberman, 1978; 1988b). These Type I fibers with larger diameters contact the inner pillar side of the IHCs where the density of afferent contacts is relatively lower and the size of the presynaptic endings is relatively smaller (Liberman, 1980a; 1980b; 1982; Liberman and Oliver, 1984; Nadol, 1983a). The important point to note is that each of these inner pillar-oriented Type I afferent fibers receive from 1 to 35 (Mean=11) synaptic contacts from the spiral, fine diameter lateral efferent fibers, as demonstrated in the cat (Liberman, 1980b).

Lateral Efferent Innervation Of Modiolar-Oriented Fibers: The remaining 40% of radial Type I afferent fibers are characterized by lower SDRs, higher thresholds, smaller fiber diameters (0.1 to 0.8μm), and a lower density of mitochondria. While many high SDR fibers in the auditory nerve saturate at levels 20 to 30dBSL, many of the remaining fibers with lower SDRs can exhibit dynamic ranges in excess of 60 to 70dB SL (Evans and Palmer, 1980; Liberman, 1988b; Sachs and Abbas, 1974; Salvi et al., 1983). Collectively, these Type I fibers contact the modiolar-oriented surface of each IHC, where the overall synaptic density is greater and the relative size of the presynaptic endings is larger (Liberman, 1980a; 1980b; 1982; Liberman and Oliver, 1984; Nadol, 1983a).

Approximately 67% of modiolar-oriented units (or 25 to 30% of the total) exhibit intermediate SDRs (<18 spikes/sec.), intermediate thresholds (10 to 20dB SPL), and make relatively simple synaptic contacts with IHCs (Liberman, 1978; 1980a; 1982). Important here is that each of these receives from 1 to 32 (Mean=10) lateral efferent synapses per dendrite. The remaining 33% of modiolar-oriented Type I afferents (or 10 to 15% of the total) are extremely mitochondria-poor, exhibiting relatively complex synaptic IHC junctions, marked by additional patches of specialized pre- and postsynaptic membrane apposing exceptionally long and narrow synaptic densities (Liberman, 1980a; 1980b; 1982; Pujol and Lenoir, 1986). These mitochondria-poor fibers usually exhibit low SDRs (<0.5 spikes/sec) and high thresholds, ranging from 20 to 80dB greater than low-threshold high SDR units (Liberman, 1978; 1980a; 1980b; 1982; 1988b). Fibers in this third SDR category are more heterogeneous as a group than high or medium SDR fibers, with respect to their thresholds, which may span a range of more than 50dB. This range is 20 to 80dB greater than the range exhibited by low threshold units (Liberman, 1978; 1988b). The dendrites of these neurons are contacted by 7 to 32 (Mean=20) lateral efferent synapses per fiber (Liberman, 1980b), or twice as many synapses as the more abundant inner pillar-oriented fibers.

These observations of the lateral efferent innervation of Type I fibers (Liberman, 1980b) were made from samples

obtained from near the cochlear base. Such evidence strongly suggests that where more lateral efferent synapses exist, there is also less Type I activity. This further suggests that tonic activity in lateral efferents may modulate (through inhibition) both the sensitivity and spontaneous discharge activity of Type I auditory fibers (Liberman, 1988b; 1990). Unfortunately, there are apparently no published reports giving the number of efferent synapses in the IHC region as a function of position along the length of the cochlea.

Type II Afferent Neurons: Before concluding, some mention should be made with respect to the smaller, and mostly unmyelinated Type II spiral ganglion cells (e.g. Brown, 1987a; Santi, 1988; Schwartz, 1986; Spoendlin, 1972; 1978; 1979; 1988). In most mammalian species, the Type II neurons are 8-12μm and less numerous (about 5% of the total) that the Type I afferents (Santi, 1988; Schwartz, 1986; Spoendlin, 1988). Upon crossing at the lower extremes of the tunnel (of Corti), fibers from these neurons reach the OHCs, and give rise to the outer spiral fibers of the cochlea. Each outer spiral afferent fiber, usually less than 0.5μm in diameter, then arborizes and innervates about 10 (5 to 28) OHCs (Brown, 1987a; Schwartz, 1986; Spoendlin, 1972; 1978; 1979; 1988).

Physiology And Function Of The Efferent System

Auditory Effects Of Efferent Activation

Overview: Although a comprehensive picture of the role played by the efferent OC system in hearing has yet to emerge, it is generally accepted that activation of medial efferent fibers leads to the suppression of gross neural responses, and modifies both the spontaneous and driven discharge rates of single auditory units. Medial efferent activation also simultaneously alters other stimulus-dependent potentials recorded from the periphery, as well as some cochlear resting potentials. Below is a short description of some of these auditory potentials, and a brief account of their susceptibility to medial efferent activation.

The Compound Action Potential (CAP)

The auditory nerve compound action potential (CAP) is a series of volume-conducted neural responses, representing extracellular current sources (outward) and (inward) current sinks (e.g. Buchwald, 1983; Dallos, 1973; Davis, 1976; Martin, 1985). It is well established that the size of the auditory nerve compound action potential reflects the extent of overall (whole nerve) activity and synchronization in response to an auditory stimulus. The response consists of the complex sum of the all-or-none discharges (voltages) initiated by the activation of single auditory neurons (units) which primarily innervate the basal turn of the

cochlear organ of Corti (Dallos, 1973; Davis, 1976; Glasscock, Jackson and Josey, 1981; Glattke, 1983; Jacobson, 1985; Moller, 1983b; Moller and Jannetta, 1982; 1985; Moore, 1983; Sohmer, 1989).

The CAP has two pronounced negative peaks, N1 and N2. These first and second negative N1 and N2 peaks of the auditory nerve compound action potential reflect neural activity of the auditory periphery and lower brainstem, respectively. In most mammalian species (including human), the amplitude of N1 represents neural activity and synchronization coincident with the onset discharges produced by single units within the auditory nerve in the periphery (Moller, 1983b; Moller and Jannetta, 1982; 1985; Sohmer, 1989; Starr and Zaaroor, 1990; Wada and Starr, 1983a; 1983b; 1983c). The magnitude of N2 in rodents coincides with the discharge level of neurons located within the region nearer to the ipsilateral cochlear nucleus and contralaterally, in areas such as the superior olivary complex that receive input from the trapezoid body (Wada and Starr, 1983a; 1983b; 1983c).

It is also well established that the whole nerve CAP is best obtained in response to broadband or high frequency filtered transients, such as clicks or tone pips, respectively (e.g. Davis, 1976; Glasscock et al., 1981; Glattke, 1983; Jacobson, 1985; Moller, 1983a; 1985; Moore, 1983; Sohmer, 1989; Stockard and Stockard, 1983) having very abrupt, or instantaneous rise times. Owing to the

distribution of frequencies along the basilar membrane, a suprathreshold broadband transient stimulus such as a click, will primarily activate those fibers tuned to higher frequencies (Davis, 1976; Glasscock et al., 1981; Glattke, 1983; Moller, 1985; Sohmer, 1989), and therefore the CAP response will be dominated by neurons that innervate the cochlear base. Due to basilar membrane mechanics, single units with lower CF units found within the more apical cochlear turns, will respond too asynchronously to clicks to contribute effectively to the CAP (e.g. Glattke, 1983; Moller, 1983b; Moller and Jannetta, 1982; 1985; Sohmer, 1989).

Latency Of The CAP Response: Latency of the CAP response is defined as the time interval between the stimulus onset and the onset or peak of the response (i.e. Durrant and Wolfe, 1991; Glattke, 1983). The travel time of the peak response on the basilar membrane (from base to apex) contributes greatly to the total latency of the CAP response, and therefore, to the latency of both peaks in the CAP. Latency is therefore largely a cochlear manifestation in healthy subjects (Eggermont, 1983; Glattke, 1983; Jacobson, 1985; Moller, 1985; Salvi et al., 1983). The CAP latency is also restricted by cochlear (hair cell) and neural transduction time, and therefore, is never less than about 1 to 1.5msec (Durrant and Wolfe, 1991; Glattke, 1983). When a broadband click stimulus is presented at near-threshold intensities, the preferred resonating properties

of the earphone as well as the low pass resonant filtering action of the middle ear apparatus result in an emphasis upon mid-frequencies (about 1 or 2kHz) of the broadband stimulus (Eggermont, 1983; Moller, 1983a; Weber, 1983). This generally results in a basilar membrane response exhibiting a moderately longer latency (Eggermont, 1983; Glattke, 1983; Jacobson, 1985; Sohmer, 1989).

It is well known that in the normal ear, the latencies of the CAP peaks also depend upon the stimulus intensity (e.g. Dallos, 1973; Davis, 1976; Durrant and Wolfe, 1991; Glasscock et al., 1981; Jacobson, 1985; Moller, 1985; Moore, 1983; Sohmer, 1989; Stockard and Stockard, 1983). As the sound pressure level (SPL) of a broadband click stimulus increases, more of the higher frequency energy (of the stimulus) becomes dominant (Durrant and Wolfe, 1991; Glattke, 1983; Moller, 1983a). The energy concentrated within the midfrequency region of the traveling wave, also activates a wider area of the basilar membrane (towards the base) effectively driving the neurons innervating the basal region (Dallos, 1973; Davis, 1976; Glattke, 1983; Moller, 1983a). Absolute latency of the CAP decreases about 0.4msec for each 10dB increase in stimulus intensity (Durrant and Wolfe, 1991).

Amplitude Of The CAP Response: As the stimulus intensity is increased, a larger area of the basilar membrane is displaced (e.g. Dallos, 1973; Davis, 1976). The greater IHC transduction current, leads to a progressive

addition (recruitment) of neurons to the response, an increase in the overall discharge rate, and a greater number of fibers discharging in close registration (synchronously). Therefore, the increased amplitude of the CAP in response to an increase in the SPL of the stimulus, is primarily a function of the extended range of basilar membrane displacement and the concomitant increase in neural activity (e.g. Dallos, 1973; Davis, 1976; Durrant and Wolfe, 1991; Glattke, 1983; Moller, 1983a; Salvi et al., 1983).

Effects Of Medial Efferent Activation On The Auditory Nerve CAP: Galambos (1956) first demonstrated, in cats, that electrical (shock) stimulation of the efferent fibers can reduce the N1 and N2 amplitudes of the round window-recorded whole nerve (compound) action potential to 75 μsec duration clicks, in a non-noise background. In that landmark investigation, concentric bipolar stimulating electrodes (0.3mm gap) were positioned near the midline of the medulla on the floor of the fourth ventricle, approximately 1cm (±2mm) rostral to the obex, thus defining the necessary electrode location to a block of tissue some 4X4X2mm thick. The results obtained in the Galambos (1956) investigation were foremost in establishing an inhibitory role for the medial efferent system, and have since been replicated under similar (Brown and Nuttall, 1984; Dewson, 1967; Fex, 1959; 1962; 1967) or somewhat varying conditions (Comis, 1970; Desmedt, 1962; 1975; Desmedt and LaGrutta, 1963; Desmedt, LaGrutta and LaGrutta, 1963; 1971; Gifford and Guinan, 1983;

1987; Kiang, 1984; Konishi and Slepian, 1971a 1971b; Nieder and Nieder, 1970a; Wiederhold, 1970; 1986; Wiederhold and Kiang, 1970; Wiederhold and Peake, 1966). When the medial efferent fibers in the cat are activated at midline, the ratio of the average SPL shift in N1 recorded contralaterally, to the average shift in N1 recorded ipsilaterally is about 2.6:1 (Gifford and Guinan, 1987). This amount parallels to the 2.5:1 ratio of crossed:uncrossed medial efferent fibers observed in this species (e.g. Warr et al., 1982; 1986).

The stimulus parameters for achieving optimal amplitude reduction of the CAP are known (e.g. Desmedt, 1962; 1975; Desmedt and LaGrutta, 1963; Desmedt et al., 1963; 1971; Galambos, 1956; Gifford and Guinan, 1983; 1987). The degree of neural suppression achieved by optimizing such parameters are approximately equivalent to a 17.5 to 24dB reduction (or 70%) in the SPL of the click or tone stimulus. This can also equate to a 70 to 100μV amplitude reduction, and is optimally observed within 100 to 200msec (Desmedt, 1962; 1975; Desmedt and LaGrutta, 1963; Desmedt et al., 1963; 1971; Gifford and Guinan, 1983; 1987; Konishi and Slepian, 1971a; 1971b; Wiederhold, 1970; 1986; Wiederhold and Kiang, 1970; Wiederhold and Peake, 1966). As the sound pressure level of the click or tone stimulus exceeds 60dB SPL, the percentage reduction in the CAP amplitude achieved with the same shock level begins to decline. Progressively stronger shock intensities will eventually fail to effect N1

amplitude as higher click or tone levels are employed (Desmedt, 1975; Konishi and Slepian, 1971a; Wiederhold, 1986; Wiederhold and Peake, 1966). Furthermore, the N1 response to a low intensity (less than 40dB SL) higher (10kHz) frequency filtered click (tone pips) is more susceptible to medial efferent suppression than is the auditory response to equivalent SPLs of lower-frequency (i.e. 400 Hz) filtered clicks (Wiederhold and Peake, 1966). In guinea pigs, CAP suppression by medial efferent activation is greatest for tone bursts having frequencies of 4 to 6kHz (Konishi and Slepian, 1971a).

Shock stimulation of the medial efferent fibers has very little effect on the latencies of N1 and N2 for auditory stimuli above 25dB up to 40dB SL. At stimulus levels below about 25dB SL, N1 latency may increase by $\approx$0.3 to 0.5msec (20 μsec/dB) provided that the click stimulus is not more than 24dB SL (Desmedt et al., 1963; 1971; Dewson, 1967; Wiederhold, 1986).

Lateral Efferent Effects On The CAP: Gifford and Guinan (1987) reported that selective stimulation of brainstem nuclei falling within the lateral efferent region failed to produce any detectable change in the CAP response to clicks. In an earlier study, Comis (1970) reported an occasional 100μV reduction in the round window-recorded N1 response to 100μsec clicks following the insertion of monopolar electrodes into the outlying region dorsolateral to the lateral limb of the LSO, within the DLPO nuclei). Whether

the effects obtained by Comis (1970) can be attributed to a synaptic interaction of lateral efferent fibers with Type I primary afferents or merely to current spread from the monopolar electrode tips into the medial efferent fiber regions, remains uncertain.

Medial Efferent Effects On Single Auditory Units: The principal outcome of medial efferent stimulation on Type 1 primary afferent fiber discharge rates in the absence of a background noise stimulus, is a reduction in response to low-moderate stimulus intensities (Fex, 1962). The suppression observed is always greatest for lower SPLs (5 to 10dB SL up to about 30dB SL) of the auditory stimulus and can range in SPL equivalents from 1 to 25dB. This corresponds to a reduction in spike activity of 10 to over 94%, respectively (Gifford and Guinan, 1983; 1987; Guinan and Gifford, 1988a; 1988b; 1988c; Teas, Konishi and Nielsen, 1972; Wiederhold, 1970; 1986; Wiederhold and Kiang, 1970; Winslow and Sachs, 1987). At very low (at threshold) or higher tonal levels (i.e. 15 to 25dB SL) medial efferent activation again reduces discharge rate (29% and 16% respectively), though the effects are significantly reduced (Guinan and Gifford, 1988b; Wiederhold, 1970; 1986). Indeed, medial efferent-induced suppression of single unit activity is generally lost at stimulus levels at or above about 30-40dB SL (Gifford and Guinan, 1983; Guinan and Gifford, 1988b; 1988d; Wiederhold, 1970; 1986).

Medial efferent stimulation also produces a 15 to 40% suppression in spontaneous discharge activity (Guinan and Gifford, 1988a; 1988c; Wiederhold and Kiang, 1970) and a 12 to 14dB threshold shift (Guinan and Gifford, 1988d) for primary auditory fibers. At the fiber's characteristic frequency, medial efferent stimulation also reduces the sharpness of auditory fiber tuning (Q_{10}) by 7 to 31%, and (Q_{20}) by 14.5%, for fibers exhibiting high rates of spontaneous discharge (Guinan and Gifford, 1988d; Wiederhold, 1970; 1986). The general results obtained from investigations of medial efferent activation on gross neural potentials, and on the firing rates of single auditory units (e.g. Fex, 1962; Gifford and Guinan, 1983; Guinan and Gifford, 1988a; 1988b; 1988c; 1988d; Teas et al., 1972; Wiederhold, 1970; 1986; Wiederhold and Kiang, 1970) are summarized below in Table 2.

The Cochlear Microphonic (CM)

The cochlear microphonic (CM) is an extracellular, frequency- and intensity-dependent manifestation of a response to sound generated predominantly by OHCs (Dallos, 1981; 1984; Dallos, Billone, Durrant and Raynor, 1972; Dallos and Wang, 1974; Moller, 1983a; Sellick and Russell, 1980; Pickles, 1988; Wang and Dallos, 1972), and is proportional to the instantaneous displacement pattern of the basilar membrane at the recording electrode location. The CM therefore exhibits no measurable latency between the

time of arrival of a stimulus within the cochlea (≈130μsec), and its onset (Glattke, 1983).

It is well accepted (i.e. Glattke, 1983; Pickles, 1988) that the CM is proportional to the transduction current passing through each individual hair cell (Corey and Hudspeth, 1979a; 1983a; Hudspeth, 1982). Consequently, the CM is an alternating current (AC) potential without a true threshold, that appears to follow the waveform of the stimulus (Dallos, 1973; 1981; 1984; Dallos et al., 1972; Dallos and Cheatham, 1976; Sellick and Russell, 1980). The mammalian CM has therefore been used reliably as an assay of the integrity of the mechanoelectric transduction currents at the apex of the OHCs, located within the first cochlear turn (i.e. Dallos, 1973; Honrubia & Ward, 1969; 1970; Patuzzi and Thompson, 1991; Patuzzi, Yates and Johnstone, 1989; Sohmer, 1989; Yates, Geisler, Patuzzi and Johnstone, 1989).

Effects Of Medial Efferent Activation On The CM: In contrast to an observed suppression of neural activity, electrical stimulation of the medial efferent bundle potentiates the amplitude of an intracochlear (Gans, 1977; Konishi and Slepian, 1971a; 1971b; Mountain, Geisler and Hubbard, 1980; Teas et al., 1972) or round window-recorded CM by as much as 13 to 16μV, which is about a 30% change (Brown and Nuttall, 1984; Desmedt, 1962; Desmedt et al., 1971; Dewson, 1967; Fex, 1959; 1962; 1967; Gifford and Guinan, 1987; Wiederhold, 1986; Wiederhold and Peake, 1966)

or an equivalent SPL of approximately 3-6dB, though much greater (175μV) effects have also been reported (Konishi, 1972).

The parameters producing optimal CM enhancement by medial efferent stimulation are known and are similar or identical to those producing maximal neural changes (e.g. Brown and Nuttall, 1984; (Desmedt, 1962; Desmedt et al., 1971; Dewson, 1967; Fex, 1959; 1962; 1967; Konishi, 1972; Konishi and Slepian, 1971a; 1971b; Teas et al., 1972; Wiederhold, 1986; Wiederhold and Peake, 1966). In guinea pigs, CM enhancement following medial efferent activation is greatest (3dB) for 1kHz tone bursts, and is not observed at tone burst frequencies much above 5kHz (Konishi and Slepian, 1971a; 1971b; Teas et al., 1972).

The Summating Potential (SP)

Another stimulus-dependent extracellular potential that has been attributed to endolymphatic transduction current shunting through both the OHCs and IHCs (Dallos, 1981; 1984; Dallos and Cheatham, 1976; Dallos and Wang, 1974; Glasscock et al., 1981; Moller, 1983a; Pickles, 1988) appears as a small-i.e. $\approx$0.05 to 0.5μV when round window recorded-negative shift in the baseline of the recorded signal. The summating potential (SP^-) is thought to represent a negative, direct current deviation in the scala media (Johnstone and Johnstone, 1966; Pickles, 1988) relative to the more positive shift produced by the depolarizing direct

current within hair cells and the scala tympani, during acoustic stimulation (Dallos, 1973; 1984). It has been referred to as a rectified or DC variation of the CM that, like the CM, persists for the duration of the stimulus (Dallos, 1973; 1984; Pickles, 1988).

The negative summating potential (SP^-) can be observed with a round window electrode, but is recorded best with an intracochlear electrode placement. Overall, optimal recordings are obtained when electrodes are differentially placed (i.e. non-inverting and inverting) between the scala vestibuli and scala tympani, respectively (Dallos, 1973; 1984; Pickles, 1988).

Medial Efferent-Induced Changes In SP^-: Fex (1959) initially noted a modification in the round window recorded SP^- following medial efferent stimulation that was coincident with a reduction of N1 and augmentation of the CM. In general, activation of the medial efferent system leads to a reduction (or an approximate 100μV positive shift) in the amplitude of the SP^-.

Hair Cell Potentials

The resting potentials from (unstimulated) mammalian outer and inner hair cells tend to be unequal. The OHC resting membrane potential is -70mV (range = -60 to -80mV) and is roughly double the -30 to -45mV potential recorded from the IHCs (Dallos, 1973; 1981; 1985a; 1985b; 1986; Gitter et al., 1986; Nuttall, 1986; Pickles, 1988; Russell

and Sellick, 1978; 1983; Santos-Sacchi, 1988; Sellick and Russell, 1980). Lower IHC resting potentials (-67mV) have also been reported (Kros and Crawford, 1989). It may be possible that the comparatively lower IHC resting membrane potential is the result of a greater continuous flow of transduction current (K^+ or Ca^{++}) into the hair cell across the apical hair cell surface (Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a) generating the spontaneous release of neurotransmitter, and producing spontaneous discharge activity in Type I fibers (Nuttall, 1986). When stimulated with tone bursts near their CF, both an AC and a positive DC potential (Nuttall, 1986; Patuzzi and Sellick, 1983) can be recorded from within both inner and outer hair cells (Pickles, 1988).

Medial Efferent Effects On IHC Potentials: The magnitude of the IHC depolarizing DC and AC potentials are reduced by activation of the medial efferent fibers, which is equivalent to a tonal stimulus reduction of roughly 9.5 to 24dB SPL (Brown and Nuttall, 1984; Brown et al., 1983a; Nuttall, 1986). Maximum suppression is often observed at low to moderate tonal stimulus levels (50dB SPL or less) at the CF of the hair cell, and is less effective at higher stimulus levels, where IHC output is nearly saturated. Another outcome of stimulating the medial efferents is the reduction in IHC sensitivity. IHC sensitivity is reversibly reduced by an amount roughly 5dB to 17dB SPL. In addition to a reduction in IHC sensitivity, the IHC Q_{10} is reversibly

reduced by as much as 33% following stimulation of the medial efferent fibers (Brown and Nuttall, 1984; Brown et al., 1983a; Nuttall, 1986).

The Endocochlear Potential (EP)

When a microelectrode enters the fluid-filled space of the mammalian scala media, a static, direct current (DC) potential having a relative magnitude of approximately +80 to +100mV is recorded (e.g. Dallos, 1973; 1984; Desmedt and Robertson, 1975; Konishi and Kelsey, 1973; Pickles, 1988; Salt and Konishi, 1986; Salt and Thalmann, 1988; Santi, 1988). It is well accepted that the high positivity of this endocochlear potential (EP) is the direct result of an especially high concentration (about 30X that of perilymph) of potassium cations (K^+) in the cochlear endolymph (e.g. Anniko and Wroblewski, 1986; Ashmore, 1991; Konishi and Kelsey, 1973; Marcus, 1986; Pickles, 1988; Santi, 1988; Smith, Lowry and Wu, 1954; Salt and Konishi, 1986; Salt and Thalmann, 1988), and that a high endolymphatic K^+ concentration is critical for maintaining normal cochlear function (e.g. Konishi, Kelsey and Singleton, 1966; Pickles, 1988; Russell, 1983; Santi, 1988; Tasaki and Spyropoulos, 1959).

Considerable evidence indicates that the low levels of Na^+, and high levels of endolymphatic K^+ are maintained by active homeostatic mechanisms located primarily within the stria vascularis (e.g. Anniko and Wroblewski, 1986; Marcus,

1986; Pickles 1988; Smith et al., 1954). The EP polarizes the apical surface of the IHCs in mammals. When combined with the intracellular IHC resting potential, both are believed to form the major driving force which moves current into the cell (Dallos, 1973; 1984; Mountain, 1986; Pickles, 1988; Santos-Sacchi, 1988), and potassium (K^+) ions carry the apical hair cell transduction current in vertebrate species (e.g. Corey and Hudspeth, 1979a; Brownell, Zidanic and Spirou, 1986; Dallos, 1973; 1981; Gitter et al., 1986; Gitter and Zenner, 1988; Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a; Nuttall, 1986; Pickles, 1988; Santos-Sacchi, 1988; Zenner, 1986a; 1986b).

Results from wide range of investigations (Brown et al., 1983b; Nuttall, 1984; 1985; Sewell, 1984a; 1984b) have all suggested an important role for the EP in regulating frequency selectivity and the sensitivity of IHCs, via alterations in electrochemical transduction currents (Mountain, 1986; Nuttall, 1986). Lowering the EP also produces an elevated auditory nerve threshold (Sewell, 1984a) and reduces spontaneous discharge rates in single auditory fibers >100 fold (Liberman and Dodds, 1984a; Sewell, 1984b).

Medial Efferent Effects On The EP: Activation of medial efferent fibers will alter cochlear resting potentials like the EP (Brown and Nuttall, 1984; Desmedt and Robertson, 1975; Fex, 1962; 1967; Gifford and Guinan, 1987; Konishi and Slepian, 1971a; 1971b; Mountain et al., 1980; Teas et al.,

1972). Medial efferent activation produces anywhere from a 0.75, to a 4-6mV (or a 6 to 7%) drop from 80mV in a scala media-recorded EP. The suppression develops relatively slowly (over 200msec) beginning 10 to 40msec after the onset of the stimulus, and exhibits a recovery-time course similar to the effects on the N1, CM and SP following medial efferent stimulation (e.g. Gifford and Guinan, 1987).

Cochlear Distortion Products

When two tones of frequencies f_1 and f_2 where $f_2>f_1$ are presented simultaneously to the ear, distortion products, or 'combination tones' are generated in the cochlea at frequencies $mf_1 \pm nf_2$, and can be recorded from the external auditory meatus, the round window, or from single auditory units (Brown and Kemp, 1984; Buunen and Rhode, 1978; Gibian and Kim, 1982; Javel, 1986; Kemp and Brown, 1984; Pickles, 1988; Kiang, 1984; Kim et al., 1980; Mountain, 1986; Rhode, 1984; Siegel and Kim, 1982; Siegel et al., 1982).

These combination tones can be attributed to the intrinsic mechanical nonlinear properties of the cochlea (e.g. Buunen and Rhode, 1978; Gibian and Kim, 1982; Glattke and Kujawa 1991; Javel, 1986; Kim, Molnar and Matthews, 1980; Pickles, 1988; Rhode, 1984; Siegel, Kim and Molnar, 1982). Though many exist, some combination tones are more salient than others. The second-order combination tones, or distortion products, consist of the difference ($f_2 - f_1$) and summation tones ($f_2 + f_1$), with the difference tone the more

salient (Javel, 1986), and consequently the most investigated. Similarly, there are third-order combination tones consisting of $2f_1 + f_2$, $2f_1 - f_2$, $2f_2 + f_1$, and $2f_2 + f_1$. The $2f_1 - f_2$ combination tone, the so-called "cubic difference tone", is the most salient and most studied (e.g. Buunen and Rhode, 1978; Glattke and Kujawa, 1991; Javel, 1986; Pickles, 1988; Rhode, 1984). In general, cochlear and neural responses to the second order and cubic difference tone distortion components behave as though tones at the distortion frequencies were actually introduced as separate stimuli along with their primaries, f_1 and f_2 (Buunen and Rhode, 1978; Dallos, 1981; Glattke and Kujawa, 1991); Javel, 1986; Kim et al., 1980; Siegel et al., 1982). Depending upon their frequency and intensity, the cubic and second order difference tone can range from 15 to 45dB below the SPL of the f_1 and f_2 primary frequencies (Buunen and Rhode, 1978; Kim et al., 1980; Mountain, 1980; Siegel and Kim, 1982).

Medial Efferent Effects On Cochlear Distortion Products: Stimulation of the medial efferent fibers alters the second order ($f_2 - f_1$) and cubic difference tone ($2f_1 - f_2$) intermodulation-distortion components recorded from the cochlea (Mountain, 1980; (Siegel and Kim, 1982)). For instance, medial efferent stimulation can lead to a 20 to 66% magnitude reduction in the second order difference tone distortion product (700Hz), for primary (f_1 and f_2) frequencies of 8.0 and 8.7 kHz, respectively, recorded from

the outer ear canal (Mountain, 1980). The general results obtained from investigations of medial efferent activation on cochlear potentials, are summarized below in Table 2.

TABLE 2

Summary table of medial efferent effects on the cochlear and neural activity in mammalian species, as presented in the text.

MEDIAL EFFERENT EFFECT	MAGNITUDE OF EFFECT	DIRECT SUPPORTING EVIDENCE
↓ Distortion Products	20 to 66% reduction	Mountain, 1980
↓ IHC DC Potential	9.5–24dB equival.	Brown & Nuttall,1984
↓ IHC Tuning	5 to 17dB or 33%	Brown & Nuttall,1984
↓ SP–	100μV reduction	Konishi & Slepian, 1971b Fex, 1959
↑ CM	13 to 16μV or 30% 3 to 6dB equiv.	Brown & Nuttall,1984 Wiederhold, 1986. Wiederhold & Peake, 1966
↓ EP	4–6mV; or 6 to 7% reduction	Gifford & Guinan, 1987 Konishi, 1972
↓ N1	70 to 100μV or 70% reduction 17.5 to 24dB equivalent	Galambos, 1956; Gifford & Guinan, 1983; 1987 Wiederhold,1970;1986 Wiederhold & Peake, 1966
↓ Single Unit SDR	15 to 40% reduction	Guinan & Gifford, 1988a; 1988c; Wiederhold & Kiang, 1970.
↑ Single Unit Threshold	12.3 to 14.3dB shift	Guinan & Gifford, 1988a; 1988d.
↓ Single Unit Tuning	7 to 31% reduction	Wiederhold,1970;1986 Guinan & Gifford, 1988d.
↓ Single Unit Driven Discharge	29% at threshold 94% or 25dB at the rising phase 16% at plateau	Guinan & Gifford, 1988b; Wiederhold, 1970; Wiederhold & Kiang, 1970.

CHAPTER 2

Medial And Lateral Efferent Neurotransmitters

Overview: There is considerable evidence in support of acetylcholine (ACh) as a neurotransmitter for both the lateral and medial divisions of the descending efferent system (e.g. Altschuler and Fex, 1986; Bledsoe, 1986; Bledsoe et al., 1988; Eybalin and Altschuler, 1990; Eybalin and Pujol, 1987; Gacek, 1972; Godfrey, Wiet and Ross, 1986; Guth and Melamed, 1982; Klinke, 1981; 1986; Wenthold, 1980). There are as yet no published reports demonstrating the presence of ACh in presynaptic efferent vesicles, though much of the evidence in support of an efferent neurotransmitter role for ACh has been inferred from the presence of associated enzymatic neurochemicals, such as acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) within the cochlea.

Neurotransmitter Evidence For ACh

Within cholinergic neurons, the enzyme ChAT catalyzes the final step in ACh synthesis, the acetylation of choline with acetyl coenzyme-A (e.g. Cooper et al., 1986; Lefkowitz, Hoffman and Taylor, 1990; Weiner and Taylor, 1985). Since ChAT serves no other known biochemical function (Cooper et al., 1986) it is generally regarded as a reliable indicator of cholinergic activity, and its presence within the perikarya, axon and axon terminals of neurons is widely accepted as unequivocal evidence for the production of ACh

(Altschuler and Fex, 1986; Cuello and Sofroniew, 1984; Eckenstein and Sofroniew, 1983; Fex and Altschuler, 1985; 1986; Godfrey et al., 1986; Morley, Farley and Javel, 1985; Schwartz, 1985a).

Histochemical investigations in the guinea pig (Fex and Wenthold, 1976; Godfrey et al., 1986), cat (Jasser and Guth, 1973; Wiet, Godfrey, Dunn and Ross, 1989) and rat (Eybalin and Altschuler, 1990; Eybalin and Pujol, 1987; Godfrey et al., 1984; Godfrey, Park, Dunn and Ross, 1982; Godfrey and Ross, 1985; Godfrey et al., 1986; Wiet, Godfrey, Ross and Dunn, 1986) have demonstrated high levels of the choline-specific enzyme ChAT within the descending medial and lateral efferent fiber bundles, and medial and lateral fibers within the organ of Corti. In rats, the activity level of ChAT within the organ of Corti is more than 10X higher than the average ChAT activity found either in whole brain, or in non-neural cochlear structures in that species. Indeed, cochlear/efferent fiber levels of ChAT are equivalent to values obtained for the facial nucleus and nerve root (Godfrey and Ross, 1985; Godfrey et al., 1982; 1984; 1986). Total transection of the efferent fibers leads to a significant peripheral depletion of ChAT (Guth, Jasser and Daigneault, 1972; Jasser and Guth, 1973) that exceeds 90% by 2 post-operative days, and is complete by 7 days (Godfrey et al., 1982; 1984; 1986; Godfrey and Ross, 1985; Wiet et al., 1986) suggesting that all ChAT activity within the organ of Corti can be attributed to the efferent OC

system. Indeed, immunoreactivity to ChAT is not observed in the organ of Corti following complete transection of the cochlear efferent bundle (Altschuler, Kachar, Rubio, Parakkal and Fex, 1985b).

Immunocytochemical investigations conducted in rats and guinea pigs also demonstrate ChAT immunoreactivity within both the lateral and medial efferent cells of origin (Altschuler et al., 1984a). High levels are detected within the organ of Corti in fibers and terminals ascribed to the efferent system (Altschuler and Fex, 1986; Altschuler et al., 1985b; Eybalin and Pujol, 1984a; Fex and Altschuler, 1985; 1986). For example, ChAT immunoreactivity is observed within: (a) the inner and tunnel spiral bundles; (b) puncta (patches) near the IHC bases; (c) the tunnel crossing fibers; and (d) within large puncta at the OHC base and circumnuclear OHC region (Altschuler and Fex, 1986; Altschuler et al., 1985b; Eybalin and Pujol, 1984a; 1987; Fex and Altschuler, 1985; 1986; Pujol and Lenoir, 1986). Other investigations employing immunoelectron microscopy in the rat cochlea have demonstrated axodendritic immunoreactive varicosities presynaptic to IHCs. Axosomatic densities presynaptic to OHCs are also immunostained for ChAT, within the lateral and medial efferent fibers and terminals, respectively (Eybalin and Altschuler, 1990; Eybalin and Pujol, 1987).

In both the guinea pig and rat, the distribution of ChAT is considerably greater within the inner vs the OHC

region (Fex and Wenthold, 1976; Godfrey and Ross, 1985; Godfrey et al., 1986). Unlike the relatively even distribution from the second to third cochlear turn in the guinea pig, overall ChAT distribution in the rat cochlea is notably enhanced within the second turn (Fex and Wenthold, 1976; Godfrey and Ross, 1985; Godfrey et al., 1986). In both rodent species, the distribution of ChAT consistently declines towards the cochlear apex (Eybalin and Altschuler, 1990; Fex and Wenthold, 1976; Godfrey and Ross, 1985; Godfrey et al., 1986).

In cats, ChAT activity within the IHC region is uniformly distributed in all 3 cochlear turns (Wiet et al., 1989). In the guinea pig, anatomical evidence has shown that the inner and tunnel spiral bundles of the lateral efferent system do not extend to the cochlear apex (e.g. Wright, 1975; Wright and Preston, 1973). Similarly, cochlear ChAT ascribed to the lateral efferent system in this species is only observed as patches of immunoreactivity near the IHC region in the extreme cochlear apex (Altschuler and Fex, 1986; Altschuler et al., 1985b; Fex and Altschuler, 1985; 1986).

In cats, ChAT activity within the OHC region is highest in the first cochlear turn (Wiet et al., 1989). Consistent with the known medial efferent innervation pattern in the guinea pig cochlea (i.e. Brown, 1987b; Smith, 1975) is the observation that immunoreactive puncta are found at the basal end of the cochlea in all three OHC rows, averaging

about nine puncta per OHC (Altschuler and Fex, 1986; Altschuler et al., 1985b; Fex and Altschuler, 1985; 1986). The total number of observable immunoreactive puncta, however, progressively declines towards the cochlear apex in this species. For example, the apicalward decline in ChAT immunoreactive medial efferent puncta begins in the third OHC row (1st turn), advances to the second row (2nd to 3rd turn), and by the beginning of the fourth turn, the number of large ChAT immunoreactive puncta are restricted to the first row of OHCs (Altschuler and Fex, 1986; Altschuler et al., 1985b; Fex and Altschuler, 1985; 1986).

These studies have all provided direct evidence for the presence within the efferent system, of the specific enzyme ChAT, necessary for the synthesis of ACh. These studies have also demonstrated the existence of ChAT within presynaptic terminals, or within close proximity to the site of presumed efferent OC action.

In the guinea pig, electrical stimulation of the medial efferents (200μA; 400 shocks/sec) produces a 4-fold release of ACh into perilymph, as indicated by recovery with push-pull perfusion pipettes (Norris and Guth, 1974; Norris, Guth and Stockwell, 1972). Such quantities were found to be sufficient to produce a contractile response in isolated samples of guinea pig ileum. Furthermore, immunohistochemical investigations have detected the presence of cholinergic receptors on isolated mammalian OHCs

(Plinkert and Zenner, 1989; Zenner et al., 1989), which are nicotinic (Canlon et al., 1990; Plinkert and Zenner, 1991; Plinkert et al., 1991). There is also some evidence for the existence of muscarinic M_3 receptors within the organ of Corti (Guiramand et al., 1990a; 1990b). Such evidence has indicated that ACh is released presynaptically within the cochlea, in amounts sufficient to produce a postsynaptic alteration. The evidence also supports the existence of specific postsynaptic receptors for ACh located within close proximity to presynaptic terminals.

It was the initial investigation in the guinea pig conducted by Gisselsson (1960) that first suggested that an intracochlear infusion of ACh could mimic the known effects of efferent OC activation. In that study, intracochlearly applied ACh produced an increase in the CM amplitude resembling the microphonic effects of efferent stimulation.

Subsequent investigations in the guinea pig have shown that intracochlear infusion of ACh at doses between $5x10^{-5}$ to $3x10^{-4}$ M (or 50 to 300μM) in combination with the anti-AChE agent (Taylor, 1990b) eserine (physostigmine) at doses of 10 to 20μM ($2x10^{-5}$ M) produces a 150 to 200μV increase in the amplitude of the basal promontory-recorded CM response (Bobbin and Konishi, 1971; 1974). The same doses of ACh and eserine cited above also produced a 70μV suppression of the basal promontory-, or intracochlear-recorded N1 potential, elicited by low intensity tone pips, 10msec in duration

(Bobbin and Konishi, 1971; 1974). They also produced a 25 to 30dB loss in tuning curve threshold sensitivity, together with a 70 to 75% reduction in the (rising phase) discharge of single auditory units. Indeed, maximal ACh effects are observed in fibers stimulated at low (10 to 30dB SL) stimulus intensities (Comis and Leng, 1979; Robertson and Johnstone, 1978). Finally, ACh and eserine given at the above-cited dose leads to a significant reduction in single auditory fiber spontaneous discharge (Comis and Leng, 1979).

The interpretation of investigations using anti-acetylcholinesterases like eserine (Taylor, 1990b) are complicated by the finding that AChE is involved in the proteolytic processing of neuropeptide precursors like proenkephalin-A (Chubb, Ranieri, White and Hodgson, 1983; Dowton and Boelen, 1988). While intracochlear pharmacologic investigations of putative efferent neurotransmitters have not been conducted in the cat, intra-arterially administered ACh at a dose of 20 to 25μg (without eserine) in one study did produce a 25% reduction in the N1 amplitude just 30 to 35sec post-infusion in this species (Brown, Daigneault and Pruett, 1969).

Additional evidence for cholinergic-mediated medial efferent effects comes from the demonstration that iontophoretic application of ACh to the basal synaptic region of isolated guinea pig OHCs produces cell shortening similar to the contractile effects produced by a depolarizing current (Brownell et al., 1985). This ACh-

induced hair cell response was reported to develop within approximately 33msec. It was also observed that ACh failed to produce a response if administered at locations distal to the basal synaptic region (Brownell et al., 1985). These studies have all demonstrated that the direct application of ACh in reasonable concentrations into synaptic regions of the cochlea produces a postsynaptic response nearly identical to the effects produced by the presumed release of neurotransmitter, during presynaptic activation by shock stimuli.

The enzyme AChE, which catalyzes ACh hydrolysis, is a less specific marker for cholinergic neurons, since it is found not only in presynaptic cholinergic neurons, but also postsynaptically within cholinergic and non-cholinergic neurons (Cooper et al., 1986; Cuello and Sofroniew, 1984; Eckenstein and Sofroniew, 1983; Morley et al., 1985; Weiner and Taylor, 1985). Indeed, AChE exhibits low substrate specificity. As alluded to earlier, in addition to its role in rapidly destroying extracellular ACh, it is also involved in the proteolytic processing of neuropeptide precursors (Chubb, Ranieri, White and Hodgson, 1983; Dowton and Boelen, 1988).

Histochemical staining for the nonspecific cholinergic inactivating enzyme AChE in cats (Gacek, 1972; Osen et al., 1984; Osen and Roth, 1969; Spangler and Henkel, 1982; Warr, 1975; 1978; 1980; Warr et al., 1982), rats (Godfrey et al.,

1982; 1984; 1986; Godfrey and Ross, 1985; Osen et al., 1984; White and Warr, 1983), mice (Osen et al., 1984), monkeys (Thompson et al., 1984; 1986), chinchillas (Iurato, Luciano, Pannese and Reale, 1971; Osen et al., 1984) and guinea pigs (Altschuler et al., 1983; Brown, 1987b; Iurato et al., 1971; Thompson et al., 1984) has revealed concentrated amounts of AChE within the descending efferent fiber bundles and brainstem cells of origin, and within the intraganglionic spiral bundle (IGSB) and organ of Corti. In the guinea pig, AChE is contained within: (a) the inner and tunnel spiral bundles; (b) the tunnel crossing fibers; and (c) beneath the IHCs all three rows of OHCs (Iurato et al., 1971; Brown, 1987b). The cochlear distributions of AChE and ChAT are very similar in both the rat and guinea pig. Both AChE and ChAT exhibit a progressively greater distribution toward the cochlear base, and less towards the apex, with a considerably greater distribution within the IHC vs the OHC region (Brown, 1987b; Godfrey and Ross, 1985; Godfrey et al., 1986). The average activity of AChE within the organ of Corti in rats is more that 5X higher than the average AChE activity found either in whole brain or in non-neural cochlear structures. Indeed, cochlear efferent fiber bundle levels of AChE are greater than AChE levels observed within the facial nucleus and nerve root (Godfrey et al., 1982; 1984; 1986; Godfrey and Ross, 1985). These studies have shown that a specific enzymatic mechanism exists for the inactivation and subsequent synaptic removal of ACh

following its presynaptic release and postsynaptic action within the cochlea.

There is also evidence for antagonism of electrical stimulation-induced medial efferent effects. In cats, intracochlear perfusion of the highly specific, nicotinic receptor blocker and snake neurotoxin, α-bungarotoxin (Koelle, 1975; Lefkowitz et al., 1990; Taylor, 1990c), completely and reversibly blocks the medial efferent-induced round window recorded changes in the EP, and N1 and CM evoked by low intensity click stimuli (Fex and Adams, 1978). In another investigation in cats (Galley, Klinke, Oertel, Pause and Storch, 1973), intracochlear perfusion of a number of nicotinic and muscarinic cholinergic antagonists completely blocked the medial efferent-induced N1 suppression and CM potentiation. These antagonists included the: (a) nicotinic ganglionic/neuromuscular antagonist d-tubocurarine (curare); (b) the nicotinic ganglionic blockers hexamethonium (C6); and mecamylamine (c); the nicotinic depolarizing neuromuscular blocker succinylcholine; (d) the choline transport/ACh synthesis blocking agent hemicholinium (HC-3); and (e) the muscarinic cholinergic antagonist atropine sulfate (Brown, 1990; Cooper et al., 1986; Taylor, 1990a; 1990c; Weiner and Taylor, 1985). In these investigations, the reported effects: (a) began 5 to 10 minutes post-perfusion; (b) were maximally effective by 25

minutes; and (c) were reversed by wash perfusions with artificial perilymph (Galley et al., 1973).

In guinea pigs, cochlear perfusion into the scala tympani with the presynaptic autoreceptor-blocking and postsynaptic muscarinic antagonist atropine sulfate or the more potent quaternary antimuscarinic methylatropine completely reversed the medial efferent-induced: (a) 150-175μV augmentation in the basal cochlear promontory recorded CM; (b) 40-60μV positive shift in the basal cochlear promontory recorded summating potential (SP^{-}); (c) 4-6mV negative shift in the promontory- and scala media-recorded EP (Konishi, 1972), or 70-80μV suppression in the promontory-recorded N1 (Bobbin and Konishi, 1974). Identical results are obtained following intracochlear infusion with the nicotinic ganglionic antagonists, decamethonium (C10) and hexamethonium (C6), or the nicotinic ganglionic and neuromuscular blocker d-tubocurarine (Bobbin and Konishi, 1974; Konishi, 1972). Furthermore, atropine, d-tubocurarine, and all of the above mentioned drug effects are reversed by an intracochlear rinse with artificial perilymph (Bobbin and Konishi, 1974; Konishi, 1972).

There is additional evidence for antagonism of ACh-induced efferent activation effects. Anticholinergic blockade of ACh-induced efferent effects have also been demonstrated in guinea pigs. The addition of d-tubocurarine to a cochlear perfusate containing ACh and eserine, completely blocks the cholinergically-induced promontory-

recorded: (a) 150 to 200μV amplitude increase in CM; and (b) 70μV suppression of the N1 potential (Bobbin and Konishi, 1974). Robertson and Johnstone (1978) demonstrated that d-tubocurarine added to a cochlear perfusate containing ACh and eserine completely blocks the cholinergically-induced 25 to 30dB(SPL) loss in single unit tuning curves and threshold sensitivity in Type I neurons recorded from the spiral ganglia.

These studies collectively demonstrate that the exogenous application of synthetic agents with known pharmacological antagonistic properties at cholinergic receptors can produce a postsynaptic blockade of efferent effects in a predictable manner.

Neurotransmitter Evidence For Gamma (γ)-Aminobutyric Acid (GABA)

Some evidence has suggested the possibility of an efferent neorotransmitter role for the inhibitory amino acid (i.e. Bloom, 1990; Cooper et al., 1986 Davidoff and Hackman; 1985; Kandel, 1985a) -aminobutyric acid (GABA) (Altschuler and Fex, 1986; Beattie and Moore, 1991; Bobbin, Ceasar and Fallon, 1990; Drescher and Drescher, 1985; Drescher, Drescher and Medina, 1983; Eybalin and Altschuler, 1990; Eybalin, Parnaud, Geffard and Pujol, 1988; Fex and Altschuler, 1984; 1985; 1986; Fex, Altschuler, Kachar, Wenthold and Zempel, 1986; Gulley, Fex and Wenthold, 1976; Schwartz and Ryan, 1983; 1986; Schwarz, Schwarz, Hu and

Vincent, 1988; Thompson et al., 1986; Thompson, Cortez and Lam, 1985). However, within the organ of Corti of both guinea pig and rat, the relative concentrations of GABA and enzymatic GAD-activity are actually quite low (Eybalin et al., 1988; Fex and Wenthold, 1976), and are no higher than concentrations found within non-neural structures of the cochlea (Godfrey et al., 1986). Such evidence has made it is difficult to consider GABA as an important inhibitory neurotransmitter within the efferent system of the cochlea in these species (Beattie and Moore, 1991; Bledsoe, 1986; Bledsoe et al., 1988; Eybalin and Altschuler, 1990; Eybalin et al., 1988; Godfrey et al., 1986; Guth and Melamed, 1982; Klinke, 1981; Wenthold, 1980). For reviews see: Bledsoe, 1986; Bledsoe et al., 1988.

There is also considerable evidence that the lateral efferent olivocochlear system employs neuroactive opioid peptides. Therefore, what follows is a short review of this literature.

Historical Overview of Opioid Research

Terminology: The term opioid is presently taken in a generic sense to refer to all substances, natural or synthetic, having opiate or morphine-like actions via their binding to designated (opioid) receptors. Opioid also refers to the antagonists of these substances, and to the receptors that bind their ligands (Goldstein, 1984; Jaffe and Martin, 1990). These include morphine and the thebain derivative,

naloxone. They also include synthetic phenylpiperidines like: meperidine, fentanyl, sufentanil, and alfentanil (Bovill, 1987; Jaffe and Martin, 1985; 1990) and the synthetic benzomorphans like pentazocine, cyclazocine, ketocyclazocine, bremazocine, and SKF-10,047 (Chang, Hazum and Cuatrecasas, 1981; Itzhak, 1988). The term 'opioid' also refers to the biologically endogenous peptides which are opium-like in action.

Historical Perspective: Opioid peptide research was given a great impetus by the discovery in three laboratories of stereospecific opioid drug binding in the vertebrate central nervous system, using tritiated ([^{3}H]-labeled): naloxone (Pert and Snyder, 1973), etorphine (Simon, Hiller and Edelman, 1973), or dihydromorphine (Terenius, 1973). These initial discoveries introduced the possibility that the brain possessed its own analgesic/antinociceptive system. The identification in 1973 of opioid receptors instituted an active search for the opioid-like ligand endogenous to the mammalian nervous system.

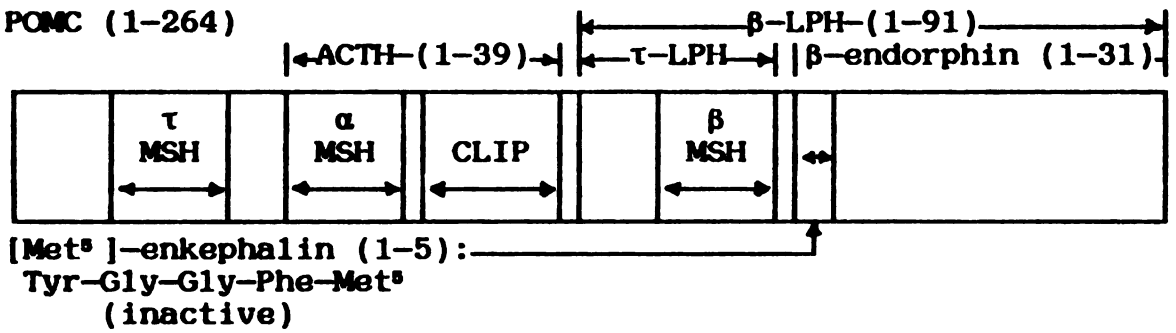
This search led to the isolation and identification in porcine brain, of the naturally-occurring opioid pentapeptides, methionine [Met^5]- and leucine [Leu^5]-enkephalin (Hughes, 1975; Hughes, Smith, Kosterlitz, Fothergill, Morgan and Morris, 1975). The application of recombinant DNA biochemistry in 1979 for the characterization of adrenocorticotropic and melanocyte-stimulating (ACTH/MSH) hormone (Nakanishi, Inoue, Kita,

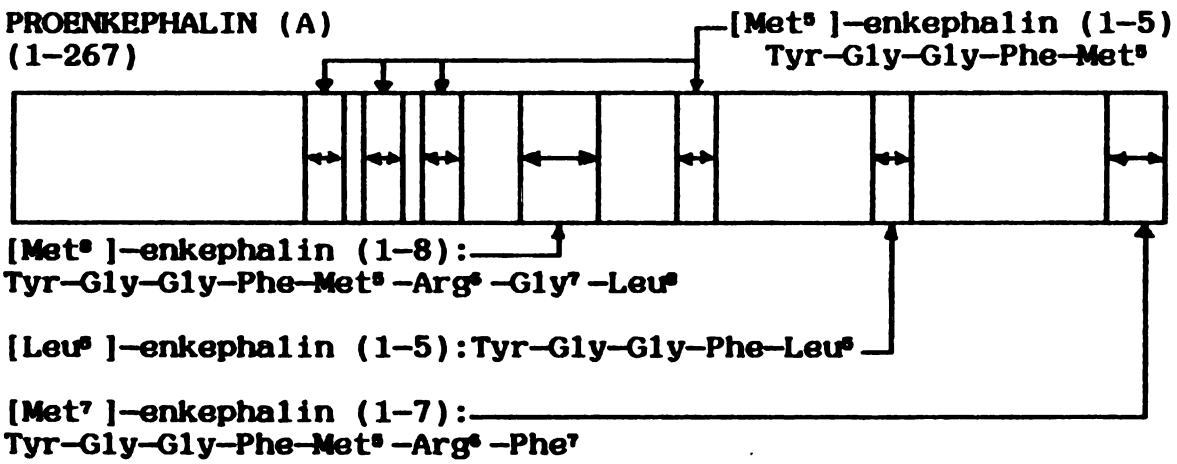
Nakamura, Chang, Cohen and Numa, 1979), and the characterization in 1982 of opioid peptides from endocrine tissue, resulted in the identification of three genetically distinct opioid peptide families (Comb, Herbert and Crea, 1982a; Comb, Seeburg, Adelman, Eiden and Herbert, 1982b; Gubler, Seeberg, Hoffman, Gage and Udenfriend, 1982; Kakidani, Furutani, Takahashi, Noda, Morimoto, Hirose, Asai, Inayama, Nakanishi and Numa, 1982; Noda, Furutani, Takahashi, Toyosato, Hirose, Inayama, Nakanishi and Numa, 1982a; Noda, Teranishi, Takahashi, Toyosato, Notake, Nakanishi and Numa, 1982b). Illustration 4 on the following page schematically depicts the three biochemical precursors and some of their metabolic products found within mammalian endocrine and nervous tissue, from which the known opioid peptides are derived. These are proopiomelanocortin (POMC); proenkephalin, derived from en kephalos meaning in the head (Hughes, 1984); and prodynorphin (proenkephalin B), derived from the Greek word dynamis for power and endorphin, from endogenous morphine (Frederickson, 1984; Goldstein, 1984; Goldstein, Tachibana, Lowney, Hunkapiller and Hood, 1979).

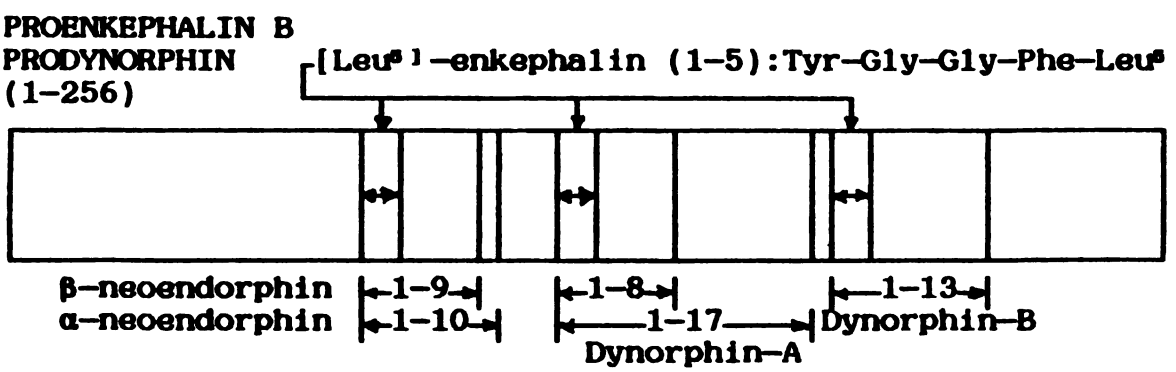
Opioid Receptors: The term receptor is operationally used to denote any cellular macromolecule to which a ligand binds in order to initiate its cellular effects (Ross, 1990). Earlier investigations of opioid binding (e.g. Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973) pri-

Illustration 4

ENDOGENOUS OPIOID FAMILIES







(Modified from: Akil et al., 1984; Dores et al., 1984; Khachaturian et al., 1985a; Lever et al., 1983)

marily identified the μ-opioid receptor. Based originally upon a detailed analysis of a wide spectrum of structure-activity relationships among analgesic ligands, it was Portoghese (1967) who first proposed the existence of multiple opioid receptor types. The pharmacological studies of Martin (1967) and associates (Martin, Eades, Thompson, Huppler and Gilbert, 1976) in the chronic spinal dog preparation, later complemented and provided a more detailed framework for this concept (Takemori and Portoghese, 1984).

Biochemical, pharmacological and anatomical evidence has accumulated supporting the existence of at least three general classes of opioid receptors. Brain opioid receptor types encompass an alkaloid (morphine-like) mu (μ) receptor, an enkephalin delta (δ) receptor, and a prodynorphin-selective (ketocyclazocine) kappa (K) receptor (Chang, 1984; Chang and Cuatrecasas, 1979; Chang et al., 1981; Goodman and Pasternak, 1984; Goodman, Snyder, Kuhar and Young, 1980; Hollt, 1986; Itzhak, 1988; Kosterlitz, 1985; Kosterlitz, Lord, Paterson and Waterfield, 1980; Kosterlitz, Magnan, Paterson and Tavani, 1981a; Kosterlitz, Paterson and Robson, 1981b; Lever, Chang and McDermed, 1983; Lord, Waterfield, Hughes and Kosterlitz, 1977; Mansour, Khachaturian, Lewis, Akil and Watson, 1987; Mansour, Khachaturian, Lewis, Akil and Watson, 1988; Mansour, Lewis, Khachaturian, Akil and Watson, 1986; Millan, 1986; Paterson, Robson and Kosterlitz, 1983; 1984). While there is no obvious endogenous opioid family associated with the best characterized opioid

receptor, the μ-site (Dores, Akil and Watson, 1984), there is evidence supporting the existence of an endorphin-selective (i.e. POMC) epsilon (ε)-receptor (Chang, 1984; Goodman, Adler and Pasternak, 1988; Goodman, Houghten and Pasternak, 1983; Houghten, Johnson and Pasternak, 1984; Law, Loh and Li, 1979; Schulz, Wuster and Herz, 1981; Wuster, Schulz and Herz, 1979).

Autoradiographic and immunohistochemical investigations have revealed separate yet interacting μ-, δ-, K- and ε-opioid receptor distributions within the rodent (e.g. Akil, Watson, Young, Lewis, Khachaturian and Walker, 1984; Goodman et al., 1980; 1983; 1988; Itzhak, Hiller and Simon, 1984; Law et al., 1979; Mansour et al., 1986; 1987; 1988) cat (Walker, Bowen, Thompson, Frascella, Lehmkuhle and Hughes, 1988), and primate CNS (Lewis, Khachaturian and Watson, 1983; Lewis, Mansour, Khachaturian, Watson and Akil, 1987; Lewis, Mishkin, Bragin, Brown, Pert and Pert, 1981). Each opioid precursor (Illustration 4) produces multiple products, and each product has a range of receptor (non) selectivities (i.e. Kosterlitz, 1985; Paterson et al., 1983; 1984). Like many other known neurotransmitters, most of these substances (especially the enkephalins) are rapidly metabolized (Hughes, 1975; Miller, 1983; Miller, Chang, Cuatrecasas and Wilkinson, 1977; Paterson et al., 1984) by peptidases (carboxy and amino) and generally, do not readily pass the blood-brain barrier (e.g. Kosterlitz, 1985; Zlokovic, Begley and Chain-Eliash, 1985).

Overall Significance: There is presently little doubt that endogenous opioid substances play an important role in gating nociceptive sensory input (Akil, 1982; Basbaum, 1984; Basbaum, Clanton and Fields, 1976; Basbaum and Fields, 1978; 1984; Fields and Basbaum, 1978; Millan, 1986). Their ubiquitous distribution however, has indicated that many other physiologic processes fall under the influence of these neuroactive transmitter/modulators. For instance, endogenous opioid peptides and their unique receptors have widespread CNS distributions within the forebrain (telencephalon; diencephalon), midbrain (mesencephalon), hindbrain (pons; medulla), and spinal cord (Cruz and Basbaum, 1985; Dores and Akil, 1987; Dores et al., 1984; Elde, Hokfelt, Johansson and Terenius, 1976; Glazer and Basbaum, 1981; 1984; Gouarderes, Cros and Quirion, 1985; Khachaturian, Lewis, Schafer and Watson, 1985a; Khachaturian, Lewis, Tsou and Watson, 1985b; Khachaturian, Lewis and Watson, 1983; Lewis et al., 1981; 1983; 1987; Mansour et al., 1986; 1987; 1988; Watson and Akil, 1987; Watson, Akil, Fischli, Goldstein, Zimmerman, Nilaver and van Wimersma Greidanus, 1982a; Watson, Akil, Khachaturian, Young and Lewis, 1984; Watson, Akil, Richard and Barchas, 1978; Watson and Barchas, 1979; Watson, Khachaturian, Akil, Coy and Goldstein, 1982b; Watson, Khachaturian, Coy, Taylor and Akil, 1982c). They are also found within the PNS (Araujo and Collier, 1987; Glazer and Basbaum, 1980; Schultzberg, 1984; Schultzberg, Hokfelt, Lundberg, Terenius, Elfvin and Elde

1978; Vincent, Dalsgaard, Schultzberg, Hokfelt, Christensson and Terenius, 1984).

It is believed that their modulatory role in biologic systems has evolved to encompass the regulation of responses to physical and psychological stress (e.g. Akil et al., 1984; Grossman and Rees, 1983) affect, mood, drive and reinforcement (Herkenham and Pert, 1980; Miller and Pickel, 1980; Panksepp, 1980; 1986) and attention (Arnsten, Neville, Hillyard, Janowsky and Segal, 1984; Arnsten, Segal, Neville, Hillyard, Janowsky, Judd and Bloom, 1983), and to the ultimate survival of species (Blank, Paneria and Friesen, 1979; Cicero, Schainker and Meyer, 1979). Evidence indicates that they enhance, or fine tune the regulatory functions exerted by other neurotransmitters or hormones (Cox, 1988; Henderson, 1983; Leander, 1983; Mudge, Leeman and Fischbach, 1979; North, 1986; Simonds, 1988; Walker, Ghessari, Peters, Watson, Seidah, Chretien and Akil, 1987a; Wood and Iyengar, 1988), and they appear to play a role in the filtering and processing of sensory information, as they are quite abundantly found within primary sensory nuclei of the visual, auditory, olfactory and somatosensory systems (e.g. Mansour et al., 1986; 1987; 1988; Walker et al., 1988).

Benzomorphans

Benzomorphan opioids, which include the racemic (±) and levorotatory (-) isomers of pentazocine, cyclazocine, ethyl-ketocyclazocine (EKC), bremazocine, and SKF-10,047, exhibit both K-receptor agonist and μ-receptor antagonist properties

(Cowan, 1981; Gillan, Kosterlitz and Magnan, 1981; Kosterlitz et al., 1981b; Lahti et al., 1985; Magnan, Paterson, Tavani and Kosterlitz, 1982). Important to the present series of investigations, however, is the overall distinction between benzomorphans which bind opioid (specifically kappa), and those which bind non-opioid receptors, such as sigma (σ). As indicated below, many dextrorotatory (+) benzomorphan isomers bind non-opioid receptors.

Sigma Receptors; Relationship To Opioid Receptors: Receptors that bind the benzomorphan (±)-SKF-10,047 (N-allylnormetazocine) were originally designated as sigma (σ), and σ-receptors were once postulated as a type of opioid receptor (Martin et al., 1976). Evidence in support of an 'opioid σ-receptor' was based upon behavioral studies in animals, using the racemates of SKF-10,047 and related benzomorphans like pentazocine and cyclazocine (Gilbert and Martin, 1976; Martin et al., 1976). The σ-receptor was thought to mediate the opioid-induced spectrum of behaviors in the dog (Martin et al., 1976) referred to as canine delirium, a condition concomitant with autonomic stimulation (e.g. mydriasis, tachycardia and tachypnea). In humans, the σ-receptor effects of benzomorphans were thought to be responsible for sedation and analgesia at low doses, and psychotomimetic effects (i.e. depersonalization, paranoid delusions, and hallucinations) at higher doses (e.g. Martin, 1967; Haertzen, 1970). Subsequent experimental evidence has

failed, however, to support the view that σ-receptors represent a class of opioid receptor.

To begin with, although the regional distribution of σ-receptors in some cases overlaps (McLean and Weber, 1988; Tam, 1983; 1985), the distributions of μ-, δ- and K-opioid receptors are nevertheless distinct from those of σ-receptors. Indeed, σ-receptors seem most concentrated within nuclei and structures associated with the expression of motor behavior. These include cranial nerve nuclei: III, V, VI, VII, IX and X, and also the cerebellum (Gundlach, Largent and Snyder, 1986; McLean and Weber, 1988; Tam, 1983; 1985; Walker, Bowen, Walker, Matsumoto, de Costa and Rice, 1990).

Compared to the classic (μ-, δ- and K-) opioid receptors, σ-receptors exhibit a reverse stereoselectivity for opioid-like compounds. Indeed, while μ-, δ- and K-opioid receptors are characteristically enantioselective for the levorotatory (-)-isomers of opioid agonists and antagonists (i.e. Goldstein, 1976), the σ-receptor is enantioselective for the dextrorotatory (+)-isomers of some opioid substances (Largent, Gundlach and Snyder, 1986b; Lahti et al., 1985; Pert and Snyder, 1973; Su, 1981; Tam, 1983; 1985; Tam and Cook, 1984; Walker et al., 1990; Zukin, 1982; Zukin and Zukin, 1988). For example, the dextrorotatory isomers of pentazocine, EKC, cyclazocine and SKF-10,047 are all devoid of opioid receptor activity (Lahti et al., 1985; Tam, 1985; Tiberi and Magnan, 1990; Walker et al., 1990; Zukin, 1982),

yet are potent σ-receptor ligands. Furthermore, the σ-receptor fails to bind the μ-opioid receptor ligands: morphine, β-endorphin, (-)-etorphine, levorphanol, and naloxone. It also fails to bind the δ-opioid receptor ligands: [Met^5]- [Leu^5]-, and [D-Ala^2,D-Leu^5]-enkephalin (DADLE); and the K-opioid receptor ligands: (-)-pentazocine, (-)-cyclazocine, (-)-ethylketocyclazocine, α-neoendorphin, and dynorphin A (1-17) or B (Bowen, de Costa, Hellewell, Thurkauf, Walker and Rice, 1990a; de Costa, Bowen, Hellewell, Walker, Thurkauf, Jacobson and Rice, 1989; Largent, Wikstrom, Gundlach and Snyder, 1987; Su, 1981; Tam, 1983; 1985; Tam and Cook, 1984; Tam and Zhang, 1988).

The universal μ, δ and K-opioid antagonist (-)-diprenorphine (i.e. Chang, 1984; Chang et al., 1981) also fails to bind the σ-receptor (Tam, 1983). The prototypic opioid antagonists, naloxone and naltrexone are also ineffective against the agonist effects of σ-receptor ligands both in vivo (Brady, Balster and May, 1982; Iwamoto, 1981; Vaupel, 1983) and in vitro (Largent et al., 1987; Tam, 1983; 1985; Tam and Cook, 1984). Such evidence further supports the view that the σ-receptor is non-opioid. Indeed, the σ-receptor is often referred to as 'naloxone- or etorphine-inaccessible' (Su, 1981; Tam, 1983, 1985; Walker et al., 1990). Since sensitivity to the non-specific antagonist naloxone is an important requirement for a receptor to be considered opioid in character (i.e. Quirion, Chicheportiche, Contreras, Johnson, Lodge, Tam, Woods and

Zukin, 1987) it is clear that the σ-receptor is not a type of opioid receptor (Walker et al., 1990).

Sigma-Receptor Ligands: Of the synthetic opioid-like compounds, many of the racemic and dextrorotatory isomers of benzomorphans like pentazocine, cyclazocine, phenazocine, SKF-10,047, ethylketocyclazocine (EKC) and bremazocine evidence the highest overall σ-receptor binding affinity (Largent et al., 1987; Tam and Cook, 1984; Tam and Zhang, 1988; Walker et al., 1990; Zukin and Zukin, 1988). Other potent and selective σ-receptor ligands include (+)-[^{3}H]-3-PPP (Gundlach et al., 1986; Largent, Gundlach and Snyder, 1986a; 1986b; 1987; Matsumoto, Hemstreet, Lai, Thurkauf, de Costa, Rice, Hellewell, Bowen and Walker, 1990; Walker et al., 1990; Zukin and Zukin, 1988) and the optically pure (+)-[^{3}H]-(**1b**)-pentazocine (de Costa et al., 1989). All of these are potently displaced by racemic and dextrorotatory benzomorphans, while their levorotatory isomers are, of course, without such effects.

Relationship of Sigma Receptors to Dopamine Receptors: It had also been suggested (i.e. Martin et al., 1976) that the sigma agonist activity of (±)-SKF-10,047 involved a dopaminergic mechanism. Indeed, binding of the tritiated neuroleptic butyrophenone [^{3}H]-haloperidol is potently displaced by the dextrorotatory σ-receptor ligand (+)-SKF-10,047, as well as by the pentazocine and cylazocine racemates (Tam and Cook, 1984). Furthermore, many other antipsychotic drugs bind to -receptors with high affinity

(Largent et al., 1987; Tam and Cook, 1984; Walker et al., 1990). The neuroleptic agent, haloperidol, which is completely devoid of opioid activity (Millan, 1989), produces its therapeutic and extrapyramidal side effects via blockade of dopamine (D_2) receptors (e.g. Bloom, 1990; Ellenbroek, Artz and Cools, 1991) while also exhibiting a very high binding affinity for σ-receptors (Bowen, Moses, Tolentino and Walker, 1990b; Largent et al., 1987; Matsumoto et al., 1990; Su, 1981; Tam, 1983; Tam and Cook, 1984; Walker et al., 1990).

Haloperidol has been shown to be the most potent inhibitor of the σ-receptor ligands: (+)-[^{3}H]-SKF 10,047 (Bowen et al., 1990a; Gundlach, Largent and Snyder, 1985; Largent et al., 1986a; 1986b; Su, 1981; Tam, 1983; 1985; Tam and Cook, 1984; Walker et al., 1990); (+)-[^{3}H]-3-PPP (Gundlach et al., 1986; Largent et al., 1986a; 1986b; 1987; Tam and Zhang, 1988; Walker et al., 1990); and the optically pure ([^{3}H]-**1b**) (+)-[^{3}H]-pentazocine (Bowen et al., 1990a; de Costa et al., 1989; Walker et al., 1990). Indeed, only the optically pure ([^{3}H]-**1b**)-pentazocine exhibits an affinity for the σ-receptor, equal to that of haloperidol (Bowen et al., 1990a; de Costa et al., 1989). Such data might suggest that (+)-SKF-10,047, (+)-pentazocine, or other benzomorphans label dopamine receptors. However, additional evidence indicates that this is not the case.

To begin with, the binding affinity of haloperidol at the -receptor is equal to its affinity at the D_2 receptor

(Bowen et al., 1990b; Tam and Cook, 1984). The butyrophenone neuroleptic and haloperidol-analog, spiperone (spiroperidol) is one of the most potent dopaminergic antagonists yet discovered, exhibiting a 10-fold higher affinity for D_2 receptors than haloperidol (Creese, Burt and Snyder, 1978). It has been shown however, that (±)-pentazocine displaces [^{3}H]-haloperidol from the guinea pig brain at least 10X more potently than does spiperone (Tam and Cook, 1984). Haloperidol has been shown to displace (+)-[^{3}H]-SKF-10,047 from guinea pig brain with a potency as great as 273X that of spiperone (Tam, 1985). Haloperidol also displaces (+)-[^{3}H]-3-PPP from the same tissue, 335X more potently than does spiperone (Largent et al., 1986b). Finally, haloperidol displaces (+)-[^{3}H]-pentazocine binding from guinea pig brain with a potency >1000 times that of spiperone (Walker et al., 1990). Such evidence strongly indicates that haloperidol displaces σ-receptor binding, and that σ-receptors are not dopamine receptors.

Additional evidence employing the dopamine (D_2) receptor ligand apomorphine also indicates that σ-receptors are not a type of dopamine receptor. Apomorphine is about 25 times more potent an agonist than dopamine itself (Bloom, 1990; Creese et al., 1978). Both apomorphine and dopamine are, however, completely devoid of σ-receptor binding activity (de Costa et al., 1989; Largent et al., 1987; Tam and Cook, 1984; Walker et al., 1990). Thus it appears that the molecular structural requirements of ligands for sigma

and dopamine receptor activity are divergent (Largent et al., 1987; Walker et al., 1990), and that the σ-receptor is not a type of dopamine receptor.

Relationship of Sigma Receptors To Phencyclidine (PCP) Receptors: It was once believed that the sigma activities of benzomorphan opioids were mediated through a common σ/PCP-receptor (Mendelsohn, Kalra, Johnson and Kerchner, 1985; Miller, 1983; Zukin, 1982; Zukin and Zukin, 1981; Zukin, Tempel, Gardner, and Zukin, 1986). The assertion that σ- and PCP-receptors are identical was based primarily upon receptor studies of [^{3}H]-PCP displacement by the prototypic sigma ligand (+)-SKF-10,047 (i.e. Mendelsohn et al., 1985; Tam, 1983; Zukin, 1982). Indeed, the racemic, levo- and dextrorotatory isomers of SKF-10,047 and cyclazocine all bind PCP receptors, albeit with very low affinity (Largent et al., 1986a; Mendelsohn et al., 1985; Rothman, Bykov, Newman, Jacobson, and Rice, 1988; Steinfels, Alberici, Tam and Cook, 1988; Tam, 1983; Tam and Zhang, 1988; Zukin, 1982; Zukin and Zukin, 1981; 1988). The potent K/σ-receptor ligand (±)-pentazocine (Gundlach et al., 1985; Largent et al., 1986a; Zukin and Zukin, 1988) or the highly selective and potent σ-receptor ligand (+)-pentazocine are, however, devoid of PCP receptor activity (Rothman et al., 1988; Steinfels et al., 1988; Tam and Zhang, 1988; Walker et al., 1990).

Several different classes of compounds can bind σ-receptors, including dissociative arylcyclohexylamine

anesthetics like the psychoactive PCP (e.g. Largent et al., 1986a; Mendelsohn et al., 1985; Tam, 1983; 1985; Walker et al., 1990; Zukin and Zukin, 1988). However, while systematic studies of PCP are generally lacking, this compound does exhibit a much greater affinity for the PCP-receptor than it does for the σ-receptor (Mendelsohn et al., 1985; Quirion et al., 1987; Tam, 1983; 1985; Walker et al., 1990; Zukin et al., 1986), on the order of 12 to 23X (Gundlach et al., 1985; Largent et al., 1986a; Steinfels et al., 1988; Tam and Zhang, 1988; Zukin and Zukin, 1988). Furthermore, the drug selectivity pattern of σ-receptor ligands: (+)-[^{3}H]-SKF-10,047, (+)-[^{3}H]-3-PPP, [^{3}H]-haloperidol, and (+)-[^{3}H]-1b-pentazocine differ considerably from that of [^{3}H]-PCP, indicating that [^{3}H]-PCP binds different receptors (Tam, 1983; Tam and Cook, 1984; Walker et al., 1990). For example, haloperidol potently displaces (+)-[^{3}H]-SKF 10,047 (Bowen et al., 1990a; Gundlach et al., 1985; Largent et al., 1986a; 1986b; Su, 1981; Tam, 1983; 1985; Tam and Cook, 1984; Walker et al., 1990), while it is weak or inactive (by a factor of 7,636 to 10,000X) against PCP-receptor binding with the potent tritiated PCP-ligands [^{3}H]-PCP (Tam, 1983), [^{3}H]-MK-801, or [^{3}H]-TCP (Gundlach et al., 1985; Largent et al., 1986a; Steinfels et al., 1988; Zukin and Zukin, 1988). Haloperidol also displaces (+)-[^{3}H]-1b-pentazocine on the order of 479 to 542X more potently than does PCP (Bowen et al., 1990a; de Costa et al., 1989).

The CNS distribution of receptors that bind the (tritiated) σ-receptor ligands (+)-[^{3}H]-SKF 10,047 and (+)-[^{3}H]-3-PPP, also differs from the distribution of sites that bind the (tritiated) PCP-receptor ligands [^{3}H]-PCP and [^{3}H]-TCP (Gundlach et al., 1985; Largent et al., 1986a; McLean and Weber, 1988; Tam, 1983; Zukin and Zukin, 1988). Such evidence supports the view that the sigma receptor is not identical to the PCP-receptor site (Walker et al., 1990). A comprehensive review of the biology and function of these non-opioid, haloperidol-sensitive σ-receptors can be found elsewhere (see: Walker et al., 1990).

Evidence For Opioid Peptide Activity Within The Cochlea

Immunocytochemical Investigations

Evidence For Proenkephalin: With the aid of immunofluorescence immunocytochemistry, Fex and Altschuler, (1981) were the first to report [Met^5]-enkephalin-like immunoreactivity within cochleae of guinea pigs and cats. In both species, immunofluorescence was localized to: (1) the unmyelinated OC efferent fibers of the intraganglionic spiral bundle (IGSB) within the osseous spiral lamina; (2) the efferent OC fibers traveling within the inner and; (3) tunnel spiral bundles; as well as; (4) the tunnel crossing fiber bundles. Immunoreactivity was also found within efferent fibers beneath the IHCs and within the efferent terminals at the OHC bases in all three rows of the first and second turns (and in the first OHC row of the third

turn) within the organ of Corti (Altschuler and Fex, 1986; Fex and Altschuler, 1981; 1985; 1986). Separate antisera developed against ß-LPH and ß-endorphin failed, however, to produce specific immunofluorescence (Fex and Altschuler, 1981).

Within the guinea pig cochlea, the effects of a weaker, or more specific antiserum having less cross reactivity with [Leu^5]-enkephalin (e.g. Fex and Altschuler, 1985) than previously observed (Fex and Altschuler, 1981) were then compared (Altschuler, Parakkal, Rubio, Hoffman and Fex, 1984b). As previously reported (i.e. Fex and Altschuler, 1981), immunofluorescence and immunoperoxidase immunohistochemistry (see: Fex and Altschuler, 1985; 1986) with this less specific antiserum, labeled both lateral (i.e. inner spiral and tunnel spiral) and medial efferent fiber bundles, and their tracts within the cochlea (Altschuler et al., 1984b). Immunoreactive puncta were again observed both at the bases of the OHCs and within the circumnuclear OHC regions, in all rows up to the third turn. Electron microscopy of the lateral and medial efferent terminal endings further confirmed the existence of enkephalin-like immunoreactivity (Altschuler et al., 1984b) over large, round, dense core vesicles commonly found together with the smaller, clear vesicles (e.g. Pujol and Lenoir, 1986). In the medial system, these dense microvesicles were observed within both the smaller circumnuclear and larger basal synaptic terminals, and in

the latter case were distinctly apposed postsynaptically by subsurface cisternae. Immunoreactivity was also observed over the dense microvesicles in the lateral efferent terminals that apposed the Type I afferent fibers (Altschuler et al., 1984b).

Both antisera in the Altschuler et al. (1984b) study permitted the identification of enkephalin-like-containing lateral efferent terminals directly apposed to primary afferent fibers. The lateral efferent fiber bundles and the smaller circumnuclear medial efferent terminals, up to the third and fourth cochlear turns were completely labeled (Altschuler et al., 1984b). However, the antisera exhibiting less cross reactivity with [Leu^5]-enkephalin failed to label the large medial efferent terminals at the base of the OHCs. Indeed, results obtained from the pre-absorption of both antisera with purified [Met^5]- and [Leu^5]-enkephalin (Altschuler et al., 1984b; Hoffman, Rubio, Altschuler and Fex, 1984), has strongly suggested a lack of multiply active proenkephalin derivatives within the medial efferent terminals which synapse at the OHC base. In fact, results from subsequent studies conducted by these investigators indicated that most antisera to [Met]-enkephalin only produced immunoreactive labeling to the lateral and circumnuclear medial efferent fibers, and failed to label the larger group of medial efferents innervating the OHC base (Altschuler and Fex, 1986; Altschuler et al., 1983;

1984a; 1984b; Eybalin and Altschuler, 1990; Fex and Altschuler, 1985; 1986).

Additional immunofluorescence and immunoperoxidase investigations further corroborated these initial studies by demonstrating the existence of enkephalin-like immunoreactivity within the lateral efferent brainstem nuclei in rats, guinea pigs (Abou-Madi et al., 1987; Altschuler et al., 1983; 1988), and chinchillas (Hoffman, Hassett, Landry and Brimijoin, 1991). Both [Met^5]- and [Leu^5]-enkephalin-like activity are also found within the IGSB and in cochlear fiber bundles associated with the lateral efferent system (Eybalin and Altschuler, 1990; Eybalin, Cupo and Pujol, 1984; Eybalin and Pujol, 1984b; Lehtosalo, Ylikoski, Eranko, Eranko and Panula, 1984). Consistent with previous studies, was the report that lateral efferent enkephalin-like immunoreactivity is greatest from the base to middle cochlear turns, gradually diminishing toward the apex (Lehtosalo et al., 1984). However, lateral efferent enkephalin-like immunoreactivity extending throughout the full length of the organ of Corti has also been reported (i.e. Eybalin et al., 1984; Eybalin and Pujol, 1984b). Presynaptic enkephalin-like immunoreactive terminal varicosities have been visualized making synaptic contacts with afferent fibers within the IGSB, and in the regions beneath the IHCs (Eybalin and Altschuler, 1990; Eybalin et al., 1984). Enkephalin-like immunoreactivity was not observed within any OHC regions, in

two of these cited investigations (e.g. Eybalin et al., 1984; Lehtosalo et al., 1984).

The octapeptide proenkephalin derivative [Met^8]-enkephalin, has additionally been localized within the lateral efferent brainstem nuclei in rats and guinea pigs (Abou-Madi et al., 1987). Both the hepta- and octapeptide proenkephalin derivatives [Met^7]- and [Met^8]-enkephalin, respectively, are also found within the cochlea. As expected, these proenkephalin derivatives are found within the IGSB and within the lateral efferent fiber bundles of the organ of Corti (Eybalin and Altschuler, 1990; Eybalin et al., 1984; Eybalin, Cupo and Pujol, 1985b; Lehtosalo et al., 1984). Hepta- and octapeptide immunoreactive lateral efferent varicosities have been visualized making synaptic contacts with afferent fibers within the IGSB, and in the regions beneath the IHCs (Eybalin and Altschuler, 1990; Eybalin et al., 1984; Eybalin, Cupo and Pujol, 1985b). Furthermore, total efferent fiber transection by evulsion of the vestibular nerve close to the vestibulocochlear (Oorts) anastomosis eliminates the enkephalin-like immunoreactivity found within the cochlea (Altschuler et al., 1984b; Eybalin and Altschuler, 1990; Hoffman et al., 1984; Hoffman, Zamir, Rubio, Altschuler and Fex, 1985).

Evidence For Prodynorphin: The deca-, trideca- and heptadeca-peptide prodynorphin derivatives, α-neoendorphin, dynorphin-B and dynorphin-A, respectively, also occur within the brainstem superior olivary lateral efferent nuclei, in

guinea pigs and rats (Abou-Madi et al., 1987; Altschuler et al., 1988). Both α-neoendorphin and dynorphin-B have also been localized to the lateral efferent (i.e. inner spiral and tunnel spiral) fiber bundles, and terminals near the IHC base in this species. Prodynorphin immunoreactivity has been observed in all cochlear turns within the organ of Corti (Altschuler, Hoffman, Reeks and Fex, 1985a). Tunnel crossing and outer spiral fibers of the medial efferent system also exhibit prodynorphin immunoreactivity. However, immunoreactive labeling to both derivatives is observed only within the medial efferent terminals which synapse at the circumnuclear level of the OHCs. Similar to the enkephalin-like distribution described earlier, prodynorphin immunoreactive circumnuclear OHC puncta were often seen in the third and lower-fourth turns of the cochlear spiral (Altschuler et al., 1985a).

Since the prodynorphin antisera used above exhibited no cross reactivity to proenkephalin derivatives, the similarity of the prodynorphin distribution with previously reported proenkephalin distributions strongly suggested the co-localization of products derived from these two distinct opioid families (Altschuler et al., 1985a). Similar to the cochlear distribution observed for ACh, enkephalin immunoreactivity, unlike prodynorphin immunoreactivity (i.e. Altschuler et al., 1985a) is generally not observed at the extreme cochlear apex (Altschuler and Fex, 1986; Fex and Altschuler, 1981; 1985; 1986). For reviews, see: Altschuler

and Fex (1986); Eybalin and Altschuler (1990); Fex and Altschuler (1985; 1986).

Summary: With the exception of the prodynorphin-containing efferent terminals at the circumnuclear level of the OHCs, the available evidence has indicated that the medial efferent system is relatively unresponsive to proenkephalin/prodynorphin antisera, when compared to the lateral efferent fiber system. Indeed, immunocytochemical investigations have further suggested that the enkephalin-like immunoreactivity earlier observed in medial efferent terminals at the base of OHCs (i.e. Altschuler et al., 1984b; Eybalin and Pujol, 1984b; Fex and Altschuler, 1981; Hoffman et al., 1984) may indicate the presence of some other structurally homologous enkephalin peptide or peptides sharing antigenic sites with methionine enkephalin (e.g. Altschuler et al., 1984a; 1984b; Eybalin, Abou-Madi, Rossier and Pujol, 1985a; Eybalin and Altschuler, 1990; Hoffman et al., 1984; Lehtosalo et al., 1984). It has even been suggested that proenkephalin-derived opioids are completely restricted to the lateral efferent fibers and terminals (Eybalin et al., 1985a; Lehtosalo et al., 1984).

Additional HPLC/RIA Investigations: Additional evidence for the existence of multiple opioid neuropeptide products within the cochlea begins with the results obtained from a high-performance liquid chromatography (HPLC) analysis of neuromodulator candidates within the perilymph of the guinea pig cochlea. During exposure to wide-band noise at 80 to

115dB SPL, [Met^5]-enkephalin-like levels were significantly elevated relative to control values obtained in quiet (Drescher and Drescher, 1985; Drescher et al., 1983). However, in another experiment (70, 90 and 110 dB SPL; 60 minutes exposure) there was a decrease of cochlear [Met^5]-enkephalin-like levels as a function of broadband noise intensity (Eybalin, Rebillard, Jarry and Cupo, 1987).

In guinea pigs, [Met^5]-enkephalin has also been identified by a combined HPLC and subsequent radioimmunoassay (RIA) analysis of whole or partial cochleae sonicates (Eybalin, et al., 1984; Hoffman, 1986; Hoffman, Altschuler and Fex, 1983; Hoffman et al., 1984). Also identified, has been the pentapeptide [Leu^5]-enkephalin (Hoffman, 1986; Hoffman et al., 1984), the heptapeptide [Met^7]-enkephalin (Eybalin et al., 1984), and the octapeptide [Met^8]-enkephalin (Eybalin et al., 1985b; Hoffman et al., 1985). RIA of guinea pig cochlear sonicates has also demonstrated the presence of immunoreactivity to dynorphin-B within the cochlea (Hoffman, 1986; Hoffman et al., 1985). Total efferent fiber transection by evulsion of the vestibular nerve close to the vestibulocochlear (Oorts) anastomosis eliminated the enkephalin-related peptides identified by HPLC-RIA (Hoffman, 1986; Hoffman et al., 1984; 1985).

Evidence That ACh Coexists With Proenkephalin And Prodynorphin Gene Products Within Efferent Neurons

Evidence Within Lateral Efferent Neurons: In the present context, the term 'coexistence' refers to the presence of more than a single neuroactive substance within a neuron, and is much more difficult to demonstrate than the 'colocalization' of the same substances within a group of neurons (Chan-Palay and Palay, 1984a; 1984b). Following the initial investigation of Fex and Altschuler (1981), [Met^5]-enkephalin-like immunoreactivity was found to coexist with immunoreactivity for AChE within the same brainstem lateral efferent olivary cells of origin in the guinea pig (Altschuler et al., 1983). Indeed, and more importantly, both [Met^5]- and [Met^8]-enkephalin-like immunoreactivities coexist with ChAT-immunoreactivity (Abou-Madi et al., 1987; Altschuler et al., 1984a) within the same lateral efferent perikarya in rats and guinea pigs (Altschuler and Fex, 1986; Fex and Altschuler, 1985; 1986). [Met^5]-enkephalin-like immunoreactivity, however, does not coexist with AChE or ChAT-immunoreactivities within medial efferent brainstem olivary cells of origin (Abou-Madi et al., 1987; Altschuler et al., 1983; 1984a; Eybalin and Altschuler, 1990). However, proenkephalin activity has been detected within brainstem medial efferent cell bodies of origin in gerbils (Ryan, Simmons, Watts and Swanson, 1988).

Immunoreactivities to the prodynorphin derivatives, α-neoendorphin and dynorphin-A have also been found to coexist

with ChAT-immunoreactivity within the same lateral efferent perikarya in rats and guinea pigs (Abou-Madi et al., 1987). Furthermore, the gene products of prodynorphin synthesis: α-neoendorphin, dynorphin-A and dynorphin-B, coexist with enkephalin-like immunoreactivity within the same cell bodies of lateral efferent fiber origin, within the brainstem in guinea pigs and rats (Abou-Madi et al., 1987; Altschuler et al., 1988). Such evidence has indicated that ACh, enkephalins and dynorphins coexist within the lateral efferent neurons which project their terminals into the cochlea. Though some cell to cell variability may exist in the respective distributions of these neuroactive substances (Abou-Madi et al., 1987; Altschuler et al., 1988), even their co-localization suggests potentially complex modulatory interactions.

Evidence For Opioid Receptors Within The Cochlea

The specific opioid receptors of the mammalian efferent system within the cochlea, are presently unknown. The existence of opioid receptors within the cochlea has, however, been confirmed by a nonspecific opioid binding assay conducted in the guinea pig cochlea (Hoffman, 1986). The possibility of the co-distribution of both μ and δ cochlear receptors has some gained support from the demonstration (Eybalin, et al., 1987) of naloxone-reversible opioid (morphine; DAGO; DSLET; [Leu^5]-enkephalin) inhibition of adenylate cyclase within guinea pig cochlear homogenates.

Indeed, it is well known that morphine and the μ-receptor, as well as [Met^5]- and [Leu^5]-enkephalin and the δ-receptor, can be negatively coupled to adenylate cyclase (Childers, 1988; Chang, 1984; Cooper, Londos, Gill and Rodbell, 1982; Law, Wu, Koehler and Loh, 1981; Sharma, Nirenberg and Klee, 1975; Simonds, 1988). The available evidence (Eybalin et al., 1987) has indicated that both μ and δ-cochlear opioid receptors may be coupled to the same pool of cochlear adenylate cyclase.

The suggestion that μ- and K, or μ- and δ-opioid receptor types may be coupled to the same terminals is quite consistent with what is presently known regarding the distribution of opioid receptors (e.g. Egan and North, 1981; North, 1986). Products of proenkephalin biosynthesis are found within the cochlea, and all proenkephalin-related opioids are known to exhibit δ-receptor activity. This activity ranges from the K-receptor-inactive pentapeptides [Leu^5]-, which are predominantly δ-selective, and [Met^5]- which is slightly more potent but less selective at δ (Barnard and Demoliou-Mason, 1983; Dores et al., 1984; Kosterlitz, 1985; Kosterlitz et al., 1981b; Paterson et al., 1983; 1984; Zukin and Zukin, 1984), to the heptapeptide [Met^7]- and octapeptide [Met^8]-enkephalins, which seem to interact equally well at both the μ- and δ-receptors (Chang, 1984; Kosterlitz, 1985; Miller, 1983; Watson et al., 1984).

Although the K-receptor has yet to be linked to adenylate cyclase, products of prodynorphin biosynthesis are

found within the cochlea, and these products are known to bind K-receptors (Chavkin, James and Goldstein, 1982; Corbett, Paterson, McKnight, Magnan and Kosterlitz, 1982; James, Chavkin and Goldstein, 1982). Furthermore, the coexistence of opioid derivatives within the same or associated lateral efferent neurons additionally suggests that K-receptors may reside pre- or postsynaptically with δ-receptors within the same or adjacent neurons or structures within the mammalian cochlea.

Overall Objectives

Virtually nothing is known regarding the function of the lateral efferent system. It is well known that lateral efferent neurons co-contain proenkephalin and prodynorphin derivatives together with ACh. In spite of this, systematic attempts to demonstrate opioid-induced modulation of efferent activity as indexed by cochlear output employing δ- or K-opioid receptor ligands, have been lacking.

The anatomical evidence reviewed earlier (Liberman, 1980b) has suggested that tonic input from lateral efferent neurons might be required to establish or modulate or maintain spontaneous activity levels and therefore, may influence the sensitivities and thresholds of Type I auditory afferent fibers (Liberman, 1988b; 1990). This evidence further indicates that the proposed modulation via the lateral efferent neurotransmitter ACh is inhibitory.

Additional evidence supporting an inhibitory role for the lateral efferent/IHC system can be gleaned from developmental studies of stimulus-induced neural suppression and microphonic changes occurring prior to the ontogeny of OHCs and/or their medial efferent innervation (Pujol, 1985; Pujol et al., 1978; 1979; 1980). Perhaps ACh serves a hyperpolarizing role at the lateral efferent synapse. Indeed, ACh may produce postsynaptic inhibition of Type I afferent activity in a manner similar to the parasympathetic inhibition observed by ACh at the preganglionic vagal-cardiac synapse, by an increased K^+ channel (efflux) conductance acting through muscarinic (M_2) receptors (Lefkowitz et al., 1990; Little, 1981; Weiner & Taylor, 1985).

Experimental Hypothesis: It is hypothesized that: (a) presynaptic (autoreceptor) K-opioid receptors modulate the release of ACh from lateral efferent terminals, just as presynaptic K-receptors inhibit the release of ACh onto the postsynaptic parikarya of neurons within the guinea pig myenteric plexus, via a direct reduction in a Ca^{++} conductance (influx) at the presynaptic terminal (Cherubini and North, 1985; North, 1986). Alternatively: (b) perhaps K-receptors are allosterically coupled with a macromolecular cholinergic receptor complex, providing noncompetitive postsynaptic antagonism of ACh (Henderson, 1983). In either case, introduction into the cochlea of a K-opioid agonist could act to antagonize either the presynaptic release (a),

or the postsynaptic effects (b) of ACh, which are presumed to be inhibitory. A disinhibition of Type I fibers would result in the addition of more fibers to the overall CAP at lower stimulus intensities (i.e lower thresholds) and therefore to increased CAP response amplitudes.

Alternatively, many K-opioid receptor ligands have been shown to produce postsynaptic hyperpolarization via direct suppression of a voltage-dependent N-type Ca^{++} current. Such effects are observed within neurons of the mouse dorsal root ganglia (Gross and MacDonald, 1987; North, 1986; Shen and Crain, 1990a; 1990b; Werz and MacDonald, 1985). The N-type Ca^{++} current normally contributes both to the peak and duration (plateau) of the postsynaptic action potential (see: Tsien, 1987). In this case, introduction into the cochlea of a K-opioid agonist could result in the postsynaptic reduction of CAP amplitude.

It is difficult to predict the direction of CM effects following the introduction into the cochlea of a K-opioid agonist. This is partly due to the difficulty in knowing which ion channel(s) would be effected, and more importantly, when and under what conditions the medial efferents are naturally activated. Indeed, the medial efferent system plays no tonic role in determining auditory threshold sensitivity (Littman, Cullen and Bobbin, 1991; Rajan, Robertson and Johnstone, 1990), and most medial efferents fibers (86 to 89%) lack spontaneous activity (Liberman and Brown, 1986; Robertson & Gummer, 1985). The

thresholds for many (monaural) efferent fibers range 15 to 80dB above low-threshold (~10 to 15dB) CF matched afferents from the same ear (Cody and Johnstone, 1982a; Liberman, 1988b; Liberman and Brown, 1985; 1986; Robertson & Gummer, 1985), and most fail to respond to stimuli below 40dB SPL (Fex, 1962; 1965; Liberman and Brown; 1986). Finally, medial efferent fibers are not activated by short duration stimuli such as 100μsec clicks, independent of stimulus intensity (Liberman and Brown, 1986).

Experimental Goals: In view of these considerations, the primary goals of the present set of investigations were to: (a) demonstrate receptor specific opioid-mediated changes in waves N1 and N2 of the CAP, using the K-opioid receptor ligands like U-50488H, U-69593, (±)-pentazocine, and (-)-pentazocine at half the dose of the racemate. The purpose was to document the magnitude rather than the specific duration of such effects. Another goal was; (b) to differentiate potential opioid effects from non-opioid, by comparing the CAP effects of the non-opioid, optical isomer (+)-pentazocine. A third goal was; (c) to further characterize the active receptor as K-, and to localize such effects to the cochlea by demonstrating a blockade of opioid effects using a specific and potent K-receptor antagonist, carefully and noninvasively placed upon the round window. Finally, another goal; (d) was to compare changes in the CAP with CM changes recorded simultaneously, in order to further demonstrate that any effects observed following the

administration of the K-opioid ligand (-)-pentazocine are not due simply to changes in the overall conduction of auditory stimuli by the middle ear system. Such evidence would strengthen the argument that such effects are mediated at the lateral efferent synapse. The principal dependent measures in the experiments described below were the amplitudes of the first and second negative peaks (N1 and N2) of the auditory nerve compound action potential (CAP), and the amplitude of the CM potential.

Choice of Species

The chinchilla was the mammalian species chosen for these investigations. The chinchilla is unique among rodents in that it has a life span of more than 20 years (Bohn, Gruner and Harding, 1990). In healthy animals, age-related changes in auditory function (i.e. spontaneous loss of OHCs) at age two years is less than 1%. A comparable loss in the number of IHCs is not observed until age 4 years in this species. Even during their last few years of life, healthy chinchillas exhibit only a 20% average loss in the number of OHCs, and only a 7% loss of IHCs (Bohn et al., 1990). Parametric studies of frequency sensitivity and selectivity in chinchillas have used animals as old as two years of age (e.g. Spagnoli and Saunders, 1987).

The chinchilla's hearing capabilities are similar to those found in humans (see: Heffner and Heffner, 1991; Moody and Stebbins, 1986). Indeed, minimum audibility sound

pressure (SPL) curves for both human and chinchilla obtained in behavioral investigations, indicate hearing threshold sensitivity greatest (i.e. within approximately 10dB SPL or less), for the 250Hz-500Hz to 4kHz-8kHz range (Heffner and Heffner, 1991; Moody and Stebbins, 1986). At the relatively extreme frequencies of 125Hz and 16kHz in both species, thresholds worsen to 25-30dB SPL and progressively worsen at a greater rate, for frequencies falling below and above those 'extremes' (Heffner and Heffner, 1991; Moody and Stebbins, 1986).

An electrophysiological investigation of frequency specific, round-window-recorded whole nerve action potentials elicited by tone bursts (30msec duration; delivered 4/sec) in awake chinchillas reported thresholds of 35-40dB SPL at 1-2kHz, and within 10-20 dB SPL for frequencies 3kHz-8kHz (Spagnoli and Saunders, 1987). Taken together with additional evidence obtained from single unit investigations in this species (Salvi et al., 1983), there is a fairly close correspondence between behavioral responses and whole nerve-recorded thresholds in this species (Salvi, Ahroon, Perry, Gunnarson, and Henderson, 1982; Spagnoli and Saunders, 1987).

The chinchilla has a total of about 23,554 auditory fibers, 95% of which have diameters of 2-4μm, with the remaining 5% exhibiting diameters of 1-6μm (Boord and Rasmussen, 1958). Compared to the 2 $^{3}/_{4}$ to 2 $^{5}/_{8}$ cochlear turns found in humans (e.g. Pickles, 1988; Salt and

Thalmann, 1988), there are 3 $^{3}/_{4}$ turns in the chinchilla cochlea (Harrison and Hunter-Duvar, 1988; Santi, 1986; 1988). The total volume of cochlear endolymph and perilymph in the chinchilla cochlea is probably close to the values of 2μl and 16μl, respectively, reported in the guinea pig cochlea (Salt and Konishi, 1986; Salt and Thalmann, 1988).

As in most mammalian species, the efferent OC system of the chinchilla is divided into medial and lateral pathways. Like most other mammalian species, lateral efferent fibers in the chinchilla project to the Type I auditory dendrites, and primarily those innervating the ipsilateral cochlea. Also, as expected, the medial efferent fibers project to the OHCs, and mostly to the contralateral cochlea (Iurato, 1974; Iurato et al., 1971; 1978). As in most rodents, lateral efferent nuclei are contained within (rather than in close proximity to), the brainstem LSO (Bianchi and Salvi, 1990; Osen et al., 1984). Medial efferent fibers in the chinchilla arise from cell bodies located within the dorsomedial and ventromedial periolivary nuclei (DMPO and VMPO) of the medial superior olivary region, and from within the medial and ventral nuclei (MNTB and VNTB, respectively) of the trapezoid body (Bianchi and Salvi, 1990; Osen et al., 1984).

The total number of medial and lateral efferent neurons innervating each chinchilla cochlea has been reported to range from 373 to 472, with an approximate mean value of 413 (Bianchi and Salvi, 1990). This rather low (conservative) value, based upon an HRP investigation, can be contrasted

with combined medial and lateral totals (for each cochlea) observed in the mouse, rat, primate, guinea pig, and cat (reviewed in an earlier discussion), which are approximately 475, 480, 780, 1234 and 1230-1300, respectively (e.g. Campbell and Henson, 1988; Warr et al., 1986; Robertson et al., 1987; Strominger et al., 1981). In the chinchilla, approximately 334 lateral efferent neurons innervate each cochlea. A large percentage (99%; or 331) of these are neurons contained within the ipsilateral LSO, while only about 1-2 (<0.1%) neurons have been observed arising from within the contralateral LSO (Bianchi and Salvi, 1990). This percent of uncrossed fibers is quite similar to that reported in guinea pigs (Strutz and Bielenberg, 1984), rats (White and Warr, 1983) and mice (Campbell and Henson, 1988).

In the chinchilla, only about 80 medial efferent neurons innervate each cochlea. About 70% of those neurons arise from within MSO regions on the contralateral side, while the remaining 30% arise ipsilaterally (Bianchi and Salvi, 1990). This medial efferent percentage of crossed fibers (70%) reported in the chinchilla, is similar to the 73%, 64%, 75% and 75% crossed distributions observed in the cat, rat, mouse and guinea pig, respectively, as discussed earlier (Campbell and Henson, 1988; Guinan et al., 1983; 1984; Robertson et al., 1987; Warr, 1975; Warr, 1978; 1980; Warr and Guinan, 1979; Warr et al., 1982; 1986; White and Warr, 1983). Furthermore, the overall synaptic distribution and pattern of efferent innervation within the chinchilla

cochlea most resembles the innervation pattern observed in the guinea pig (Iurato, 1974; Iurato et al., 1971; 1978).

As indicated in an earlier discussion, histochemical investigations conducted in chinchillas have detected the presence of the nonspecific, cholinergic inactivating enzyme AChE within brainstem medial and lateral efferent nuclei (Iurato et al., 1971; Osen et al., 1984). Both medial and lateral efferent terminals within the chinchilla cochlea are also selectively damaged by the choline uptake inhibitor AF64A (Smith, Mount and Callahan, 1989). Also recall that enkephalin-like immunoreactivity has been reported within lateral efferent brainstem nuclei in this species (Hoffman et al., 1991).

Background Review Of Opioid Substances Employed

Morphine: Morphine effects were not investigated in the present study. However, many descriptions of the receptor affinities and potencies of the substances which were employed, use morphine as a standard of comparison. Therefore, the classic drug alkaloids such as morphine, and related agonists (normorphine, dihydromorphine, oxymorphine, and levorphanol) are all relatively selective for μ-receptors, and exhibit comparatively less activity (~1%) at other opioid receptors (Barnard and Demoliou-Mason, 1983; Chang, 1984; Magnan et al., 1982; Paterson et al., 1983; 1984; Tam, 1985). For instance, morphine's μ-receptor affinity is about 90-125X greater than its affinity at δ-

receptors (2% cross reactivity), and about 200-1000X greater than its affinity (0.6% cross reactivity) at the K-receptor (Barnard and Demoliou-Mason, 1983; Chang, 1984; Chang and Cuatrecasas, 1979; Chang et al., 1981; Magnan et al., 1982; Paterson et al., 1983; 1984; Robson, Paterson and Kosterlitz, 1983).

Fentanyl: Fentanyl effects were investigated in the present study. The binding affinity of fentanyl for the μ-receptor is similar to morphine (Paterson et al., 1983). Fentanyl however, exhibits only a 22 fold greater affinity for μ- over δ-receptors, and a 67 fold greater affinity for μ-over K-receptors (Magnan et al., 1982; Paterson et al., 1983). Like the potent sufentanil, fentanyl is highly lipophilic (Bovill, 1987; Magnan et al., 1982), and therefore its relative potential for entering the CNS is 156X greater than morphine (Bovill, 1987). Following iv administration, the onset of action is rapid, and the duration of action is short (Bovill, 1987).

Fentanyl Potency: Compared to morphine, fentanyl (0.04 to 0.17 mg/kg sc) has been reported to exert 22 to 85-fold greater antinociception in mice (Shaw, Rourke and Burns, 1988; Upton, Sewell and Spencer, 1982), and a 70 to 100X greater antinociception in rats (Millan, 1989). Peak antinociceptive effects of fentanyl in rats (0.02-0.05 mg/kg sc) are observed within 15min post injection (Millan, 1989). Peak antinociceptive effects of iv fentanyl (0.02mg/kg) in rabbits is observed within 10min (Herz, Albus, Metys,

Schubert and Teschemacher, 1970). In animals, a lethal dose of fentanyl is usually >10 mg/kg (Bovill, 1987).

Clinically, fentanyl is 60 to 80X more potent as an antinociceptive agent than morphine (Bovill, 1987; Jaffe and Martin, 1990). The average duration of fentanyl antinociception in humans has been reported to be 1-2 hrs and the plasma half-life (t ½), defined as the duration in hours producing a 50% clearance in plasma concentrations, is 3-4 hrs (Bovill, 1987; Jaffe and Martin, 1990; Marshall and Longnecker, 1990). The longer t ½ of fentanyl, compared to morphine (1.5 to 2.0hrs) is apparently due to fentanyl's higher tissue affinity (Bovill, 1987).

Naloxone: Naloxone, which is a relatively selective μ-opioid-receptor antagonist was also employed in these investigations. Naloxone exhibits about an equal binding affinity as morphine for the μ-receptor (e.g. Barnard and Demoliou-Mason, 1983; Chang, 1984; Jaffe and Martin, 1990; Kosterlitz, 1985; Magnan et al., 1982; Paterson et al., 1984; Robson et al., 1983), and is the antagonist of choice for use in selective blockade of μ-receptor-mediated effects (Lahti et al., 1985). Unlike morphine, however, naloxone is only about 10-30X less active at δ-and K-receptors (respectively) when compared to its affinity for μ-receptors (e.g. Akil et al., 1984; Barnard and Demoliou-Mason, 1983; Chang, 1984; Chang and Cuatrecasas, 1979; Kosterlitz, 1985; Lahti et al., 1985; Paterson et al., 1983; 1984; Robson et al., 1983; Tam, 1985; Tiberi and Magnan, 1990). Therefore,

while useful as tool in generally determining the possible involvement of opioid mechanisms, naloxone is insufficiently selective to sort out actions mediated by subpopulations of opioid receptors (e.g. Barnard and Demoliou-Mason, 1983; Goldstein, 1984; Paterson et al., 1984).

Naloxone Potency: Animal studies have generally indicated that parenteral doses of between 0.05 and 0.30mg/kg can antagonize the antinociceptive effects produced by morphine (Duggan and North, 1984; Martin, 1984), though much higher doses can safely be used as well. A dose of naloxone (1mg/kg; sc) provides effective μ-antagonist effects. A dose of about 10mg/kg is required to achieve significant blockade of the δ-receptor, while K-receptors in animals appear relatively insensitive to naloxone, requiring even higher doses (Leander, 1983; Lewis et al., 1987). In humans, the average duration of naloxone effects is 1-4 hrs, and t ½ is 1.1 (± 0.6) hrs (Bovill, 1987; Jaffe and Martin, 1990).

U-50488H and U-69593: Two K-opioid receptor agonists were also investigated in a limited number of animals. These were the K-selective, non-peptide agonists U-50488H (Piercey, Lahti, Schroeder, Einspahr and Barsuhn, 1982; Von Voigtlander, Lahti and Ludens,1983) and U-69593 (Lahti, Mickelson, McCall and Von Voigtlander, 1985; Tiberi, Payette, Mongeau and Magnan, 1988). Although U-50488H is much less potent (i.e. 63X) at the K-binding site than some prodynorphin fragments (Kosterlitz, 1985; Paterson et al., 1984; Tam, 1985), it exhibits up to a 120-1300X greater

binding affinity for K- over μ-, and is devoid of δ-receptor activity (Goldstein, 1984; Kosterlitz, 1985; Lahti et al., 1985; Lever et al., 1983; North, 1986; Paterson et al., 1984). U-69593 is a more potent analog of U-50488H exhibiting a 484X greater binding selectivity for K-, relative to μ- or δ-receptors (Lahti et al., 1985). Indeed, its affinity for KAPPA is 1.3X greater than U-50488H (Lahti et al., 1985; Tiberi et al., 1988).

Onset Of Effects and Acceptable Doses: Both U-50488H and U-69593 display antinociceptive activity in a variety of assays (Chang, 1984; Lever et al., 1983; Lahti et al., 1985; Leighton, Rodriguez, Hill and Hughes, 1988; Nagase, Narita, Suzuki and Misawa, 1990; Piercey et al., 1982; Shaw et al., 1988; Takemori, Ho, Naeseth and Portoghese, 1988; Von Voigtlander et al., 1983). Peak antinociception to both heat and pressure following U-50488H (1.0 to 2.14mg/kg sc) in mice (Portoghese, Lipkowski and Takemori, 1987b), or 10mg/kg sc in rats (Millan, 1989) can occur within 15min, diminishing over a period of approximately 45min. Peak antinociception following 2mg/kg U-69593 (sc) in rats has also been observed within 15min, diminishing over a period of about 135min (Millan, 1989).

In mice and rats, U-50488H has been investigated at sc doses as high as 20 to 40mg/kg (Millan, 1989; Millan, Czlonkowski, Lipkowski and Herz, 1989; Shaw et al., 1988; Von Voigtlander et al., 1983; Von Voigtlander and Lewis, 1988), or even as high as 150.0 mg/kg (10, 20, 40, 40, 40

mg/kg) over a 5-day period (Cowan and Murray, 1990). Intravenous doses as high as 10mg/kg over a 30sec period have also been used in rats (Leighton et al., 1988). U-69593 has been administered to rats at sc doses as high as 5mg/kg sc (Millan, 1989; Millan and Colpaert, 1990), or iv as high as 10mg/kg within 30sec (Leighton et al., 1988).

Pentazocine: The K-opioid receptor binding effects of pentazocine (N-dimethylallylnormetazocine) were investigated in all three sets of experiments.

Opioid Actions: The antinociceptive (K-receptor) properties of (±)-pentazocine reside within its (-)-isomer (e.g. Brogden, Speight and Avery, 1973). Unfortunately, there are no well controlled, receptor-specific binding investigations comparing the relative K-receptor potencies of the other benzomorphans against (±)- or (-)-pentazocine. As discussed earlier, levorotatory isomers of benzomorphans typically exhibit both K-receptor agonist and μ-receptor antagonist properties. Levorotatory forms of pentazocine, however, exhibit potent K-receptor agonist, and very weak μ-receptor antagonist properties (Duthie and Nimmo, 1987; Bovill, 1987; Brogden et al., 1973; Jaffe and Martin, 1990; Lahti et al., 1985). Indeed, pentazocine exhibits μ-receptor activity which is: 87X less than (±)-cyclazocine, 43X less than (±)-bremazocine, 17X less than (-)-EKC, 13X less than (-)-SKF10,047, and 35X less than naloxone (Tam, 1985). In fact, pentazocine exhibits the lowest affinity for the morphine receptor of all the benzomorphans (e.g. Lahti et al., 1985),

and only appears to interact with 'μ' at sc doses (in rats) of about 32mg/kg (Brogden et al., 1973; Holtzman and Jewett 1972). Pentazocine is also 522X less effective than naloxone in precipitating morphine withdrawal in dogs (Martin, 1984), and is virtually devoid of δ-receptor activity (Lahti et al., 1985; Tam, 1985).

Sigma-Receptor Actions: (±)-Pentazocine appears to exhibit about an equal affinity for opioid K- and non-opioid σ-receptors (Walker et al., 1990). (±)-Pentazocine in guinea pig brain exhibits about a 53-fold higher binding affinity for σ-, compared to (+)-SKF-10,047 (Tam, 1985; Tam and Cook, 1984), and (±)- or (+)-SKF-10,047 in rat brain (Largent et al., 1987; Zukin and Zukin, 1988). The σ-receptor potency difference is even greater for (±)-pentazocine vs (+)-EKC (Tam, 1985). A similar potency difference has been observed for the dextrorotatory isomer (+)-pentazocine compared to (+)-SKF-10,047, in displacing the potent σ- receptor ligand (+)-[^{3}H]-3-PPP (Walker et al., 1990). Therefore, among the benzomorphans, both (±)- and (+)-pentazocine exhibit the highest overall specificity for the σ-receptor (Tam and Cook, 1984; Tam, 1985; Largent et al., 1987). As indicated earlier, the levorotatory (-)-pentazocine fails to bind σ-receptors (Bowen et al., 1990a; de Costa et al., 1989).

Solubility, Potency, Onset, and Acceptable Dose: Pentazocine is reported to be highly lipophilic relative to other benzomorphans (Walker et al., 1990). It is well absorbed from parenteral sites, passes the human placental barrier,

and is rapidly metabolized (Brogden, et al., 1973; Jaffe and Martin, 1990; Payne, 1973).

Compared to morphine, antinociception from pentazocine reaches a higher initial peak, and declines more rapidly (Angel, 1983; Brogden et al., 1973). Its clinical and experimental K-receptor potency for antinociception has been reported to vary from about one-third to one-tenth that of morphine (Bovill, 1987; Brogden et al., 1973; Gilbert and Martin, 1976; Martin, 1984; Payne, 1973), depending upon the particular assay (Levine, Gordon, Taiwo and Coderre, 1988; Shaw et al., 1988; Tyers, 1980). An antinociceptive dose of pentazocine (TALWIN) in humans is approximately 30-60mg/70kg, or about 0.43 to 0.86mg/kg (comparable to 10mg/70kg morphine) administered parenterally (Angel, 1983; Brogden et al., 1973; Bromm, Ganzel, Herrmann, Meier and Scharein, 1987; Jaffe and Martin, 1990).

Peak antinociception in humans occurs within 2 to 15min following 0.29 mg/kg iv, and within 15min to 1hr following 0.64 mg/kg (im or sc) administration (Angel, 1983; Brogden et al., 1973; Jaffe and Martin, 1990; Payne, 1973). In humans, the average duration of pentazocine effects can last from 1 hour (iv) to 4-6 hrs (0.64 mg/kg; im or sc) (Brogden et al., 1973; Jaffe and Martin, 1990). The plasma half life (t ½) in humans following iv (0.36 mg/kg) or im (0.64 mg/kg) administration is from 2.0 to 4.6 (± 1.0) hours respectively (Brogden et al., 1973; Jaffe and Martin, 1990).

Standard antinociceptive (sc) doses of pentazocine in rats (2mg/kg) and mice (10mg/kg) have been reported (Harkness and Wagner, 1983), as have iv doses (5mg/kg) in dogs (Vaupel, Nickel and Becketts, 1989). Pentazocine at 64mg/kg (sc) may produce seizure activity in rats (Holtzman and Jewett, 1972), however, most antinociceptive studies have successfully employed higher doses (50-80mg/kg; sc) in this species (Cowan, 1981; Tyers, 1980). In dogs, iv pentazocine doses greater than 5mg/kg produce seizure activity (Vaupel et al., 1989). The LD_{50} of pentazocine in mice is reported to be 125mg/kg (sc) and 24mg/kg (iv). In rats the LD_{50} is reported to be 175 (± 36) mg/kg (sc) and 21.5mg/kg iv (Brogden et al., 1973; Windholz, Budavari, Blumetti and Otterbein, 1983).

Nor-Binaltorphimine (nor-BNI): Some of the experiments in the present set of investigations employed the naltrexone-derived, highly K-opioid receptor-selective antagonist, nor-BNI (Portoghese, Lipkowski and Takemori, 1987a; 1987b; Portoghese, Nagase, Lipkowski, Larsson and Takemori, 1988). In tests of K-receptor selectivity for antagonism, nor-BNI exhibits a 66X greater K-receptor preference over naloxone, and a 100 fold greater K/μ-receptor preference over naltrexone (Millan, 1989; Portoghese et al., 1987b). In selectivity tests of K-receptor antagonism using EKC vs morphine or EKC vs DADLE or DSLET, nor-BNI exhibits a 170 fold greater K/μ-receptor

affinity and a 150 fold greater K/d-receptor selectivity (Portoghese et al., 1987a; 1988; Takemori et al., 1988).

Nor-BNI also exhibits a K-receptor binding affinity which is 77.5X greater than U-50488H (Takemori et al., 1988), and 20X (Tiberi and Magnan, 1990) to 44X greater than U-69593 (Smith, Medzihradsky, Hollingsworth, de Costa, Rice and Woods, 1990). Nor-BNI is effective at sc doses (20mg/kg) in reducing the antinociceptive effects of U-50488H in rats (Millan, 1989). While peak K-receptor antagonism is seen by 90min, nor-BNI antagonist activity is also apparently quite potent 5 minutes post sc administration (Millan, 1989).

MATERIALS And METHODS

Animals: Experiments were conducted in adult male pigmented chinchillas (Chinchilla laniger, 430-666g), 0.5 to 1.5 years of age. Animals were obtained from a local breeder. Auditory thresholds were estimated in all chinchillas by the use of auditory brainstem response (ABR) recording. In these brief preliminary hearing assessments, animals were anesthetised with an intramuscular injection of 20mg/kg Telazol (see: Hrapkiewicz, Stein and Smiler, 1989).

Far field auditory brainstem responses were elicited by 1200 clicks delivered at a rate of 68.3/sec. Stimuli were produced by an ER-3A ceramic microphone mounted through a form-fitting foam earplug that sealed the left external ear canal. Bioelectric signals recorded with Grass E-2 cephalic needle electrodes were amplified and band pass filtered from 150 to 1500 Hz (6dB/octave roll off). Twelve hundred click evoked responses were averaged on-line.

Standard cephalic electrode positions were used in these ABR screening procedures. That is, the positive electrode was inserted subcutaneously at the vertex, and the negative electrode was positioned near the nasion. A common ground electrode was inserted into the neck musculature. ABR electrode impedances for both active electrodes were typically 2-5kΩ. Far-field threshold estimates of no greater than 40dB SPL were taken to indicate normal hearing. Following the threshold screening procedure confirming

reasonable hearing status, chinchillas were returned to their cages and were allowed to recover from the anesthesia for at least 48 hours prior to acute surgery initiating further experimental study.

Acute Electrophysiological/Neuropharmacological Studies

Surgical Preparation: Animals were food- and water-deprived overnight prior to the surgery that initiated acute electrophysiological experiments. Unless otherwise noted, chinchillas were pre-anesthetized with an intramuscular dose (50mg/kg) of the dissociative anesthetic ketamine hydrochloride (Ketalar). With the chinchilla anesthetised, the head and neck were sheared and the eyelids closed to reduce corneal drying. A polyethylene tracheal tube was introduced following a standard tracheostomy procedure, to aid in maintaining a stable and open airway. A small-animal positive-pressure respirator was available for assisted ventilation in these experiments, but was not used unless indicated in the text or Figure legends.

The right internal jugular vein was cannulated with the aid of a stereo dissecting microscope (Wild# M-3Z) using standard procedures. A zero dead-volume three-way Hamilton stopcock valve was mounted on the end of the venous cannula tubing. Microliter syringes were mounted as needed on this stopcock in order to administer supplementary anesthesia, Ringer's control solutions, and intravenous doses of important opioid and non-opioid receptor ligands. An additional length of PE-10 tubing also extended from the

stopcock to a syringe mounted on a Harvard Apparatus multispeed perfusion pump (#600-000) system located outside of the test chamber, to maintain constant anesthetic infusion.

A steady plane of surgical anesthesia was achieved by weaning the chinchilla from Ketalar, and maintaining anesthesia with sodium pentobarbital (6.5mg/ml in sterile lactated Ringer's solution) delivered at a constant rate of 5 to 10μl/min. Additional supplemental pentobarbital anesthesia was occasionally required as indicated by regular corneal and withdrawal reflex tests, and cardiac monitoring. Heart rate was routinely monitored using the Nicolet signal averager. At the end of each experiment, chinchillas were euthanized via the jugular cannula with 0.1ml of Somlethal.

Surgical Exposure Of The Middle Ear: Following tracheal and venous cannulation, the animal's left pinna was retracted anteriorward and the skin, muscle and connective tissue overlying the left auditory bulla, in the mastoid region were surgically excised (see: Browning and Granich, 1978). Postauricular vessels were surgically ligated or cauterized when necessary. With the pinna still retracted forward, an opening into the osseous canal entrance was created with a jewelers forceps. The thin bone of the auditory bulla was perforated with a dental pick, and the bulla then opened by use of a small rongeur. Through this bulla opening, the tympanic annulus, tympanic membrane,

cochlear base, round window, and part of the incudostapedial joint were exposed.

With the cochlear exposure completed, the animal's head was placed into a restraint appliance that secured it without compromising respiration. The pinna of the contralateral ear was folded inward and taped to reduce contralateral ear stimulation by ambient noise. Each animal was maintained on a Harvard Apparatus heating blanket (30-7079) during all phases of auditory testing. Body temperature was monitored with a rectal thermistor-probe (Fluke #52 K/J), and maintained at a constant 35.5 to 36^{o} C throughout testing.

Electrophysiological Recording Procedures

Recording Equipment: The animal preparation was placed on a surgical table in a portable sound attenuating chamber that was itself located within an isolated room. A click attenuator (Grason Stadler #1292) calibrated in 1.3 ±0.1 dB steps was used to control stimulus intensities. All auditory potentials in the experiments described below were elicited by 100 μsec rectangular pulses generated by a Nicolet 1007A click generator. Click stimuli were delivered at a rate of 18.3/sec for recording near-field compound action potential (CAP) responses, and at 68.3/sec for far-field auditory brainstem response (ABR) recording. The sound generation system was capable of producing stimuli ranging from 22 to 135 dB peak sound pressure level (SPL) (i.e., re 20 μPa).

Again, in all described experiments, clicks were delivered to the animal through an Etymotic ER-3A ceramic (insert) microphone. The ER-3A device has a nearly flat frequency response from 250Hz to >3kHz (i.e. Beauchaine, Kaminski and Gorga, 1987; Musiek and Baran, 1990; Wilber, Kruger and Killion, 1988), and is especially well suited for reducing or eliminating stimulus artifacts. The 28cm-long ER-3A sound tube produces a 0.9 msec delay in the delivery of the acoustic stimulus (e.g. Beauchaine, et al., 1987). The form-fitting foam ear plug used to couple the ER-3A sound-delivery tube to the osseous ear canal reduced ambient room noise by 32-42dB (Beauchaine, et al., 1987). Taken together with the measured 31 to 33dBA SPL noise level within the test chamber, stimuli were therefore delivered to each animal in a virtually noise-free environment.

Recorded responses were amplified 100,000 times (Nicolet HGA-200A), filtered (Nicolet 501A), and averaged on-line (Nicolet Clinical Averaging System; CA-1000) with a signal averager. Data was digitized by use of an 8-bit A/D converter with a 10kHz sampling rate. In CAP recording, three hundred responses were averaged over a 5msec time window. Neuroelectric activity was band pass filtered (6dB/octave roll off) from 0.15 to 3.0 kHz. CAPs were differentially recorded, again using standard platinum-alloy Grass E2 subdermal needle electrodes. The positive electrode was positioned at the scalp vertex, and again, a common ground electrode was inserted into the neck. The second

differential electrode was positioned near the round window niche in the opened middle ear, using the dissecting microscope. This electrode consisted of a subdermal platinum-alloy needle electrode, wrapped with a fine diameter silver wire flamed at the tip to form a ≈0.5mm diameter ball. The impedance of the round window electrode was maintained during each experiment by the controlled application and/or removal of a conductive hypoallergenic gel (Aquasonic 100; Parker Labs). The impedance of this electrode was never allowed to vary more than ± 2000Ω during any experiment. The spherical electrode tip was held in place over the mastoid opening and against the surface of the cochlea under slight pressure by use of a custom-designed clamp. This clamp was adjustably secured to the head holder, allowing for easy placement, fine adjustment, and removal of this middle ear-recording electrode. During all phases of testing, the position of the middle ear recording electrode was continually monitored to further insure recording stability.

Recording Sequence: Electrophysiologically obtained far-field and near-field auditory responses at near-threshold were defined as: (a) the lowest click intensity that produced a replicable ABR response; and (b) the lowest click intensity that produced visually identifiable and replicable N1 and N2 waves in the near-field recording of the CAP. Thresholds were established by use of a tracking procedure in which the intensity was reduced in 2dB steps from +40dB

SPL, until no response (N1 or N2) could be observed. The intensity level was then increased in 2dB steps until a response was again obtained. The intensity was then again reduced to no response, and then once again increased in 2dB steps. Near-threshold estimates derived in the descending series invariably matched those made during ascending trials.

The near-threshold values obtained in initial ABR screening served as a guide for setting up these descending-ascending stimulus intensity series. The stimulus intensity defined as "near threshold" (just-suprathreshold) for each animal during pre-drug baseline periods served as a reference for all subsequent testing in that animal.

Stimulus Intensity and Wave Analysis: The first and second negative waves (N1 and N2) of the CAP were obtained just above threshold, and at +10, +30 and +50dB above threshold (dB SL) in all experiments. In Experiments 2 and 3, responses to +5dB SL were also recorded in many preparations. Latencies of each wave peak of interest were measured at their point of maximum amplitude. Response amplitudes were peak-to-peak, that is, were measured from the point of maximum negativity to maximum positivity of the following trough.

Establishing Baselines: The recording procedure described above was repeated 30-36 times at each intensity, over the full duration of each experiment. For a given animal, the

amplitude data obtained at each intensity was accepted as a baseline only when it met criterion for stabilization. Responses were regarded as stable in the absence of a consistent amplitude increase or decrease in the N1 or N2 at any stimulus intensity, over a total baseline period of not less than 90 minutes. When amplitude data obtained during an initial baseline period was judged to be stable, the acquisition time of the first "stable" response was noted. Additional responses were then recorded repetitively until a reliable baseline was seen for a period of exactly 90 min.

Baseline Treatment: Following the last 60-minute baseline recording, each chinchilla in Experiments 1 and 2 received iv-administered Ringer's solution (1µl/5g body weight; rate of 50 µl/min). In Experiment 3, each chinchilla received 1µl of an artificial perilymph solution in the absence or presence of 1-2mMolar (1-2 µM/ml; or 0.74 to 1.5µg/µl) of the specific K-opioid receptor antagonist nor-binaltorphimine (nor-BNI), which was applied directly to the round window via a microliter syringe. Recordings were then obtained thereafter in all animals, for the final 30 minutes of this baseline period.

Postbaseline Testing: At the end of the 90 minute baseline period described above, animals received either one, two, or three iv doses of an opioid or non-opioid substance, or injections of Ringer's solution in the controls. All multiple iv administrations were separated in

time from the previously administered dose by 30 minutes. All postbaseline iv solutions were given at a constant volume (1μl/5g body weight) and rate (50μl/min). Again, all postbaseline solutions were delivered through a port on the three-way Hamilton valve, using a microliter syringe. Auditory electrophysiological testing was immediately resumed after each drug injection. Recording continued for an additional 90 minute-long postbaseline period (i.e. through three additional 30min periods). The total experimental time was therefore always 180 minutes. Thus, data from each of the 3 sets of experiments described below were obtained during 3 equal-duration baseline and 3 equal-duration postbaseline time intervals, each 30 minutes long.

EXPERIMENT 1

Electrocochleography-(CAP): In the first experimental series, N1 and N2 components of the CAP were recorded before and after drug administration in 21 chinchillas. CAP responses were generated by alternating polarity clicks While threshold detection of evoked potentials is generally not influenced by click polarity (i.e. Sininger and Masuda, 1990), the advantage to using alternating polarity clicks was the elimination of distortion, especially at the intensity level of 50dB SL. A disadvantage is the elimination of the cochlear microphonic potential by cancellation.

The overall across-animal impedances of round window electrodes in this series ranged from 4500-8500Ω. Five to six N1 and N2 potential measurements were obtained for each stimulus intensity during each of the six (3 baseline; 3 postbaseline), 30 minute-long test periods. Animals studied in this first series had N1 and N2 response at near threshold, ranging from 22 to 40dB SPL, with average near threshold responses at 29dB SPL. This threshold range is fairly consistent with earlier electrophysiologic data obtained in this species (Spagnoli and Saunders, 1987).

Drug Administration: In this first experimental series, the following drugs were administered postbaseline: (±)-pentazocine at 8.0 mg/kg (n=4) and 16.0 mg/kg (n=5); U-50488H (6.0 mg/kg; n=3); fentanyl citrate (2.0 mg/kg; n=2); and naloxone HCl (1.1 mg/kg; n=2). The (±)-pentazocine given at 16mg/kg was always delivered in one dose, administered immediately after the last 90 minute-long baseline recording period.

The lower doses of (±)-pentazocine, fentanyl, naloxone and U-50488H were always delivered in multiple doses. For example (±)-pentazocine (4mg/kg), fentanyl (1mg/kg), naloxone (0.1mg/kg) and U-50488H (2mg/kg) were all given after the last 90 minute baseline recording. A second administration of (±)-pentazocine (4mg/kg), fentanyl (1mg/kg), naloxone (1.0mg/kg), or U-50488H (2mg/kg) was given 30 minutes after the first postbaseline dose. A third

administration of U-50488H (2mg/kg) was given 30 minutes after the second.

All drugs were dissolved in sterile lactated Ringer's solutions and were mixed on the day of testing. Control animals (n=5) were given equivalent postbaseline volumes of the drug vehicle, sterile lactated Ringer's solution, in lieu of solutions containing drugs. Immediately following the 90 minute baseline recording period, two of these five control animals received a single postbaseline Ringer's injection, another two of the five control animals received two postbaseline Ringer's 'doses' spaced 30 minutes apart, and one control animal received three vehicle administrations spaced 30 minutes apart.

Electrocochleography-(CM): To preliminarily investigate the possible opioid effects at the outer hair cells, cochlear microphonic (CM) potentials were also recorded in two of the five animals tested with (±)-pentazocine (16mg/kg). Microphonic activity was bandpass filtered from 150Hz to 8kHz and recorded differentially using the CAP electrodes. CM potentials were elicited by 300 negative polarity clicks (18.3/sec), and were obtained at the single intensity of 10 dBSL. In both tested animals, CMs were tracked through each successive 30-minute-long baseline and post-drug recording period.

Far Field Recording (ABR): As an added control, amplitudes and latencies of the component waves I-IV of the auditory brainstem response (ABR) were obtained in two of

the four animals tested with (±)-pentazocine (8mg/kg), and in one of the animals tested with (±)-pentazocine at 16mg/kg. Near-threshold and suprathreshold ABR recording was conducted as described earlier.

Additional Animals: Additional CAP studies were conducted using the same experimental design in ten other animals not included in the above group. U-50488H was administered to eight of the ten; one other chinchilla was tested with (±)-pentazocine; the last was administered U-69593. In seven of the eight U-50488H-animals, the K-receptor agonist U-50488H was delivered in two equal 10 mg/kg injections to receive a total dose of 20.0 mg/kg. Injections were spaced 30 minutes apart, with the first immediately following the 90 minute-long baseline period. Four of these animals succumbed from respiratory failure following the initial 10mg/kg dose. Another animal succumbed from respiratory failure following the second of two 5mg/kg administrations. These animals could not be revived using the small-animal respirator.

Three of the seven animals receiving a total dose of 20.0 mg/kg U-50488H survived. In one of these three animals, naloxone (0.1mg/kg) was co-administered with the first 10mg/kg delivery of U-50488H. In the second, naloxone (2mg/kg) was co-administered with the second delivery of U-50488H. The third surviving animal received no naloxone.

Following the baseline period, naloxone (1mg/kg) was co-administered with (±)-pentazocine (16mg/kg) in one

additional animal; U-69593 (1mg/kg) was administered alone in another. U-69593 is relatively insoluble in water and was made to enter solution by employing a minute amount of 1M HCl diluted with Ringer's, then pH adjusted with 2μl of NaOH, as suggested elsewhere (e.g. Leighton et al., 1988).

EXPERIMENT 2

In a second experimental series, CAPs and CM responses were recorded in a total of 10 chinchillas, that were administered two isomeric forms of pentazocine. CM recording was more complete in this series, as it was achieved at all stimulus intensity levels. The overall across-animal impedances of the round window electrodes in this series ranged from 8,000 to 10,000 Ω. Animals had near threshold CAP values ranging from 7.7 to 34dB SPL, with an average value of 20.8dB SPL.

Drug Administration: In determining whether or not the response amplification effects of (±)-pentazocine (16mg/kg) observed in Experiment 1 (see Results) could be ascribed to its levorotatory K-opioid rather than to its σ-receptor (dextrorotatory) binding properties, the pentazocine doses used in Experiment 2 were approximately halved from the original 16mg/kg injections. In the one group of five chinchillas, the K-opioid ligand (-)-pentazocine succinate was administered at 8mg/kg. The non-opioid, dextrorotatory isomer and -receptor ligand (+)-pentazocine succinate (8mg/kg) was injected in five other animals. Again, all

drugs were dissolved in the sterile lactated Ringer's solution, and were mixed on the day of testing.

Additional Animals: Additional CAP investigations were conducted in another three animals in the second experimental series, with the potent K-receptor agonist, U-69593. U-69593 was dissolved in an acidic solution that was later neutralized, as described earlier. U-69593 was injected immediately postbaseline with a single administration at a total dose of 10.0mg/kg and 2.0mg/kg in two of the three animals. Both animals succumbed from respiratory failure. Effects observed from one animal in this experimental series surviving a postbaseline dose of 1.0 mg/kg are described in the Results.

EXPERIMENT 3

In the third experimental series, CAP and CM responses were recorded in 15 chinchillas. In some of these animals, the K-receptor antagonist nor-binaltorphimine (nor-BNI) was introduced at the round window, prior to intravenous administration of the K-receptor agonist (-)-pentazocine. The impedances of round window electrodes in this experimental series ranged from 8,000 to 10,000 Ω., and animals had near threshold CAP values ranging from 11.6 to 32dB SPL with an average value of 24.6dB SPL.

Drug Administration: In these experiments, after 60 minutes of the baseline period, the round windows in each of these 15 animals were treated with 1μl of an artificial

perilymph solution (see Appendix A) introduced either alone (n=6), or as a vehicle solution for 1-2 mMolar (about 1-2μM/ml or 0.74 to 1.5μg/μl) nor-BNI (n=9). Note that the molecular weight of nor-BNI is 734.73. Round window solutions were applied under the stereo dissecting microscope, using a 10μl Hamilton syringe mounted with a blunt-ended 32 gauge needle. The relatively large size of the cochlear reference electrode ($\approx$0.5mm diameter) required its temporary displacement during the application of these solutions to the round window. However, the position and impedance of this electrode were restored relatively easily following this simple procedure.

The artificial perilymph solution containing the 0.74 to 1.5μg/μl of nor-BNI was mixed on the day of testing. All solutions placed onto the cochlear round window were 36^{o} C. It might be noted that the pH of guinea pig endolymphatic and perilymphatic fluid has been reported to be approximately 7.3 (Salt and Konishi, 1986; Schacht, 1986). Investigations conducted in guinea pigs involving direct perfusion of the scala tympani have typically employed artificial perilymphatic solutions having a pH of 7.4, or slightly less (Bobbin and Konishi, 1971; 1974; Comis and Leng, 1979; Robertson and Johnstone, 1978). In the present experiments, the chinchilla cochlear perilymph pH was found to be approximately 7.6. In one pilot animal, a 1μl round window application of a slightly more acidic (pH 7.2) artificial perilymph solution produced an immediate 20dB

loss in hearing that did not recover. Therefore, in the present investigation the stock solution of artificial perilymph was always mixed to a pH of 7.4 (see Appendix A). The relatively high 1-2mM concentration of nor-BNI exhibited a relatively acidic pH of 6.8, and was therefore always buffered to 7.4 with μl amounts of NaOH.

Following the 90 minute-long baseline epoch, that is 30 minutes after the application of round window solutions, the levorotatory K-opioid ligand (-)-pentazocine succinate (8mg/kg) was delivered with one postbaseline administration to thirteen of the fifteen animals. As a final control, two animals received postbaseline, post nor-BNI Ringer's solution at the same volume and rate.

DATA ANALYSES

Data matrices from all three experiments were submitted to Statistical Analysis System (SAS) multivariate analysis of variance (MANOVA) programs. The strategy used throughout each experiment (Appendix B) was a 'between treatment' analysis. The first analysis tested the stability of baselines values collapsed across drug treatment, that is, independent of animal treatment. This was accomplished by comparing the overall baseline values obtained at 0-30 and 30-60 minutes, to the values obtained during the 60-90 minute period (i.e. following i.v. Ringer's, or the round window administrations). As a rule, these procedures did not measurably alter CAP, ABR or CM responses, and responses in

control and experimental groups had equivalent baseline stabilities.

A second analysis examined possible changes in pre- vs postbaseline values collapsed across animal treatment. This was accomplished by combining all of the baseline responses measured at the 0-90 minute preinjection period, and comparing them to all postbaseline (i.e. 90-180 minute) response measures. The remaining statistical analyses took the two separate treatment groups in each experimental series into account. Thus, between-treatment differences across time were assessed, as were between-treatment baseline stabilities, and between-treatment changes in pre- vs postbaseline values. In all five analyses, statistical significance was defined by an alpha level of $p<.05$.

Experiment 1: (±)-Pentazocine effects on the amplitudes of the first and second components (N1 and N2) of the CAP were statistically evaluated in a total of 14 animals. Baseline vs postbaseline changes in these compound auditory nerve evoked responses were assessed for (±)-pentazocine effects at two doses of 16mg/kg (n=5) and 8mg/kg (n=4). Responses were compared with those derived in single (n=2) and multiply injected (n=3) vehicle-control chinchillas.

Experiment 2: In this experimental series, the CAP response amplification effects of (-)-pentazocine (n=5) at specific stimulus intensities were compared with responses recorded after administration of the non-opioid σ-receptor

ligand (+)-pentazocine (n=5), using a modified form of the statistical program (Appendix B). In addition, ANOVA tests were applied (Appendix B) to determine whether CAP response amplitude differences existed both between and within the two (i.e. (+)-pentazocine control (n=5); (-)-pentazocine experimental (n=5) treatment groups, as a function of stimulus intensity. The same MANOVA programs were used to compare the treatment effects of (-)- vs (+)-pentazocine on CM amplitudes at specific stimulus intensities.

Experiment 3: The capacity for K-receptor block of (-)-pentazocine-induced CAP amplitude changes by a single, round window-administered dose of nor-BNI was statistically evaluated at each stimulus intensity in a total of 13 (7 experimental; 6 control) animals. Data were analyzed in a single statistical run using the MANOVA programs previously described (Appendix B).

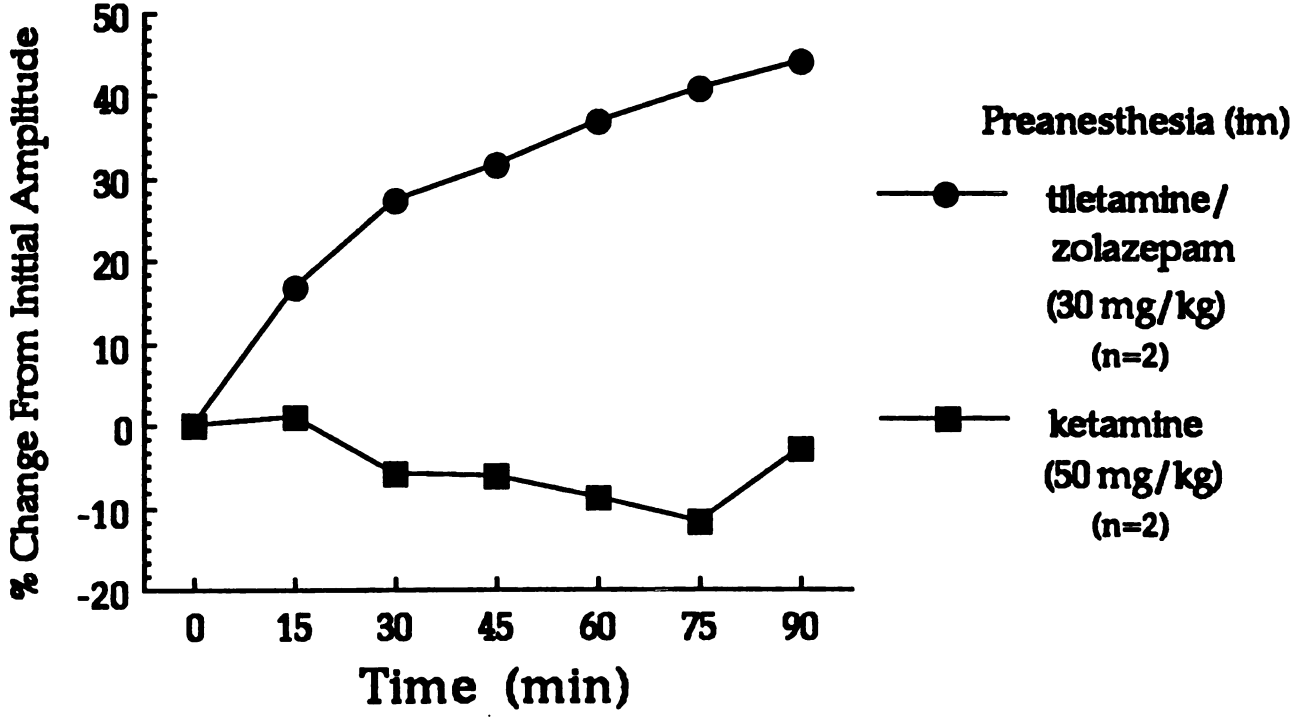
RESULTS

EXPERIMENT 1

Preanesthesia Effects: Preliminary experiments of near-field-recorded auditory potentials using 30mg/kg tiletamine/zolozepam (Telazol) as a preanesthetic to pentobarbital, revealed that it produced a consistent positive drift in CAP response amplitudes. Amplitudes at all stimulus intensities exhibited a consistent upward instability that was greatest at the relatively high suprathreshold intensity of +50dB SL. Amplitudes usually stabilized after roughly 2-3 hours of CAP testing. Including surgical preparation time, this stabilization occurred approximately >5 hours after Telazol administration. Such response instability was never observed in chinchillas that were initially anesthetised with 50mg/kg ketamine.

The different effects of these anesthetics on CAP (N1) response amplitudes are illustrated for responses evoked by +50dB SL stimuli from four representative animals (2 Telazol and 2 ketamine-treated) in Figure 1. In each of these two pairs of animals, two N1 amplitudes were initially recorded (0 min), and two additional recordings were made during each of six successive 15 minute-long time periods. Data are plotted with reference to response amplitudes recorded initially. Again note that the initial recording time in the cases shown in Figure 1 actually corresponds to an approximately 3 hour post-preanesthetic benchmark.

FIGURE 1: Telazol vs Ketalar Preanesthesia Effects On CAP (N1) Response Magnitudes At 50dB SL



Given this observed instability in baseline recordings, the use of tiletamine/zolozepam as a preanesthetic agent was discontinued. The results described and shown in the remaining figures represent experiments conducted exclusively with ketamine/pentobarbital anesthesia.

Basic Data Presentation Format: Most of the neuropharmacological data from these experimental series is presented in figures describing changes in peak-to-trough CAP (N1 & N2) and CM response amplitudes plotted as a percent change from baseline. A grand baseline mean was determined for each experimental and control group, defined as the mean of the summary amplitude values obtained during the 90 minute-long baseline recording periods. Thus, in most data presented in the Figures, each of the three baseline (control) and postbaseline 30 minute averages are presented relative to this grand baseline mean.

Consequences of IV Fentanyl, Naloxone and U-50488H Injections

Administration For Auditory Nerve Response Amplitudes: Mu-opioid effects on near-field auditory potentials were determined using fentanyl and naloxone. Multiple iv administrations of μ-opioid receptor-preferring ligands failed to significantly affect CAP N1 and N2 response amplitudes at any stimulus intensity. Those negative results applied to administration of the μ-opioid receptor antagonist naloxone (1.1mg/kg; n=2), and the potent μ-

receptor agonist fentanyl (2mg/kg; n=2), as shown in Figures 2A (N1) and B (N2) below. Multiple postbaseline iv delivery of the selective K-receptor agonist U-50488H (6mg/kg; or 2mg/kg X 3) also failed (n=3) to produce postbaseline N1 and N2 amplitude changes (see Figures 9A and B; respectively). No drug-induced changes in response latency were observed in any animal following any of the above drug treatments.

(±)-Pentazocine (16mg/kg) Injection

Effects On Compound Auditory Potential (CAP) Response Amplitudes: The administration of Ringer's solution following the initial 60 minutes of the baseline recording period failed to produce alterations in the amplitudes of CAP N1 [$F(1,8)=2.99$; p(ns)] and N2 [$F(1,8)=0.03$; p(ns)] responses. By contrast, postbaseline administration of the potent K/σ-receptor ligand (±)-pentazocine markedly increased near-threshold N1 and N2 amplitudes as shown in Figures 2A and B, respectively. Indeed, at near-threshold intensities, 16 mg/kg (±)-pentazocine (n=5) significantly amplified postbaseline N1 amplitudes [$F(1,8)=18.29$; $p<0.005$] compared to controls. In one animal, (±)-pentazocine-induced postbaseline amplitudes in N1 averages rose to 133% of baseline value. In the same animals, however, postbaseline (±)-pentazocine (16 mg/kg) effects on the amplitudes of the second CAP component, N2, were not statistically significant [$F(1,8)=4.25$; $p=.07$]. Nevertheless, (±)-pentazocine led to

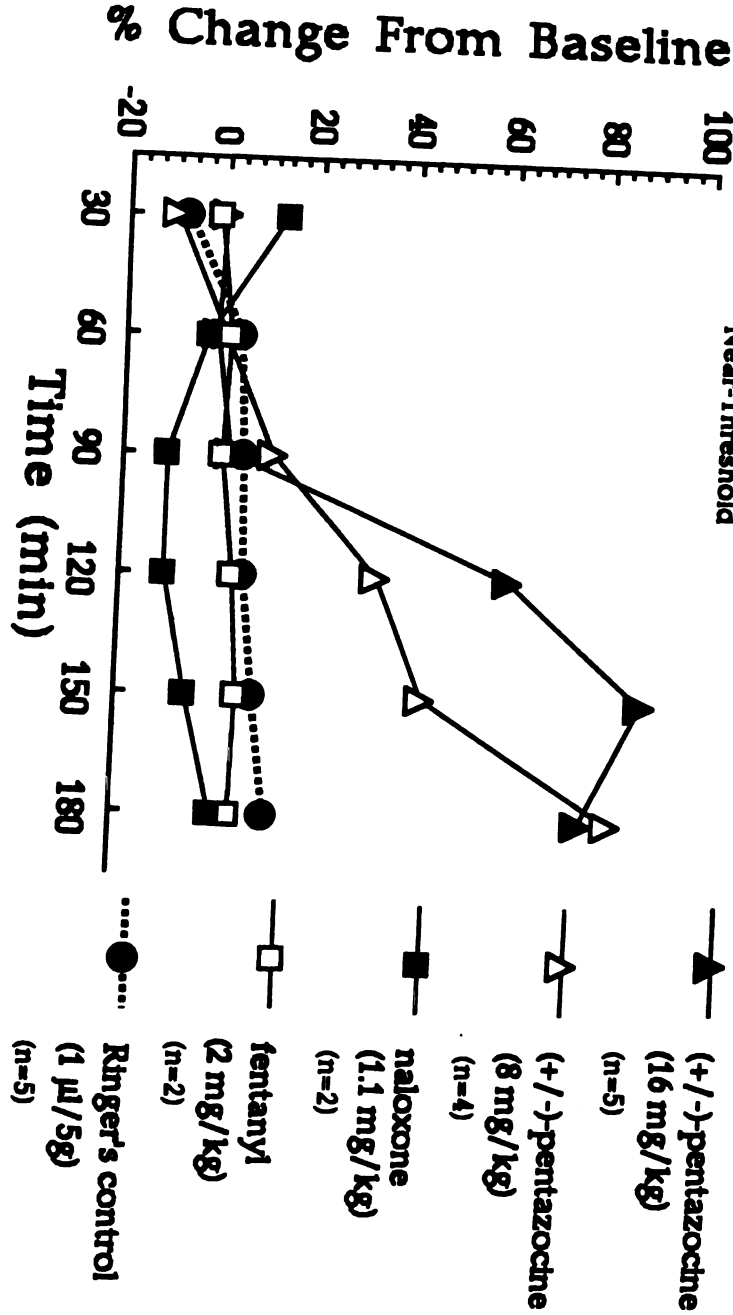
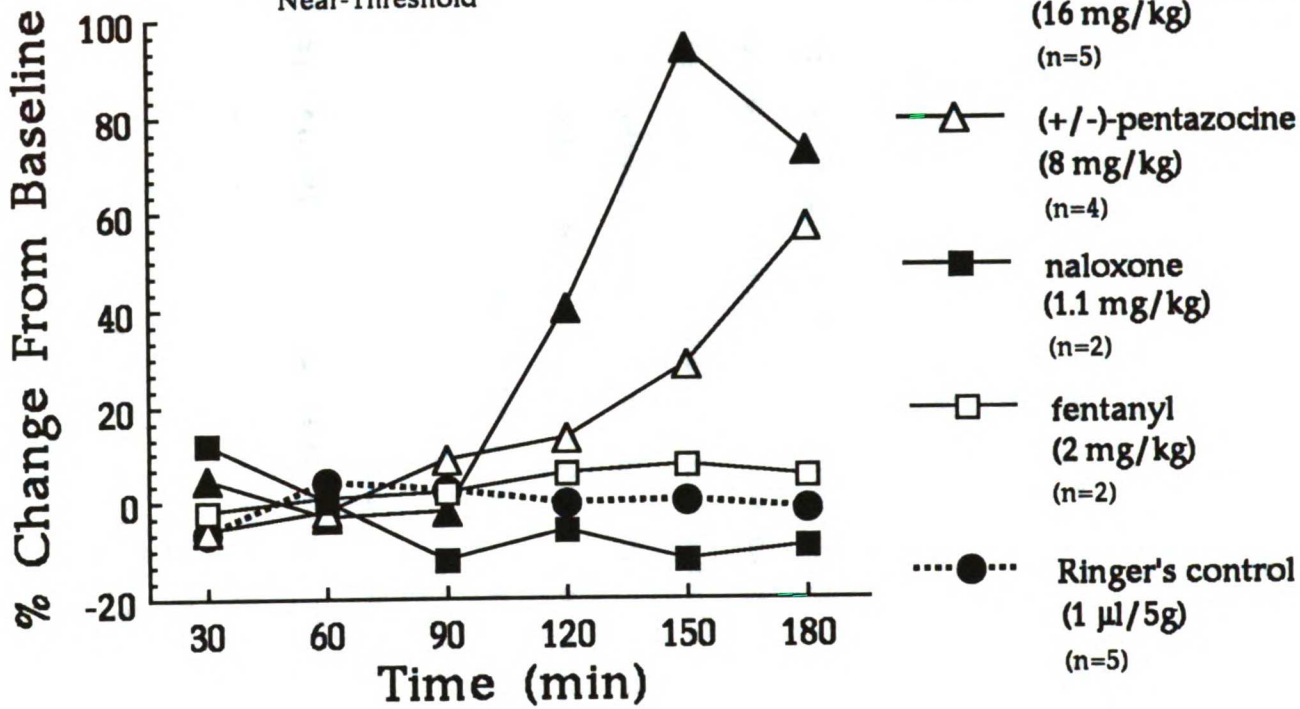


FIGURE 2A: Mu- And Kappa-Receptor Ligand Effects On CAP (N1) Response Magnitudes At Near-Threshold

FIGURE 2B: Mu- And Kappa-Receptor Ligand Effects On CAP (N2) Response Magnitudes At Near-Threshold



postbaseline amplitude changes in N2 averaging as high as 122% in one animal. Maximum effects of (±)-pentazocine on the amplitudes of these two CAP components were observed within 60 minutes of its iv administration at either dose. In one animal, CAP amplitude effects of (±)-pentazocine (16mg/kg) appeared to return to baseline values by approximately 135 minutes post administration. No measurable drug-induced changes in response latencies followed (±)-pentazocine administration.

Positive amplitude changes following 16mg/kg (±)-pentazocine seemed to apply at least primarily to responses to stimulus intensities near the defined threshold intensities for the CAP. This intensity-dependent effect on CAP N1 and N2 response amplitudes following (±)-pentazocine injection are illustrated by example in Figures 3A and B. There, responses obtained at each of the four stimulus intensities applied in one chinchilla, are shown. Representative waveforms at the baseline threshold intensity of +30dB SPL in this chinchilla are shown with a +30 minute post-pentazocine response below in Figure 4A.

Cochlear microphonic (CM) potentials derived using constant polarity clicks were also obtained in two experimental animals receiving postbaseline 16mg/kg (±)-pentazocine in this initial experimental series. Data were derived only for +10dB SL click stimuli. Unlike the observed (±)-pentazocine-induced amplitude changes in spiral ganglion-generated CAP responses, (±)-pentazocine failed to

FIGURE 3A: Kappa-Receptor Ligand Effects On CAP (N1) Response Magnitudes At Four Stimulus Levels In One Animal

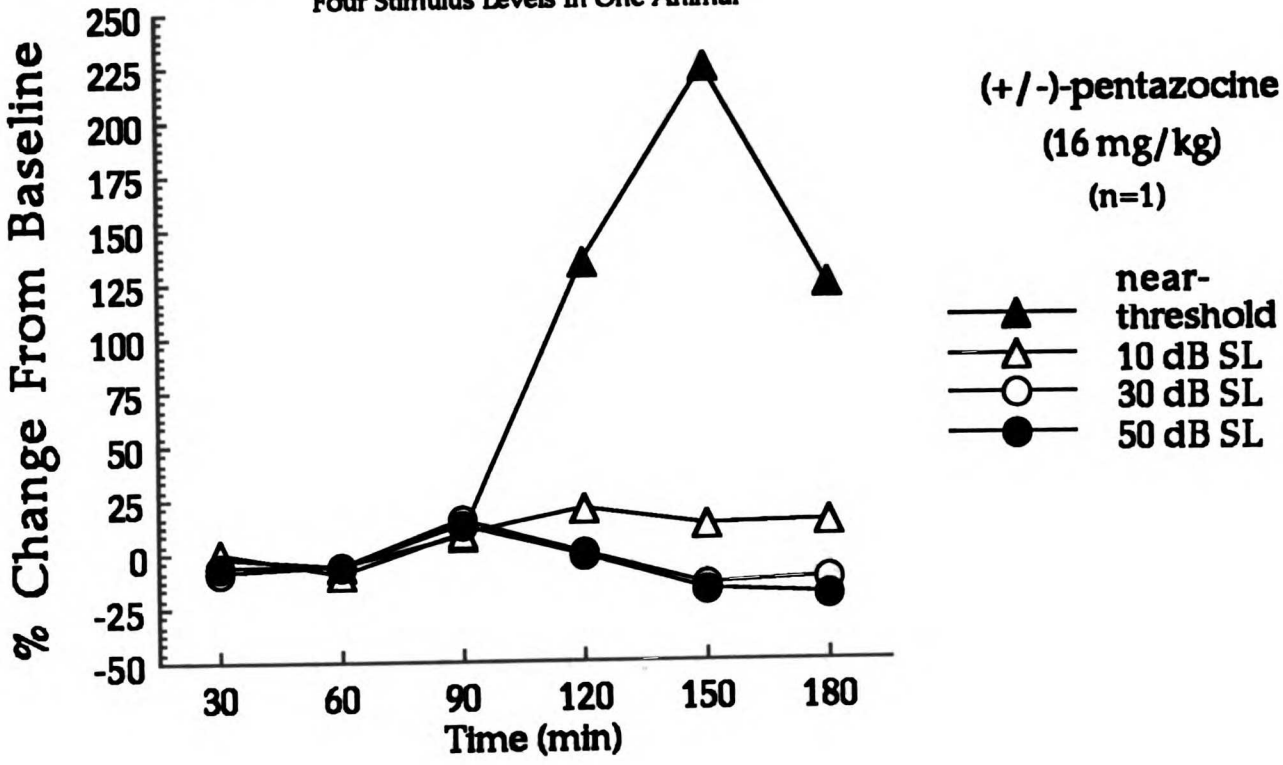


FIGURE 3B: Kappa-Receptor Ligand Effects On CAP (N2) Response Magnitudes At Four Stimulus Levels In One Animal

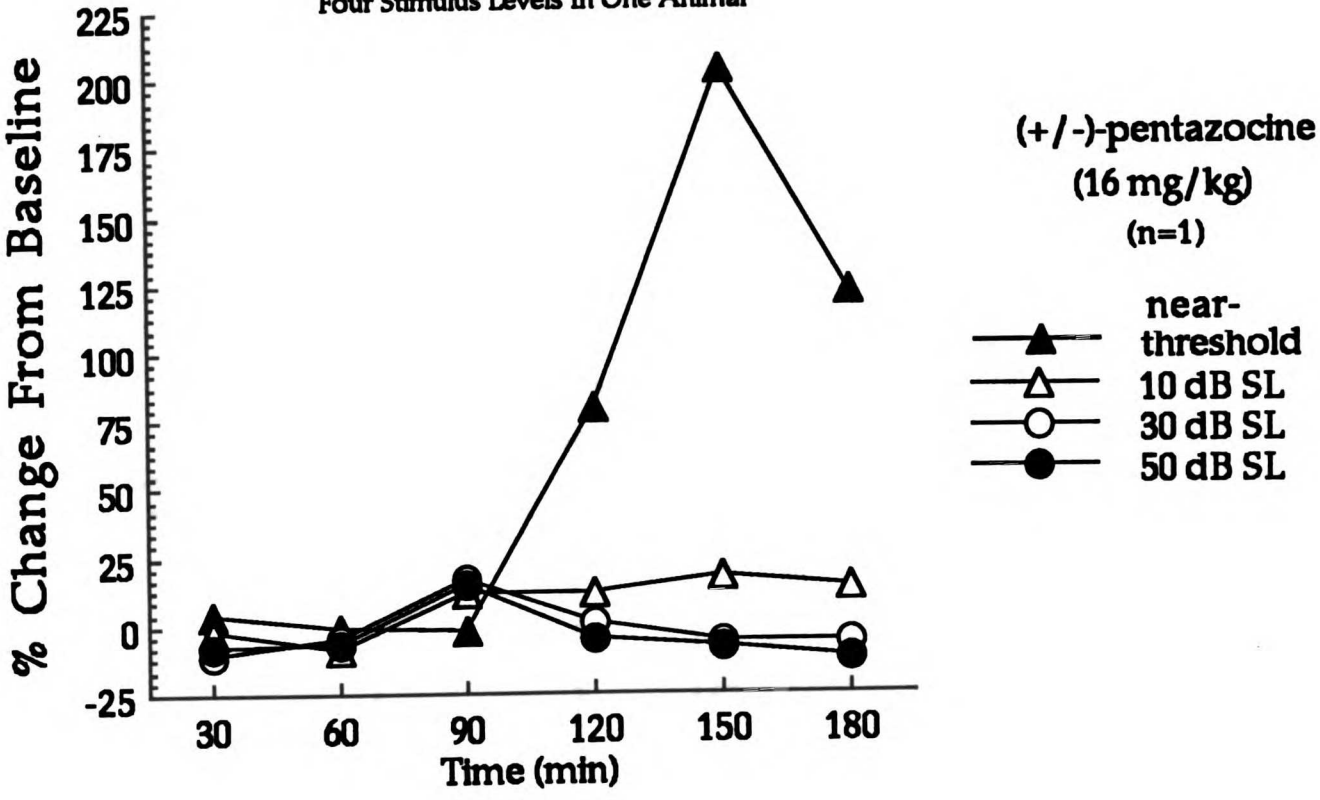
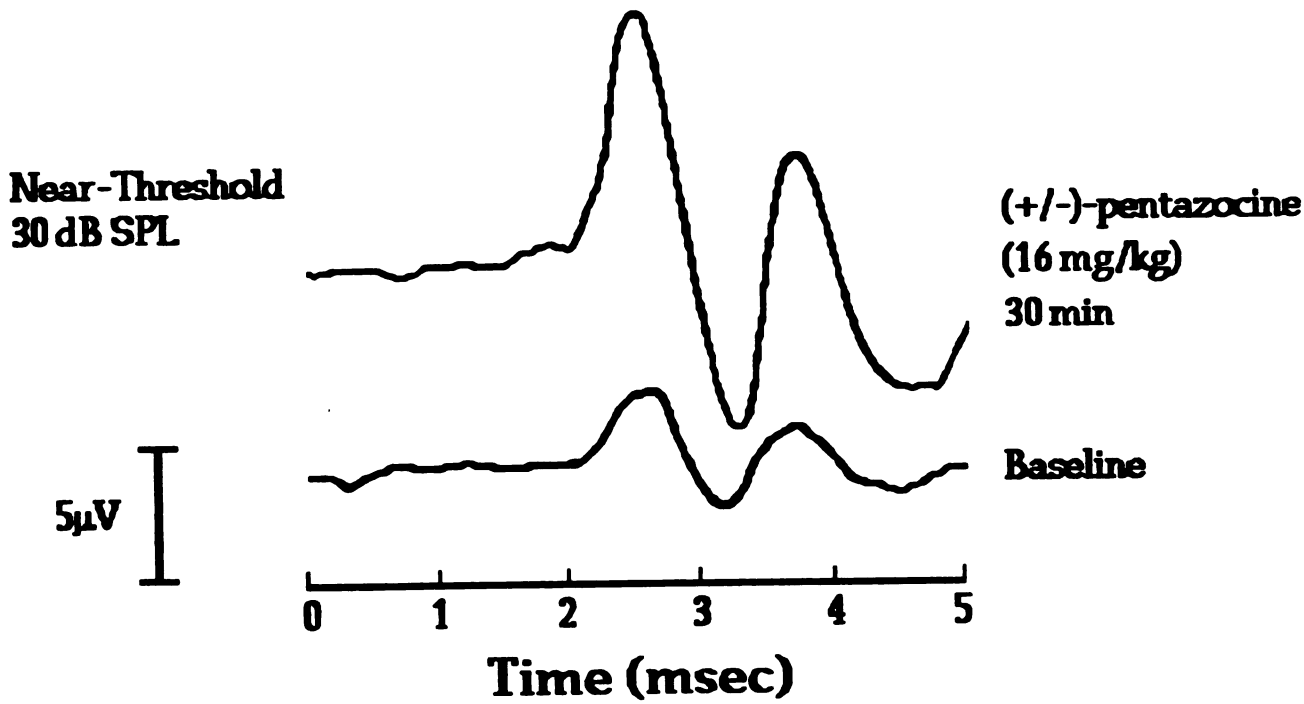


Figure 4A: CAP (N1 & N2) Responses Evoked During Baseline And 30 Minutes After (+/-)-Pentazocine Administration In The Same Animal



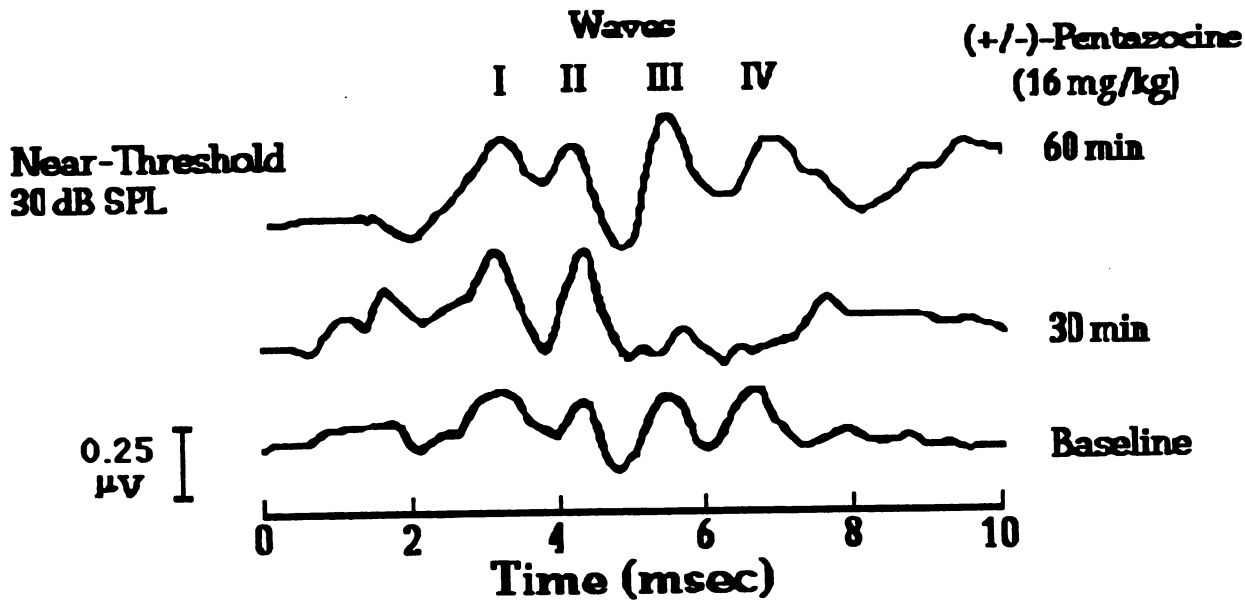
produce evidence of amplitude changes in the microphonic responses attributable to outer hair cells. Additional documentation of cochlear microphonic response stability following K-receptor activation with pentazocine will be described with the presentation of data from Experimental series 2 and 3, below.

Effects On Evoked Brainstem Responses: Limited data obtained with ABR recording in this first series indicated that all ABR wave components at near-threshold sound intensities generally demonstrated proportionally similar positive amplitude changes following postbaseline (±)-pentazocine (16mg/kg) administration. ABR potentials from the same animal whose near-field data are also illustrated in Figures 3A, B and 4A, are shown below in Figure 4B. The ABRs shown were obtained at a near-threshold intensity of 30dB SPL throughout baseline, and during the 0-60 minute postbaseline epoch. Again, no detectable drug-induced ABR latency changes followed (±)-pentazocine administration at either dose level, in any animal so tested.

(±)-Pentazocine (8mg/kg) Injection

Postbaseline administration of (±)-pentazocine at a lower dose level of 8 mg/kg (n=4), given 4mg/kg in two successive doses, each separated by 30 minutes, produced similar amplitude effects on the CAP at near-threshold stimulus intensities (Figures 2A and B). CAP response amplitudes were consistently further increased by the second 4mg/kg (±)-pentazocine dose. After the second 4mg/kg dose,

Figure 4B: ABR Responses Evoked During Baseline, And At 30 & 60 minutes After (+/-)-Pentazocine Administration In The Same Animal



average N1 and N2 amplitude changes were > +50% overall, and about 2X baseline values in one animal. However, overall postbaseline N1 [F(1,5)=3.35; p(ns)] and N2 [F(1,5)=2.30; p(ns)] amplitudes were not statistically different when postinjection responses were compared with control animal responses in these lower-dose chinchillas.

Additional Animals: Some results from additional but less complete studies conducted with U-50488H (20mg/kg; n=3), U-69593 (1mg/kg; n=1) and (±)-pentazocine (16mg/kg; n=1) are summarized in Figures 9A, B and C (pp. 160-162). In one of the three animals surviving a 20mg/kg dose of U-50488H, a 3 fold average positive change in N1 amplitude was observed (Figure 9A; p.160). Changes observed in N2 were more variable (Figure 9B; p.161). In a second animal, naloxone (0.1mg/kg) was co-administered with the first 10mg/kg delivery of U-50488H following the last 90 minute baseline recording. The positive change in the amplitude of N1 was eliminated or drastically reduced, even after the second 10mg/kg delivery of U-50488H given 30 minutes later (Figure 9C; p.162). Such data suggests a possible blockade of the K-opioid effects of U-50488H by the μ-receptor antagonist naloxone. In a third special preparation, naloxone (2mg/kg) co-administered with the second 10mg/kg delivery of U-50488H appeared to suppress the amplitude of N1.

The potent K-receptor agonist U-69593, administered at the non-lethal dose of 1mg/kg in one animal failed to

produce an observable change in CAP (N1) response amplitudes (Figure 9A and B). Finally, naloxone (1mg/kg) co-administered with (±)-pentazocine appeared to antagonize the positive N1 effects of the pentazocine racemate (Figure 9C). These limited examples again suggest a possible naloxone antagonism of (±)-pentazocine response amplification effects on compound auditory nerve potentials. Results of the first experimental series demonstrated significant changes in CAP responses following administration of the potent K/σ-receptor ligand, (±)-pentazocine. Experiment 2 was therefore designed to determine whether the amplitude changes observed in Experiment 1 were mediated by K-opioid, or non-opioid σ-receptors.

EXPERIMENT 2

(-)-Pentazocine vs (+)-Pentazocine; Effects On CAP Responses: Consistent with the results obtained in Experiment 1, postbaseline administration (8mg/kg) of the K-opioid receptor ligand (-)-pentazocine (n=5) produced significant and positive changes both in N1 [$F(1,8)=15.76$; $p<0.005$] and N2 [$F(1,8)=15.06$; $p<0.005$] CAP response component amplitudes at near-threshold stimulus levels. Administration of the non-opioid, σ-receptor ligand (+)-pentazocine was without effect (Figures 5A and B, Tables 3 and 4). As indicated in Figure 5A and Table 3, postbaseline (-)-pentazocine effects on the near-threshold N1 amplitude over the 30-60 minute period after injection, averaged a

FIGURE 5A: Opioid (-) Kappa- And Non-Opioid (+) Sigma-Receptor Effects Of Pentazocine On CAP (N1) Response Magnitudes At Four Stimulus Levels

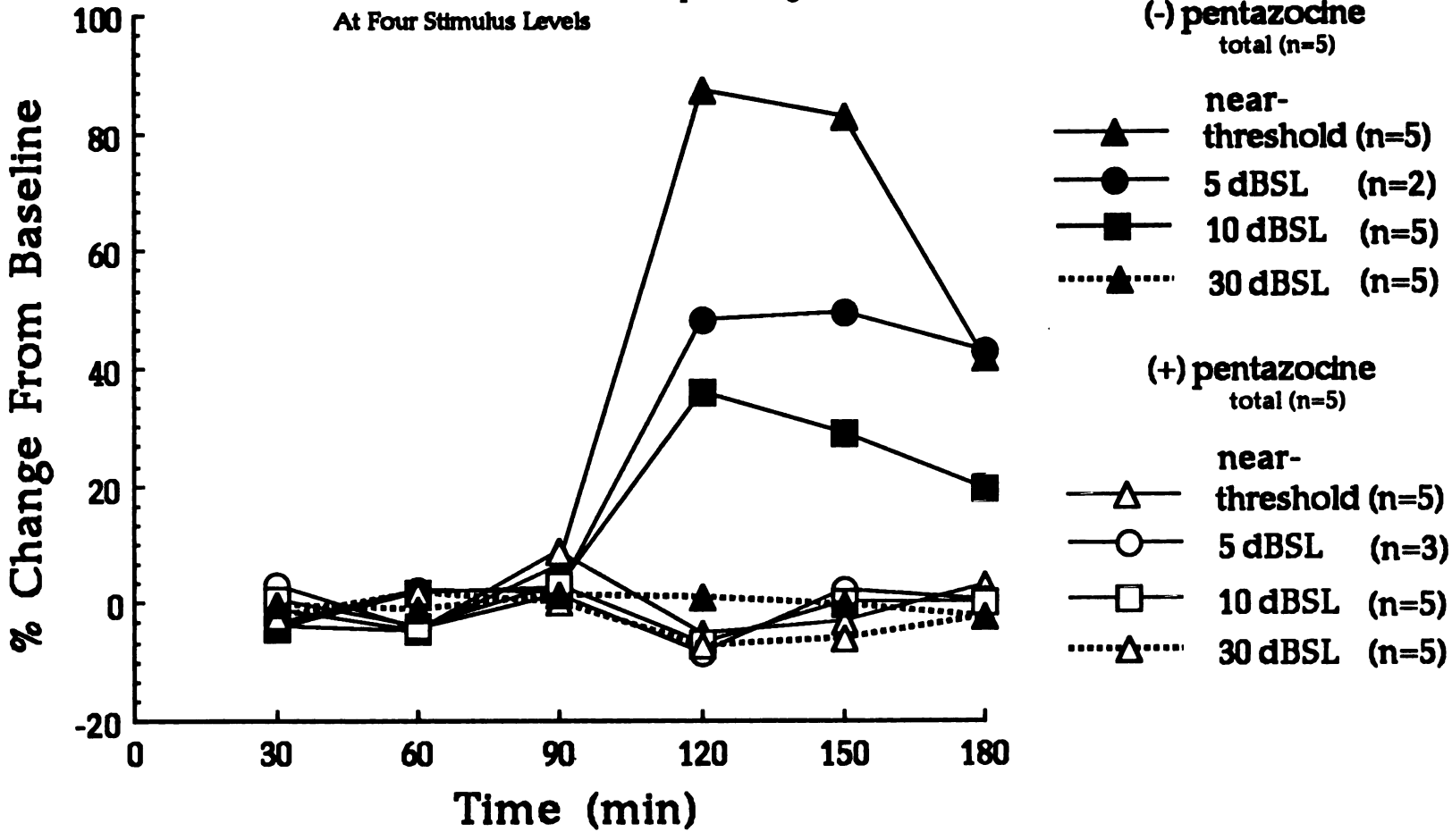


FIGURE 5B:

Opioid (-) Kappa- And Non-Opioid (+) Sigma-Receptor Effects
Of Pentazocine On CAP (N2) Response Magnitudes
At Four Stimulus Levels

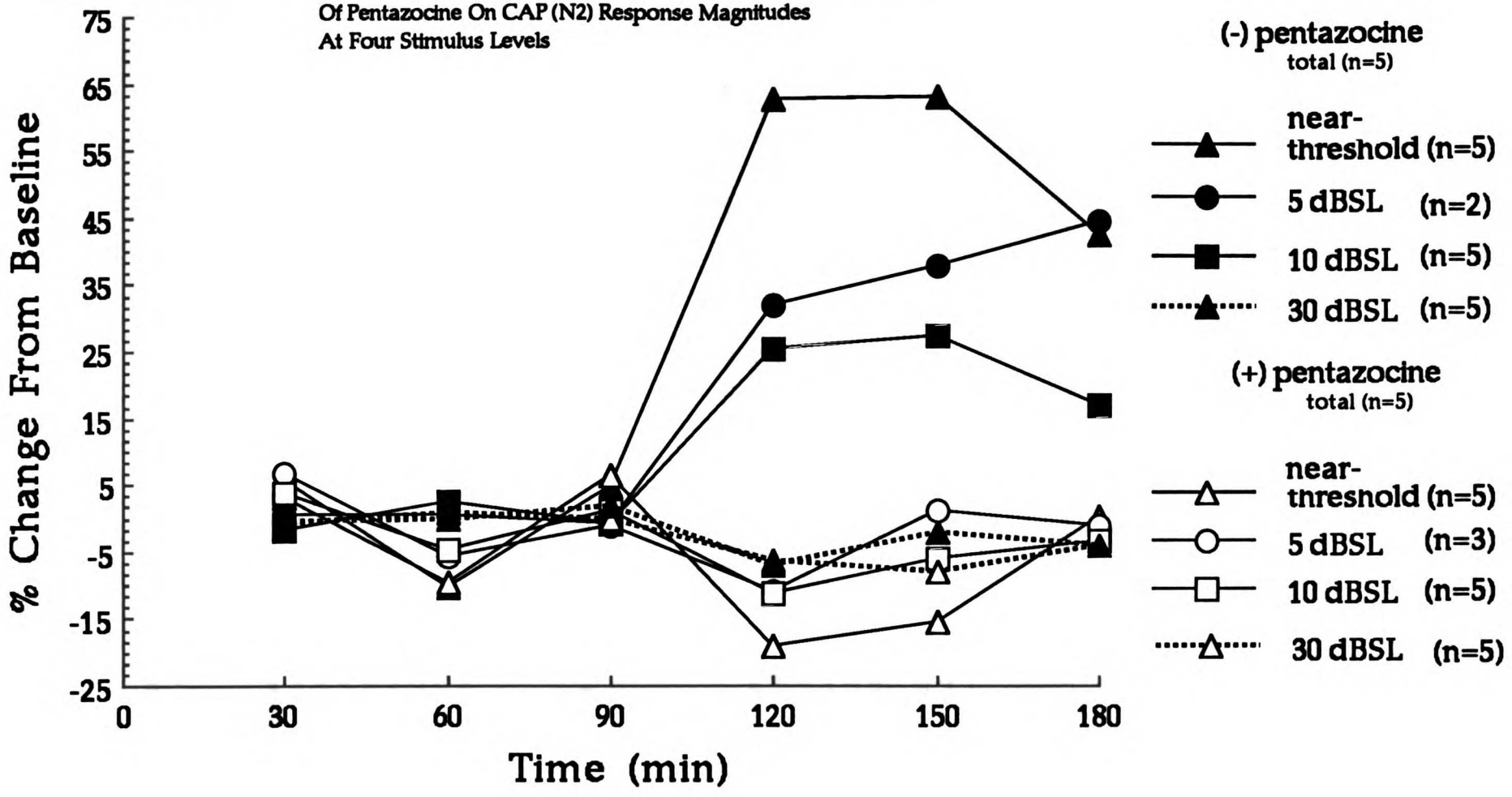


TABLE 3

Experiment 2

Data Matrix Of Mean CAP Amplitudes (In Microvolts) In Response To Near- Threshold
Stimulus Levels Before And After A Single (-)-Pentazocine (8 mg/kg; iv) Administration

N1 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
1 (1/29)	3.43	3.53	3.90	8.08	8.48	7.05
2 (3/8)	3.30	3.48	3.42	6.58	4.38	2.58
5 (3/11)	2.75	2.45	3.70	4.95	4.70	3.53
7 (3/14)	3.62	2.86	3.37	6.60	6.58	5.68
8 (3/20)	6.56	6.62	6.85	11.08	12.22	9.36
MEAN=	3.93	3.79	4.23	7.46	7.27	5.64
SD=	1.50	1.65	1.48	2.31	3.22	2.72
BASELINE GM= 3.96						
PLOT %=	-1.25%	-4.89%	6.14%	87.29%	82.56%	41.63%

N2 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
1 (1/29)	2.30	2.52	2.56	4.02	4.73	4.28
2 (3/8)	2.58	2.05	2.37	3.60	2.26	1.53
5 (3/11)	0.97	0.87	1.37	1.75	1.60	1.70
7 (3/14)	3.24	2.32	2.85	4.44	4.75	4.08
8 (3/20)	2.08	1.68	1.87	3.32	3.82	3.38
MEAN=	2.22	1.89	2.20	3.43	3.43	2.99
SD=	0.88	0.65	0.59	1.03	1.44	1.30
BASELINE GM= 2.11						
PLOT %=	5.64%	-10.32%	4.69%	62.73%	63.01%	42.21%

TABLE 4

Experiment 2

Data Matrix Of Mean CAP Amplitudes (In Microvolts) In Response To Near- Threshold Stimulus Levels Before And After A Single (+)-Pentazocine (8 mg/kg; iv) Administration

N1 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
3 (1/30)	4.18	3.95	4.45	4.18	4.58	4.48
4 (3/4)	8.57	8.60	10.98	9.27	8.53	10.40
14 (4/17)	4.59	3.36	4.25	4.27	4.92	5.08
15 (4/18)	4.84	5.40	5.22	4.23	4.28	3.73
16 (4/19)	4.57	5.17	5.35	4.45	4.64	4.98
MEAN=	5.35	5.30	6.05	5.28	5.39	5.73
SD=	1.81	2.03	2.80	2.23	1.77	2.66
BASELINE GM= 5.57						
PLOT %=	-3.87%	-4.84%	8.72%	-5.13%	-3.17%	3.03%

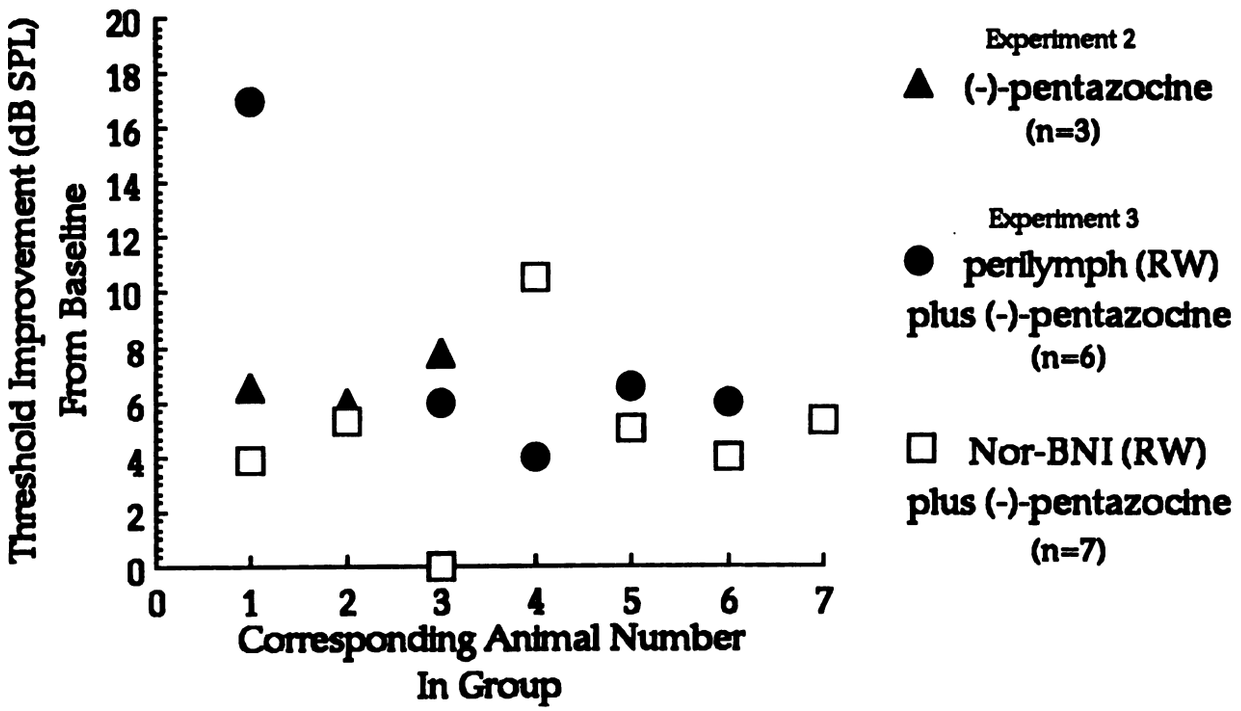
N2 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
3 (1/30)	1.23	1.25	1.33	1.08	1.10	0.94
4 (3/4)	4.60	4.03	5.42	3.92	3.73	4.93
14 (4/17)	2.89	2.27	2.90	2.57	2.86	3.36
15 (4/18)	2.44	2.18	1.95	1.33	1.38	1.50
16 (4/19)	1.87	1.70	1.83	1.30	1.62	1.92
MEAN=	2.61	2.29	2.69	2.04	2.14	2.53
SD=	1.28	1.06	1.63	1.20	1.12	1.61
BASELINE GM= 2.53						
PLOT %=	3.17%	-9.50%	6.33%	-19.24%	-15.36%	0.16%

87.3% and 83.0% positive change relative to baseline amplitudes. This corresponded to a mean positive response amplitude change in near-threshold N1s from a baseline level of 3.98μV to postbaseline values of 7.46-7.27μV (Table 3). In one animal, average N1 amplitude effects in this same time epoch revealed a 136% positive change relative to the animal's baseline values. Average postbaseline (-)-pentazocine effects on the amplitudes of N2s at near-threshold were also observed as a 62.7% to 63% change from overall baseline levels (Figure 5B, Table 3). This corresponded to a positive amplitude change in N2 from a baseline of 2.1μV, to postbaseline means of about 3.4μV (Table 3). In one animal, average positive postbaseline N2 amplitude changes were as great as 92% from respective baseline values.

The clear shift in the near-threshold response that occurred within 60 minutes after (-)-pentazocine (8mg/kg) administration was carefully measured during peak drug effects in three of these experimental cases. The recorded shift was the amount of sound attenuation required to produce postdrug CAP response amplitudes that approximately matched predrug (baseline) amplitude values. The mean change amounted to a 6.77 ± 0.93dB improvement in near-threshold stimulus levels. Threshold improvements during peak drug effects in each of these three animals are plotted in the scatter diagram of Figure 6 below. The CAP waveforms from each of these three animals are used in Figures 7A, B and C

Figure 6: Change In The CAP Response At Near-Threshold Intensities Following (-)-Pentazocine Administration In Experiments 2 and 3

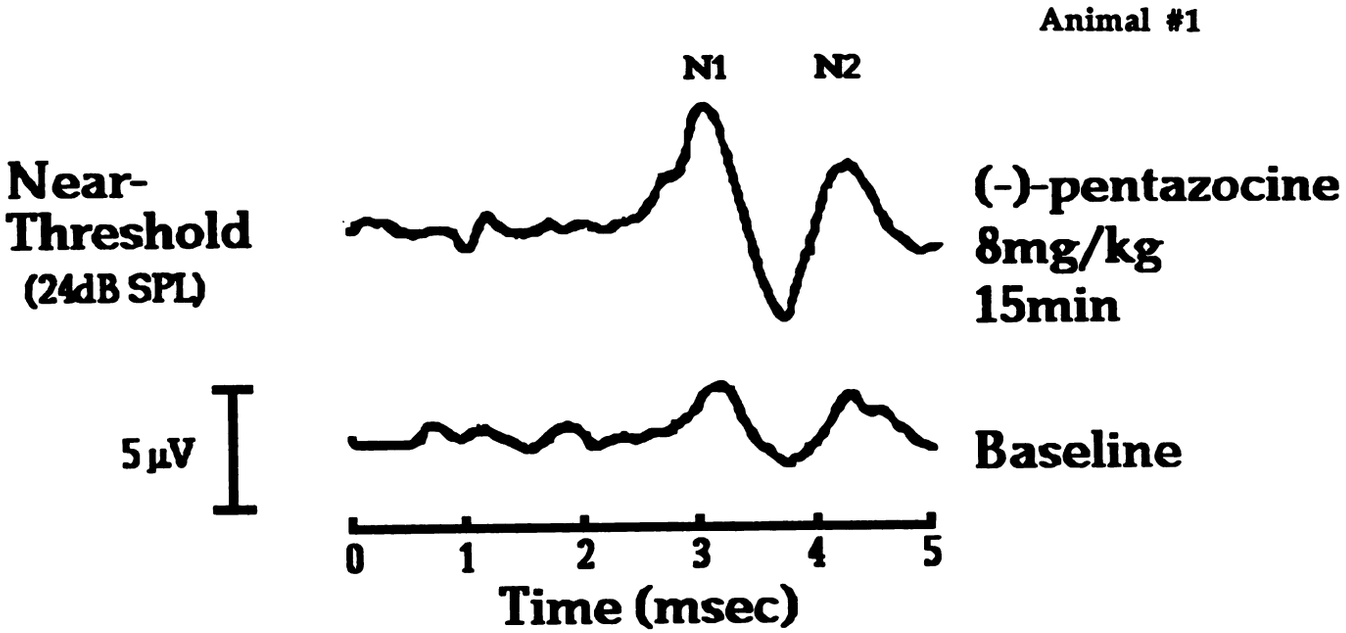


to illustrate these postbaseline (-)-pentazocine effects at near-threshold stimulus intensities. Baseline and +15-minute postinjection waveform responses shown below in Figure 7A were obtained at 24dB SPL. Baseline and +40 minute postinjection waveforms obtained for 7.7dB SPL are shown below in Figure 7B, as are baseline and +45 minute postinjection waveforms for 18.1 dB SPL in Figure 7C.

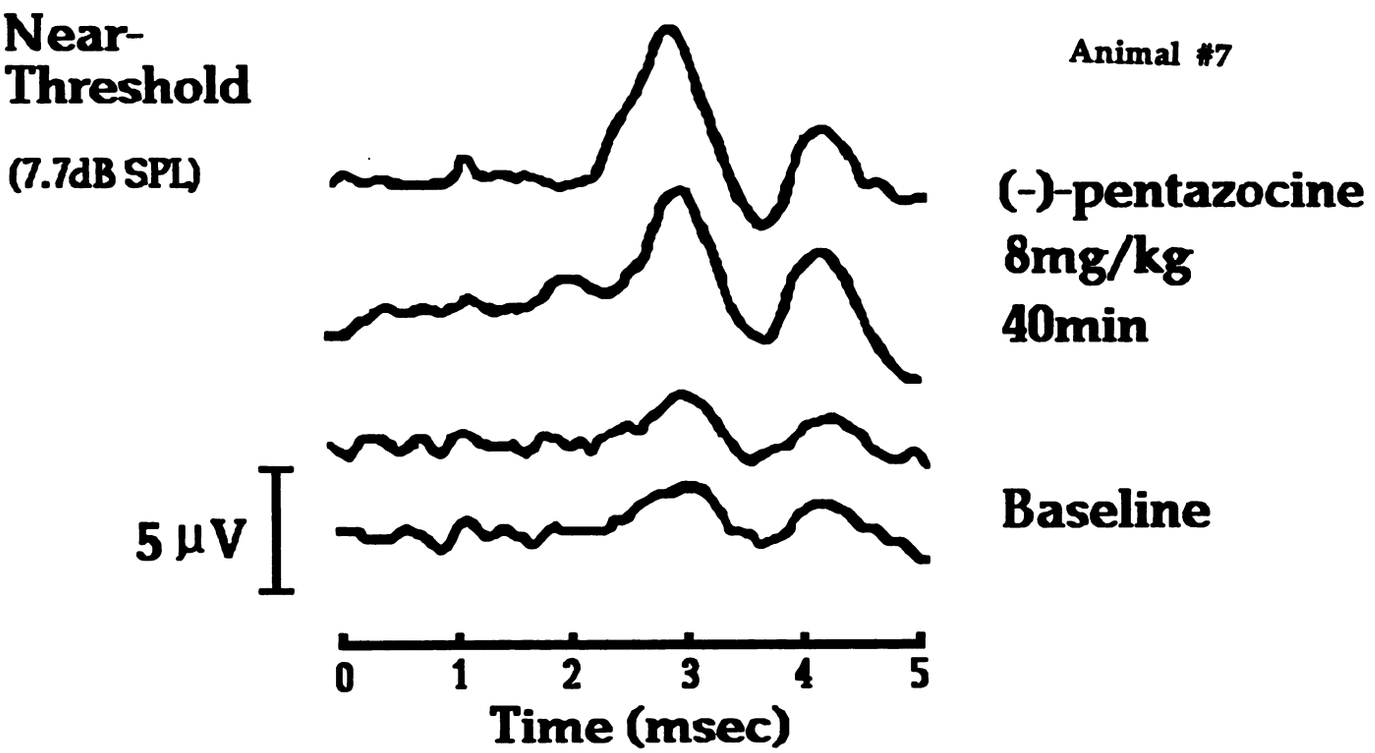
Similar though less dramatic (-)-pentazocine induced positive changes in the amplitudes of N1 and N2 were observed at the stimulus intensities of +5 and +10dB SL (Figures 5A and B). Indeed, postbaseline amplitude changes were significantly different from baseline in N1 for +5 dB [$F(1,3)=10.08$; $p=0.05$] and +10dB [$F(1,8)=10.00$; $p<0.02$], and in N2 for +5dB [$F(1,3)=172.08$; $p=0.001$], and +10dB [$F(1,8)=8.59$; $p<0.02$]. Suprathreshold CAP waveforms from animals used to illustrate drug amplification of near-threshold responses, are also shown below for responses to +5 (Figures 7D and E) and +10dB SL (Figure 7F). As indicated earlier in Figures 5A and B, these positive effects of (-)-pentazocine on CAP amplitudes were not evident at +30dB SL for N1 [$F(1,8)=0.47$; p(ns)] or N2 amplitudes [$F(1,8)=0.25$; p(ns)]. Again, no drug-induced changes in N1 or N2 latencies followed either (-)- or (+)-pentazocine administration at any stimulus intensity, in any tested animal. This negative effect on N1 and N2 latencies is also summarized below in Tables 5 and 6, respectively.

Figure 7: **Averaged CAP (N1 & N2) Responses In Representative (-)-Pentazocine-Injected Chinchillas, At The Indicated Stimulus Levels And Post-Injection Times**

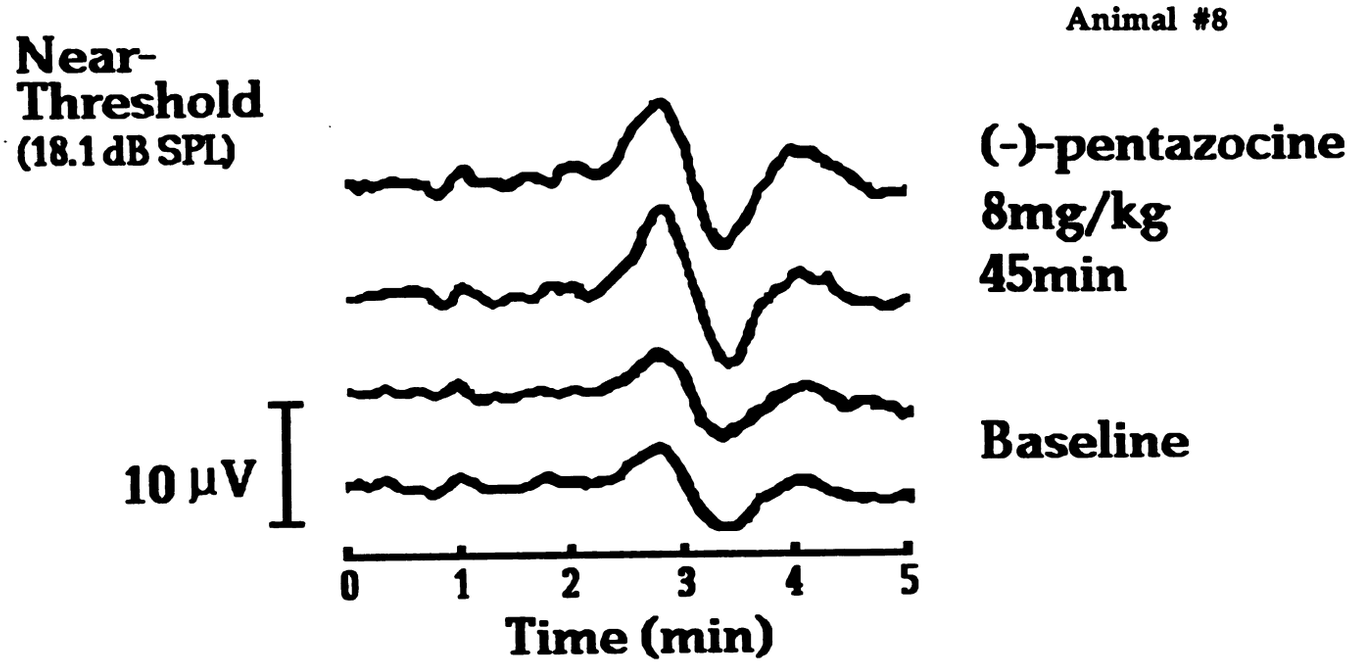
A

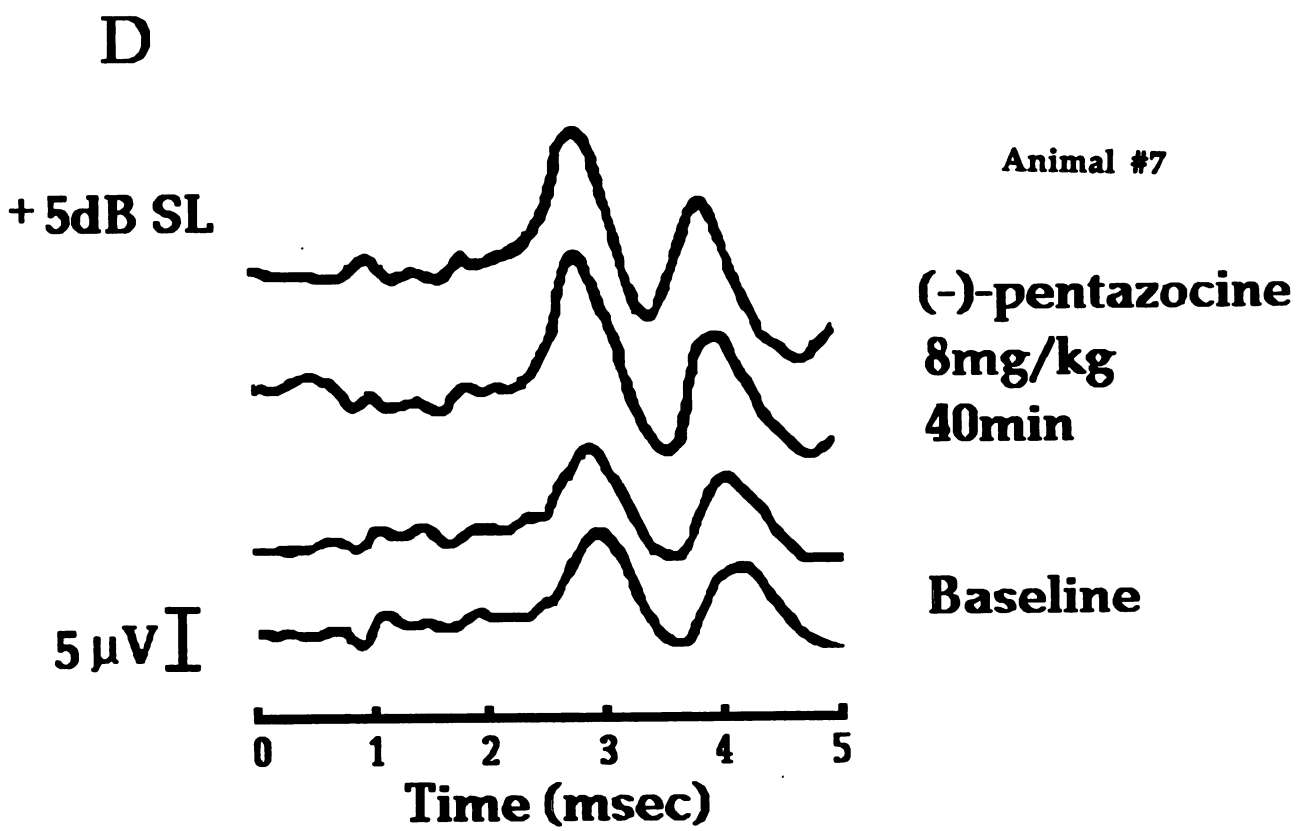


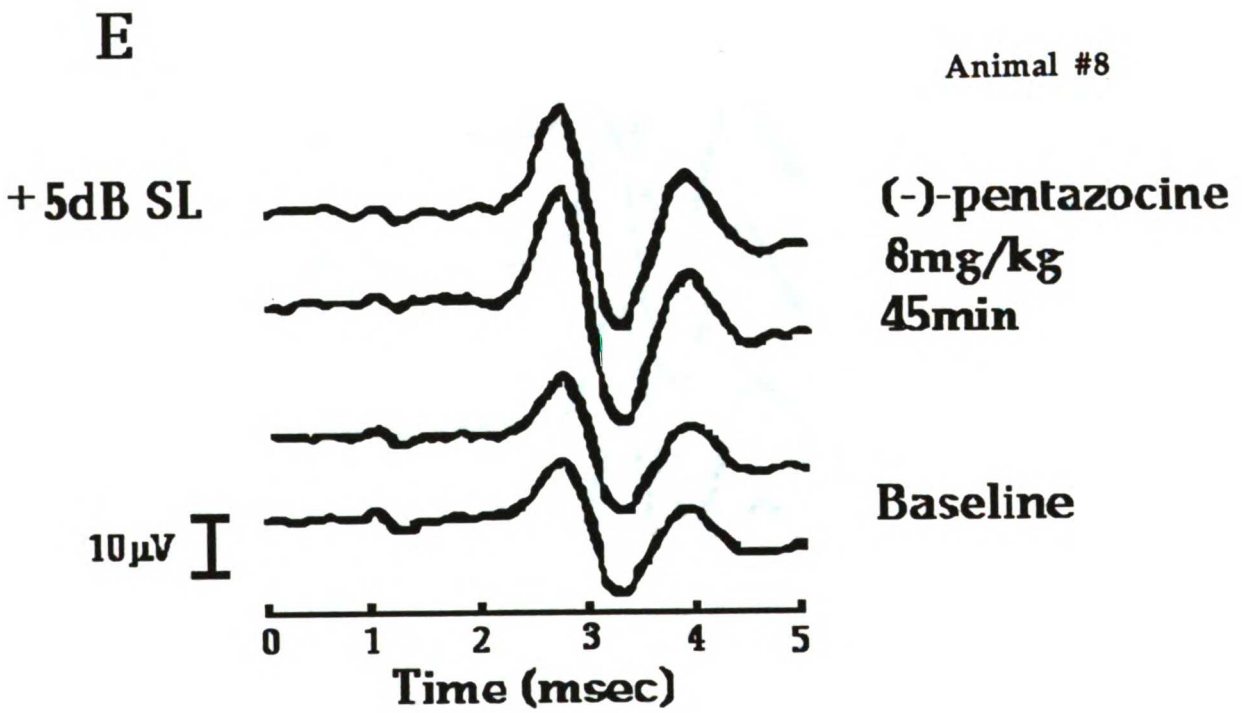
B


Near-
Threshold
(7.7dB SPL)
Animal #7
(-)-pentazocine
8mg/kg
40min
Baseline
5 µV
0
1
2
3
4
5
Time (msec)

C


Animal #8
Near-
Threshold
(18.1 dB SPL)
(-)-pentazocine
8mg/kg
45min
Baseline
10 µV
0
1
2
3
4
5
Time (min)


D
+5dB SL
Animal #7
(-)-pentazocine
8mg/kg
40min
Baseline
5 μV
0
1
2
3
4
5
Time (msec)


E
Animal #8
+5dB SL
(-)-pentazocine
8mg/kg
45min
Baseline
10μV
0
1
2
3
4
5
Time (msec)

F

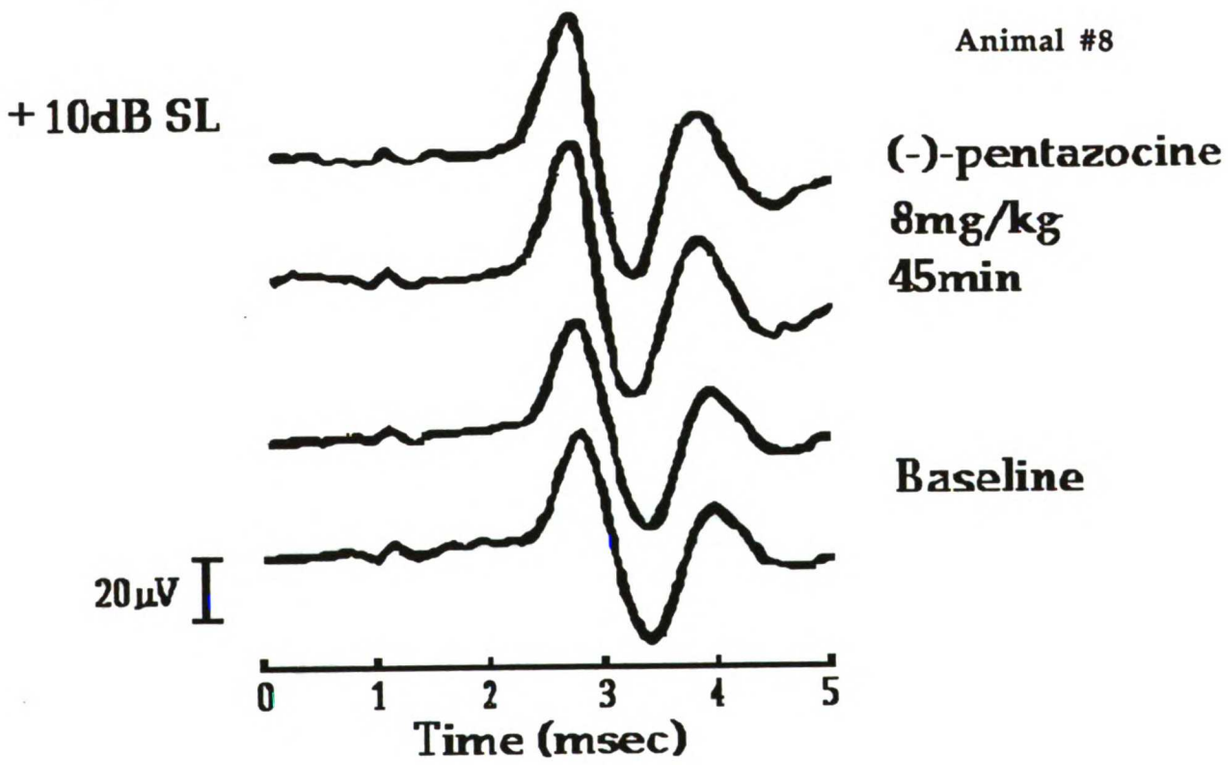

Animal #8
+10dB SL
(-)-pentazocine
8mg/kg
45min
Baseline
20μV
0
1
2
3
4
5
Time (msec)

TABLE 5

Experiment 2

Data Matrix Of Mean CAP Latencies (in Milliseconds) In Response To Near- Threshold Stimulus Levels Before And After A Single (-)-Pentazocine (8 mg/kg; iv) Administration

N1 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
1 (1/29)	3.05	3.08	3.08	3.04	3.03	3.05
2 (3/8)	3.03	3.06	3.09	3.04	3.09	3.04
5 (3/11)	2.94	2.98	2.95	3.01	2.95	3.01
7 (3/14)	3.05	3.03	3.04	3.02	3.03	3.03
8 (3/20)	2.83	2.86	2.91	2.85	2.88	2.88
MEAN=	2.98	3.01	3.01	2.99	3.00	3.00
SD=	0.10	0.09	0.08	0.08	0.08	0.07
BASELINE GM= 3.00						
% Change=	-0.67%	0.20%	0.47%	-0.27%	0.07%	0.07%

N2 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
1 (1/29)	4.01	4.08	4.07	4.06	4.05	4.04
2 (3/8)	4.06	4.14	4.10	4.11	4.06	4.13
5 (3/11)	4.07	3.98	4.04	4.07	4.13	4.04
7 (3/14)	4.08	4.09	4.06	4.06	4.08	4.07
8 (3/20)	3.96	3.97	4.01	3.97	3.93	3.94
MEAN=	4.04	4.05	4.06	4.05	4.05	4.04
SD=	0.05	0.07	0.03	0.05	0.07	0.07
BASELINE GM= 4.05						
% Change=	-0.30%	0.10%	0.20%	0.15%	0.05%	-0.10%

TABLE 6

Experiment 2

Data Matrix Of Mean CAP Latencies (in Milliseconds) In Response To Near- Threshold Stimulus Levels Before And After A Single (+)-Pentazocine (8 mg/kg; iv) Administration

N1 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
3 (1/30)	2.90	2.92	2.91	2.94	2.92	2.92
4 (3/04)	2.98	3.01	3.00	3.02	3.02	2.99
14 (4/17)	3.10	3.11	3.12	3.07	3.10	3.10
15 (4/18)	3.20	3.18	3.21	3.21	3.20	3.21
16 (4/19)	3.45	3.46	3.46	3.44	3.45	3.45
MEAN=	3.13	3.14	3.14	3.14	3.14	3.13
SD=	0.21	0.21	0.21	0.20	0.20	0.21
BASELINE GM= 3.13						
% Change =	-0.26%	-0.06%	0.19%	0.06%	0.13%	0.00%

N2 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 (Ringers)	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
3 (1/30)	3.91	3.99	3.95	4.00	4.04	4.02
4 (3/04)	3.87	3.90	3.92	3.92	3.91	3.89
14 (4/17)	4.21	4.21	4.18	4.17	4.18	4.15
15 (4/18)	4.45	4.44	4.42	4.42	4.44	4.40
16 (4/19)	4.47	4.48	4.48	4.49	4.49	4.47
MEAN=	4.18	4.20	4.19	4.20	4.21	4.19
SD=	0.29	0.26	0.26	0.25	0.25	0.25
BASELINE GM= 4.19						
% Change =	-0.24%	0.29%	-0.05%	0.19%	0.48%	-0.14%

Tests For Trends: Significant amplitude changes in N1 [F(1,8)=31.70; p<.001] and N2 [F(1,8)=16.50; p<.005] were found between the two treatment groups as a function of intensity. The statistically significant 'between-treatment' difference at each intensity justified separate 'within-treatment' analyses for linear intensity-dependent trends. Significant linear trends (see Methods) were found in the postbaseline amplitude change for both N1: (-)-pentazocine= [F(1,4)=44.14; p<0.003]; and the N2: (-)-pentazocine= [F(1,4)=15.33; p<0.02] CAP responses for experimental animals. Thus, the postbaseline amplitude increments observed were quantitatively different as a function of stimulus intensity in this group. No such intensity trends were observed for animals within the non-opioid pentazocine control group, for N1: [(+)-pentazocine= [F(1,4)=0.49; p(ns)], or N2: (+)-pentazocine= [F(1,4)=1.03; p(ns)].

Pentazocine Effects On CM Responses: There were no obvious or consistent postbaseline changes in the amplitudes of cochlear microphonic responses following the postbaseline administration of either the opioid (-)- , or the non-opioid (+)-pentazocine control, at near-threshold, +5, +10 and +30dB SL stimulus levels. Near threshold, however, significant pre- vs postbaseline amplitude differences were observed between the two treatment groups [F(1,8)=13.35; p<.01]. That occurred in part because CM amplitudes in (+)-pentazocine-injected animals were lower on the average in the postbaseline periods. Note that both pre- and

postbaseline CM amplitudes in the experimental group were somewhat variable in this experimental series (see Figure 8 below).

Figure 8 directly contrasts the postbaseline neural (N1) and cochlear microphonic potential (CM) effects of (-)-pentazocine administration recorded simultaneously at three stimulus intensities, in the same animals. These data again indicate that postbaseline effects of (-)-pentazocine are not simply due to factors that might alter the overall level of acoustic input to the organ of Corti, as such factors would affect the response amplitudes of both the mass neural (CAP) and mass hair cell (CM) potentials in parallel. Moreover, they again demonstrate pentazocine effects on CAP responses in parallel with stable receptor (hair cell) potential responses in the organ of Corti.

Additional Animal: Included in this series are results from an additional but less complete experiment on CAPs derived with constant polarity clicks, following 1.0 mg/kg U-69593 in one chinchilla. U-69593 appeared to produce a positive amplitude change in both the N1 (Figure 9A) and N2 potentials (Figure 9B). Generally, however, the results of Experiment 2 using (-)-pentazocine indicated that the observed drug induced shifts in near-threshold responses, and concomitant CAP amplitude changes, were K-opioid mediated. Experiment 3 was designed to determine whether such effects could be further characterized

FIGURE 8: Relative Changes In CAP (N1) And CM Response Magnitudes At Four Stimulus Levels Before And After 8 mg/kg (-)-Pentazocine Administration In The Same Animals

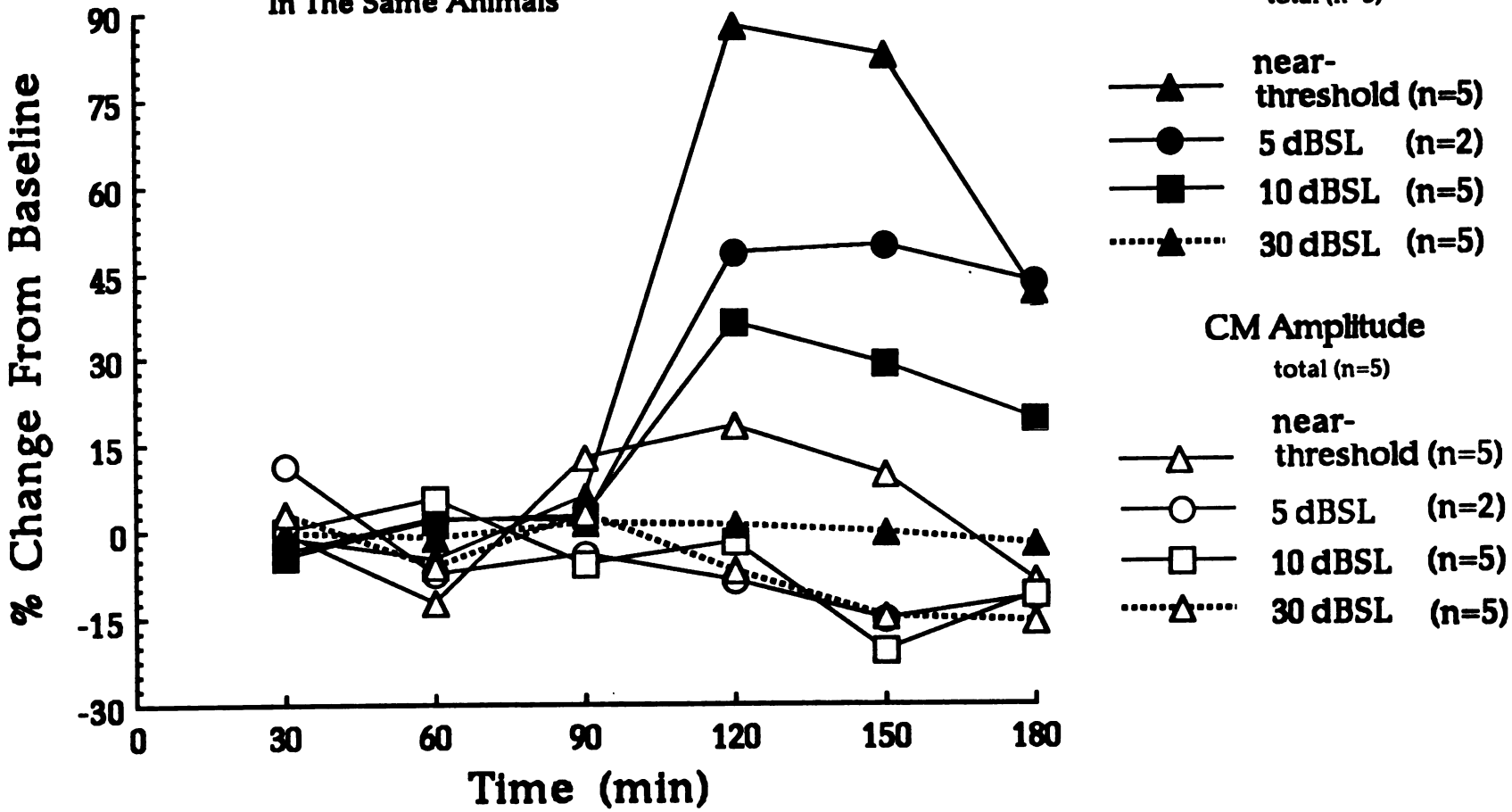


FIGURE 9A: Additional Kappa-Receptor Ligand Effects On CAP (N1) Response Magnitudes At Near-Threshold

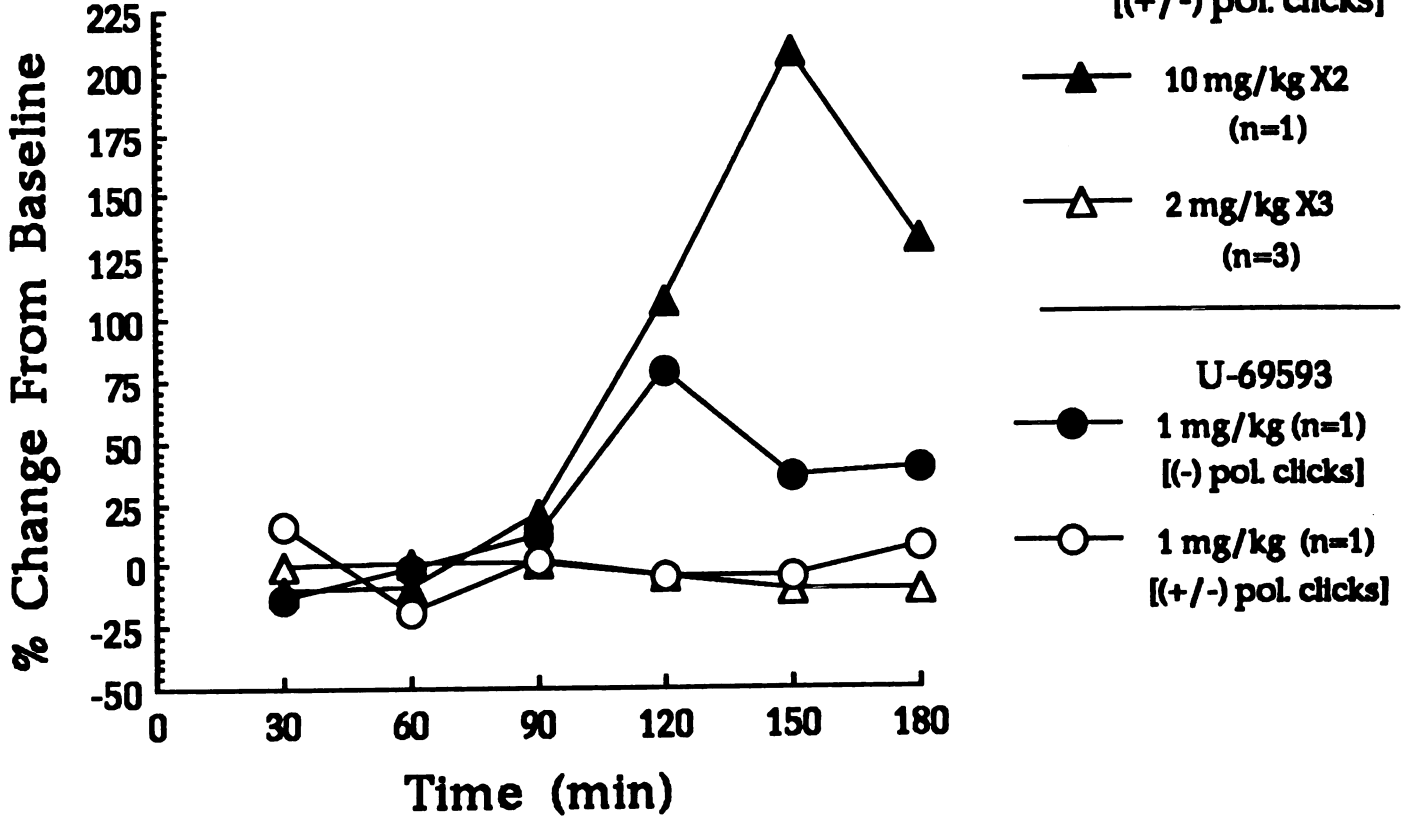


FIGURE 9B: Additional Kappa-Receptor Ligand Effects On CAP (N2) Response Magnitudes At Near-Threshold

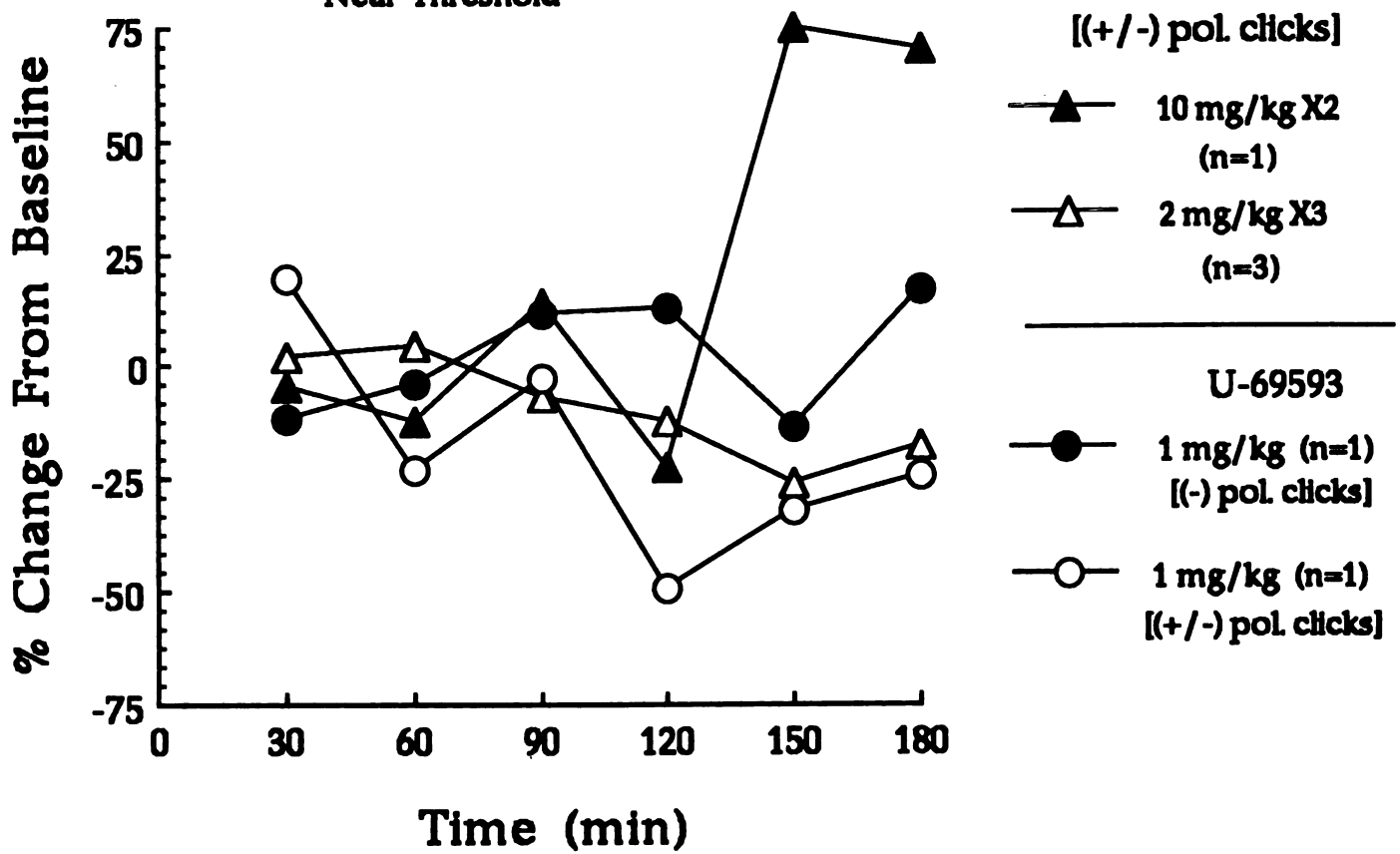
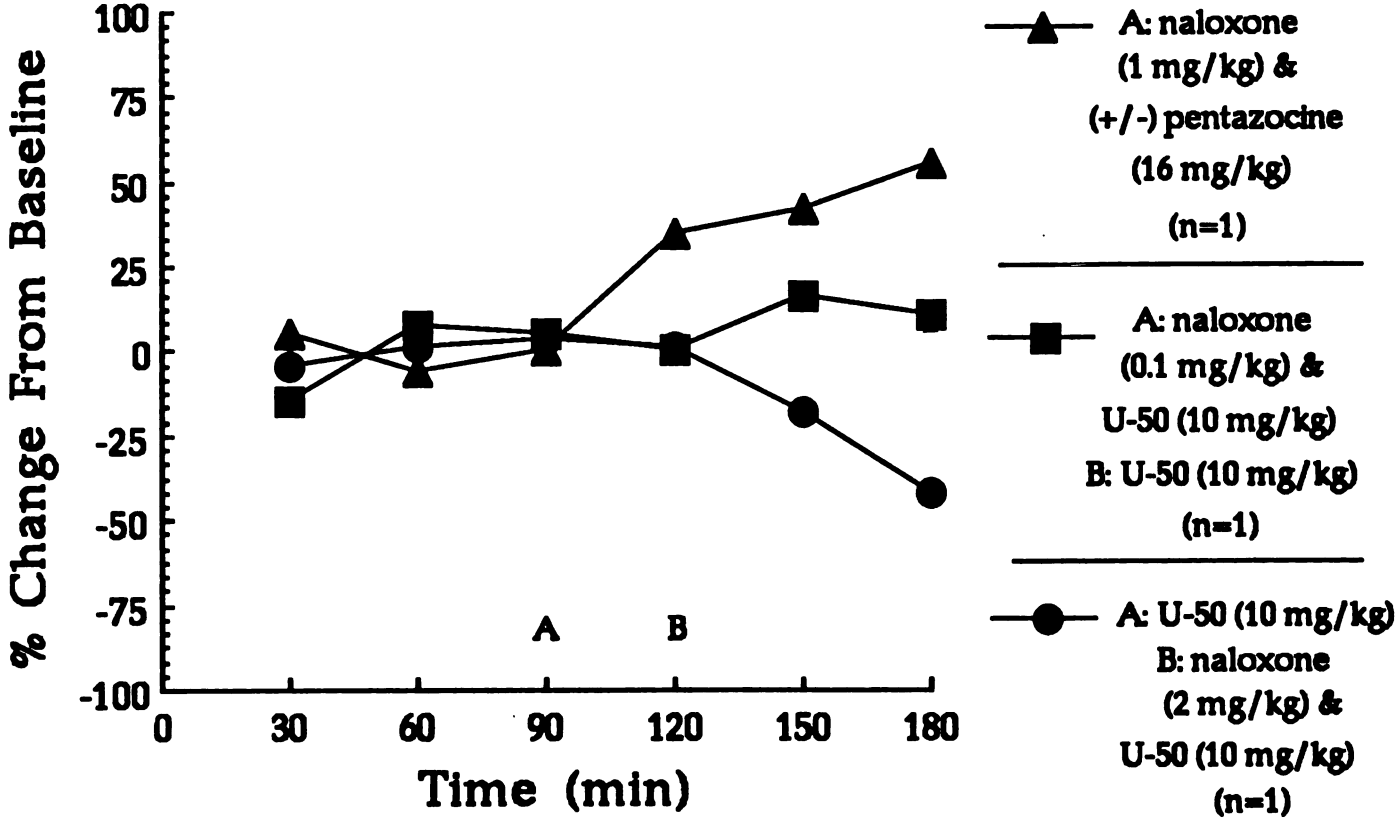


FIGURE 9C: Preliminary Studies Of Possible Opioid Blockade With Naloxone Of Kappa-Receptor Ligand Effects On CAP (N1) Response Magnitudes At Near-Threshold



pharmacologically with K-receptor-specific blockade at the cochlea.

EXPERIMENT 3

Combined Nor-BNI and (-)-Pentazocine (8mg/kg); Effects On CAP Responses: In the third experimental series, the objective was to determine whether the response amplification effects of (-)-pentazocine could be blocked by the potent and specific K-receptor antagonist nor-BNI. Control animals (n=6) were administered an artificial perilymph vehicle at 1μl, topically applied to the round window (RW). Experimental animals (n=7) were administered the artificial perilymph vehicle (1μl) containing 1-2mM of nor-BNI to the RW. Baseline response amplitudes remained stable throughout the 30 minutes following the topical application of these 36^{o} C solutions to the RW. Consistent with results obtained in earlier described experiments, 8mg/kg (-)-pentazocine injections produced positive, statistically significant postbaseline changes in CAP component amplitudes recorded at three of the five stimulus intensities tested, with greatest effects at the near-threshold intensities (i.e. overall N1= [$F(1,11)=4.80$; $p=.05$]. As illustrated below in Figure 10A and Table 7, average (-)-pentazocine effects on near-threshold N1 amplitudes in the control condition (i.e. perilymph on the RW; n=6), were seen as a 182% - 191% postbaseline change in the 120-150 minute epoch (i.e. 30-60 minutes after

FIGURE 10A: Relative CAP (N1) Response Magnitude Effects At Three Stimulus Levels Of 8 mg/kg (-)-Pentazocine With Or Without Prior Round Window Application Of The Specific Kappa-Receptor Antagonist Nor-BNI

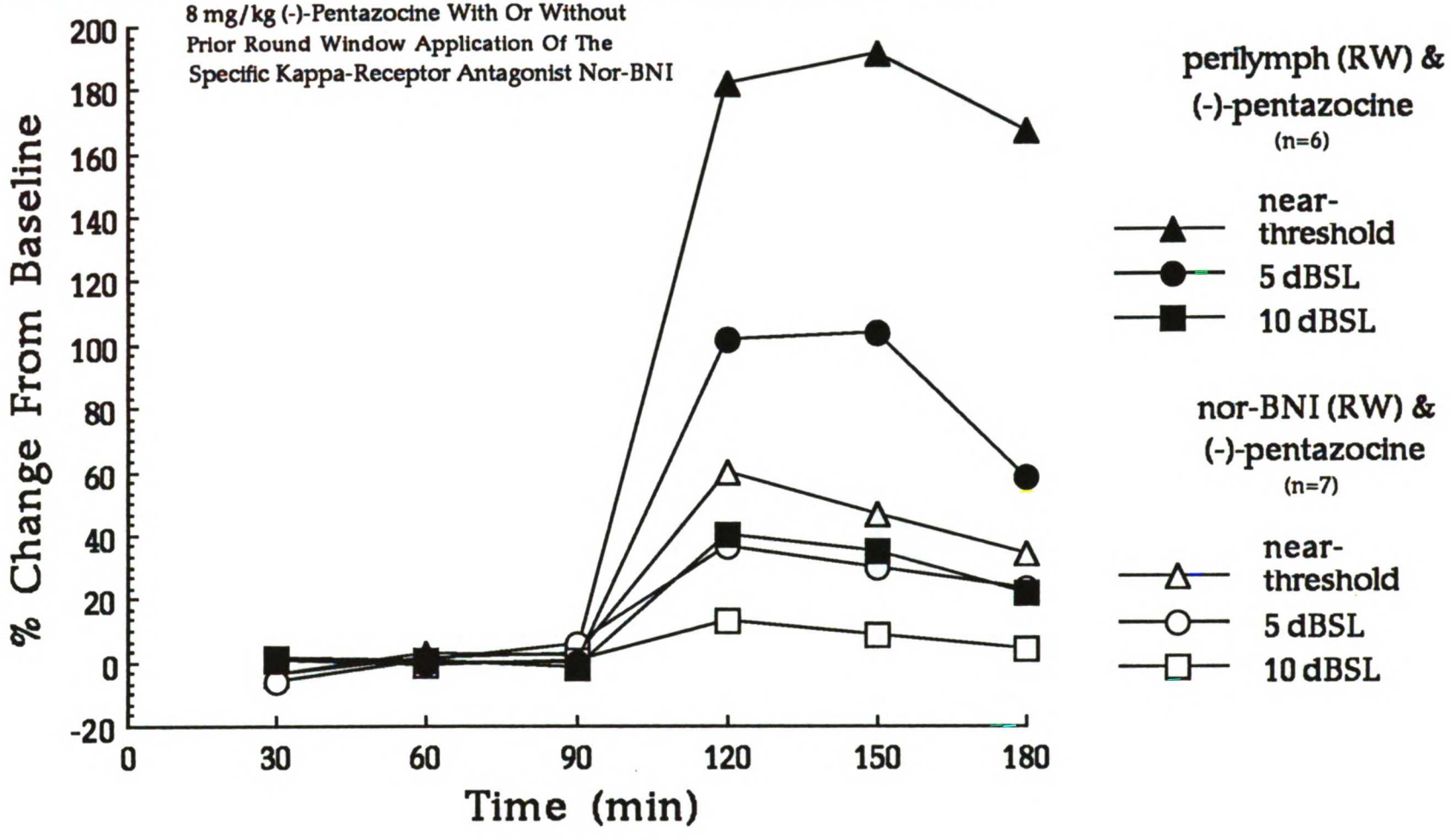


TABLE 7

Experiment 3

Data Matrix Of Mean CAP Amplitudes (In Microvolts) In Response To Near- Threshold
Stimulus Levels After Round Window Treatment With 1ul Perilymph Followed By
A Single (-)-Pentazocine (8 mg/kg; iv) Administration

N1 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline 3 (1 ul) RW PERI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
19 (5/13)	3.66	4.46	4.48	30.13	36.60	35.96
20 (5/17)	4.36	4.00	4.22	6.58	5.52	4.80
23 (6/27)	3.06	3.42	3.46	7.63	6.03	4.90
24 (6/07)	4.90	4.28	4.88	7.65	6.63	7.33
25 (6/08)	3.30	4.18	3.82	5.20	5.74	4.68
26 (6/09)	3.58	3.68	3.58	9.80	8.75	5.95
MEAN=	3.81	4.00	4.06	11.17	11.55	10.61
SD=	0.69	0.39	0.55	9.41	12.33	12.47
BASELINE GM= 3.96						
PLOT %=	-3.81%	0.96%	2.83%	181.63%	191.21%	167.54%
w/o # 19						
MEAN=	3.84	3.91	4.00	7.37	6.53	5.53
SD=	0.77	0.36	0.57	1.69	1.31	1.13
BASELINE GM= 3.92						
new PLOT %=	-1.87%	-0.14%	2.01%	88.19%	66.80%	41.22%

N2 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline 3 (1 ul) RW PERI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
19 (5/13)	1.68	2.28	2.48	11.05	14.88	13.90
20 (5/17)	3.80	2.88	3.92	4.43	4.26	4.72
23 (6/27)	0.96	1.60	1.43	1.88	1.97	1.57
24 (6/07)	2.94	3.02	2.80	3.55	3.93	4.10
25 (6/08)	3.20	3.08	3.20	3.27	4.18	3.72
26 (6/09)	1.26	1.22	1.18	1.48	1.73	1.72
MEAN=	2.31	2.25	2.45	4.28	5.16	4.96
SD=	1.16	0.93	1.04	3.49	4.89	4.57
BASELINE GM= 2.34						
PLOT %=	-1.21%	-3.78%	5.00%	83.15%	120.91%	112.21%
w/o # 19						
MEAN=	2.43	2.24	2.45	2.92	3.21	3.17
SD=	1.25	1.04	1.16	1.22	1.25	1.43
BASELINE GM= 2.37						
new PLOT %=	2.50%	-5.59%	3.09%	23.15%	35.46%	33.44%

injection). Average (-)-pentazocine effects on near-threshold N2 amplitudes in controls were 83% to 121% over responses recorded during the baseline period.

The positive change in N1 amplitudes near threshold corresponded to an overall shift from a baseline mean of 3.96μV to postbaseline means of 11 to 12μV (Table 7). Corresponding postbaseline positive changes in N2 amplitude recorded near-threshold, ranged from means of 4 to 5μV, from an overall baseline of 2.34μV (Table 7). In one animal included in the group average, mean N1 amplitude effects at time 120-150 minute epoch were observed as a 771% positive postbaseline change with near-threshold responses rising to 36.6μV from the baseline level of 4.2μV. Similar changes were recorded for the N2 component. From Table 7 it can be seen that data from this animal (#19) were not generally representative of the magnitudes of (-)-pentazocine effects observed in other pentazocine-treated chinchillas.

A downward postbaseline threshold shift (dB SPL) was documented in all of these (n=6) control chinchillas (Figure 6). It was greatest within 60 minutes of (-)-pentazocine (8mg/kg) administration. The mean change in these non-nor-BNI chinchillas (which included data from animal #19) was 7.47 ±4.75dB SPL. The threshold shift excluding data from chinchilla #19 (i.e. n=5), indicated a mean relative threshold improvement in this (-)-pentazocine-only group, of 5.56 ±0.97 dB SPL.

As illustrated in Figures 10A above and 10B (below), round window pre-pentazocine treatment in experimental animals with nor-BNI (1-2mM) followed 30 minutes later by (-)-pentazocine (8mg/kg) again, but not in all cases (see Figure 6 and Table 8) resulted in positive postbaseline amplitude changes of CAP potentials. These were observed in the N1 and N2 amplitudes recorded in response to near-threshold stimuli, and at +5dB and +10dB SL. However, the magnitudes of the amplitude changes from baseline were generally lower than those recorded following (-)-pentazocine administration in control chinchillas (Figures 10A, B, and Table 8). CAP (N1) amplitude changes recorded at near-threshold during postinjection epochs 0-30 and 30-60 minutes were 59.8% to 46.8% over baseline values (Figure 10A, Table 8). Postinjection N2 amplitudes were 27.5% to 47% over baseline values (Figure 8B, Table 8). Again, these changes compared to relatively stronger postbaseline amplitude effects (182% - 191% for N1; 83% to 121% for N2) seen at near-threshold in the (-)-pentazocine control chinchillas in this experimental series, suggesting that some blockade of (-)-pentazocine effects was achieved by prior round window administration of nor-BNI (see Figures 6, 10A and B).

As in the control chinchillas, the amount of postbaseline threshold shift (dB SPL) was greatest in the 30 to 60 minute epoch following (-)-pentazocine (8mg/kg) injection in these seven nor-BNI / (-)-pentazocine

FIGURE 10B: Relative CAP (N2) Response Magnitude Effects At Three Stimulus Levels Of 8 mg/kg (-)-Pentazocine With Or Without Prior Round Window Application Of The Specific Kappa-Receptor Antagonist Nor-BNI

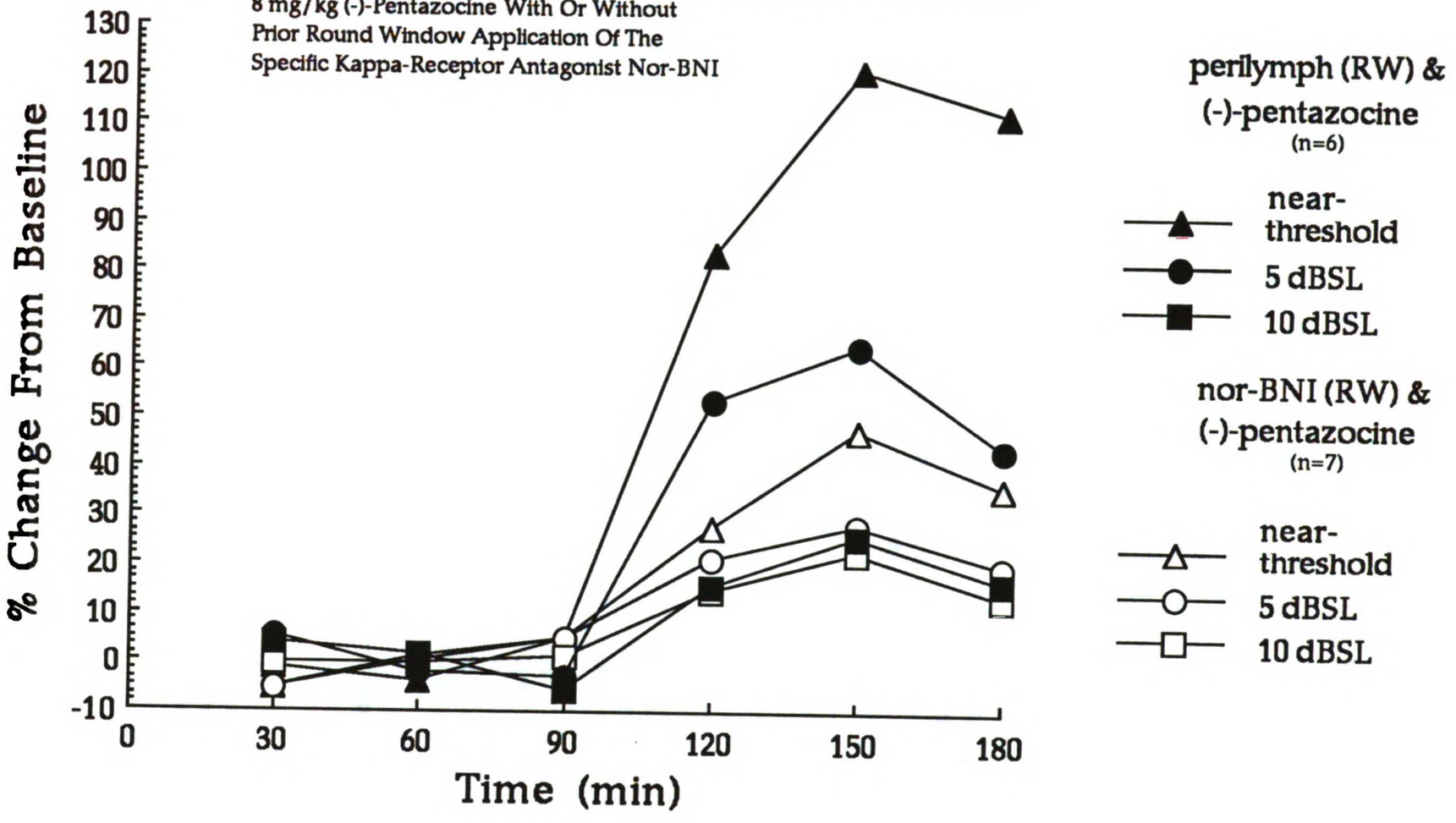


TABLE 8

Experiment 3

Data Matrix Of Mean CAP Amplitudes (In Microvolts) In Response To Near- Threshold
Stimulus Levels After Round Window Treatment With 1-2mM Nor-BNI (1ul) Followed By
A Single (-)-Pentazocine (8 mg/kg; iv) Administration

N1 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline 3 1-2mM@1ul RW BNI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
9 (4/2)	6.93	7.67	8.97	11.40	10.95	11.08
10 (4/4)	5.38	5.00	4.78	7.34	6.70	5.88
11 (4/9)	3.93	4.27	3.13	3.48	4.05	3.20
12 (4/10)	3.60	3.90	3.53	10.33	8.30	6.73
13 (4/12)	4.36	4.84	4.65	7.68	8.00	6.50
17 (5/15)	3.90	4.15	4.63	6.83	5.67	6.57
18 (5/16)	3.90	4.38	4.35	6.35	5.40	4.80
MEAN=	4.57	4.89	4.86	7.63	7.01	6.39
SD=	1.19	1.28	1.91	2.62	2.29	2.42

BASELINE GM= 4.77

PLOT %= -4.23% 2.38% 1.85% 59.85% 46.86% 33.93%

N2 Amplitudes

ANIMAL #	Baseline1	Baseline2	Baseline 3 1-2mM@1ul RW BNI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
9 (4/2)	2.62	3.08	3.00	3.80	3.90	3.58
10 (4/4)	2.58	1.82	1.53	2.58	2.26	1.83
11 (4/9)	2.38	2.92	3.30	2.70	6.00	4.73
12 (4/10)	2.40	2.66	2.55	5.10	5.00	4.23
13 (4/12)	2.26	2.60	3.25	3.43	3.87	4.03
17 (5/15)	1.50	1.35	1.80	1.80	1.67	2.57
18 (5/16)	2.85	3.30	2.98	3.00	3.17	2.87
MEAN=	2.37	2.53	2.63	3.20	3.70	3.41
SD=	0.43	0.70	0.71	1.05	1.50	1.03

BASELINE GM= 2.51

PLOT %= -5.61% 0.87% 4.74% 27.50% 47.18% 35.63%

(experimental) animals. The mean (±SD) shift in threshold following (-)-pentazocine (8mg/kg) in this group was 4.86 ±3.1, which compares to the greater 7.47 ±4.75dB SPL mean threshold change in the non-nor BNI chinchillas (Figure 6). It might be noted that application of the potent K-receptor antagonist nor-BNI on the RW probably contributed little by itself to the observed postbaseline alterations in N1 and N2 amplitudes. This is suggested by results from two additional control animals (Figures 11A and B below) that received nor-BNI, then Ringer's solution in place of (-)-pentazocine, postbaseline. Such evidence is again consistent with a partial blockade of (-)-pentazocine effects by nor-BNI.

Finally, baseline response latencies of the CAP remained stable at all stimulus intensities throughout the 30 minutes following the topical application of the 36^{o} C artificial perilymph solutions to the RW. Postbaseline latencies of CAP responses also remained relatively stable at all stimulus intensities in both groups, following the administration of (-)-pentazocine. To illustrate this stability, near-threshold N1 and N2 response latencies for the six control and seven experimental animals are presented below in Tables 9 and 10, respectively.

Statistical Analyses: No significant between-treatment differences were observed in CAP response amplitudes at any applied stimulus intensity, indicating a statistically nonsignificant blockade of (-)-pentazocine-induced amplitude effects by round window-application of

FIGURE 11A: Relative CAP (N1) Response Magnitude Effects At Three Stimulus Levels Of A Round Window Application Of The Specific Kappa-Receptor Antagonist Nor-BNI With Or Without Postbaseline (-)-Pentazocine Administration

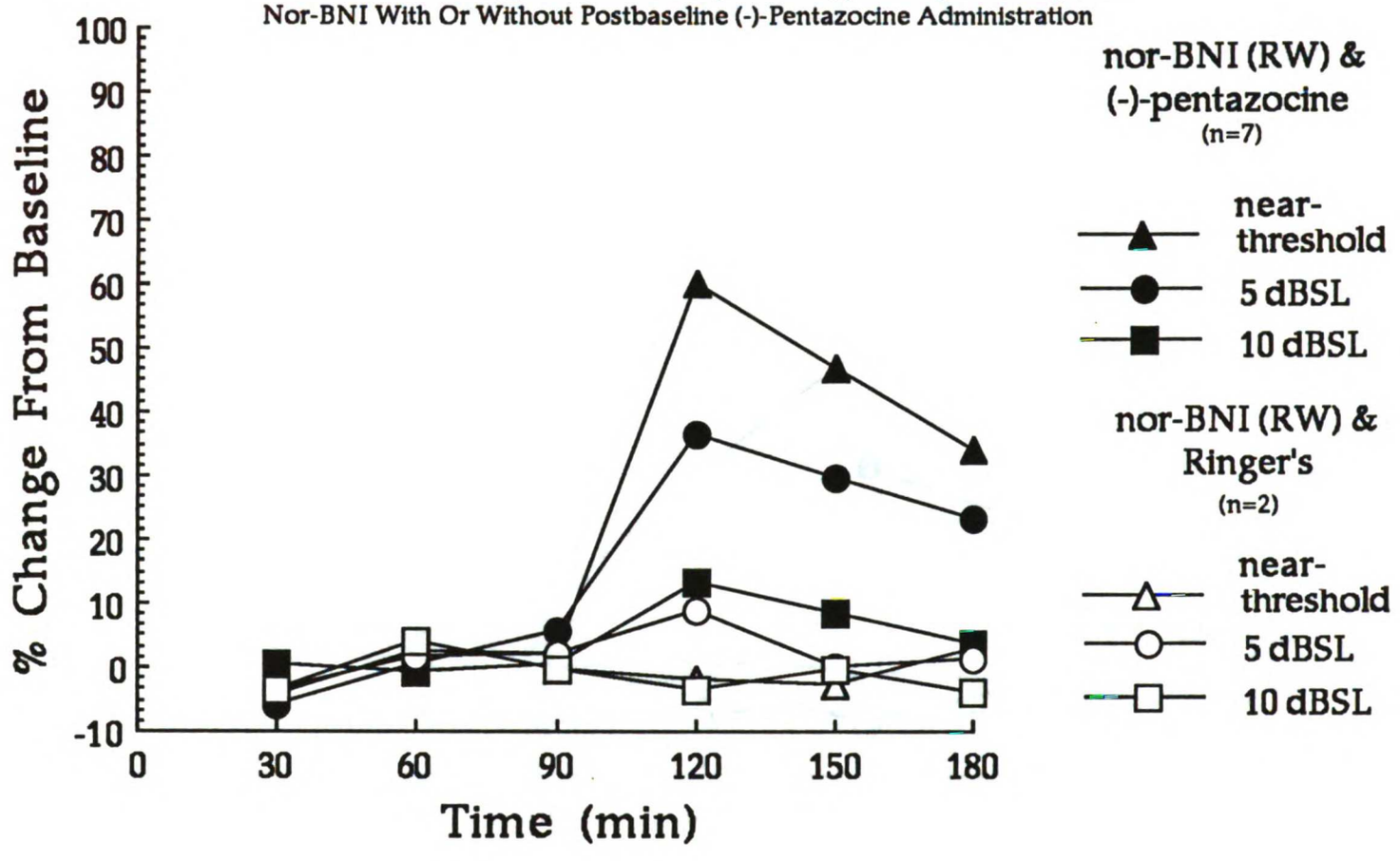


FIGURE 11B:

Relative CAP (N2) Response Magnitude Effects At Three Stimulus Levels Of A Round Window Application Of The Specific Kappa-Receptor Antagonist Nor-BNI With Or Without Postbaseline (-)-Pentazocine Administration

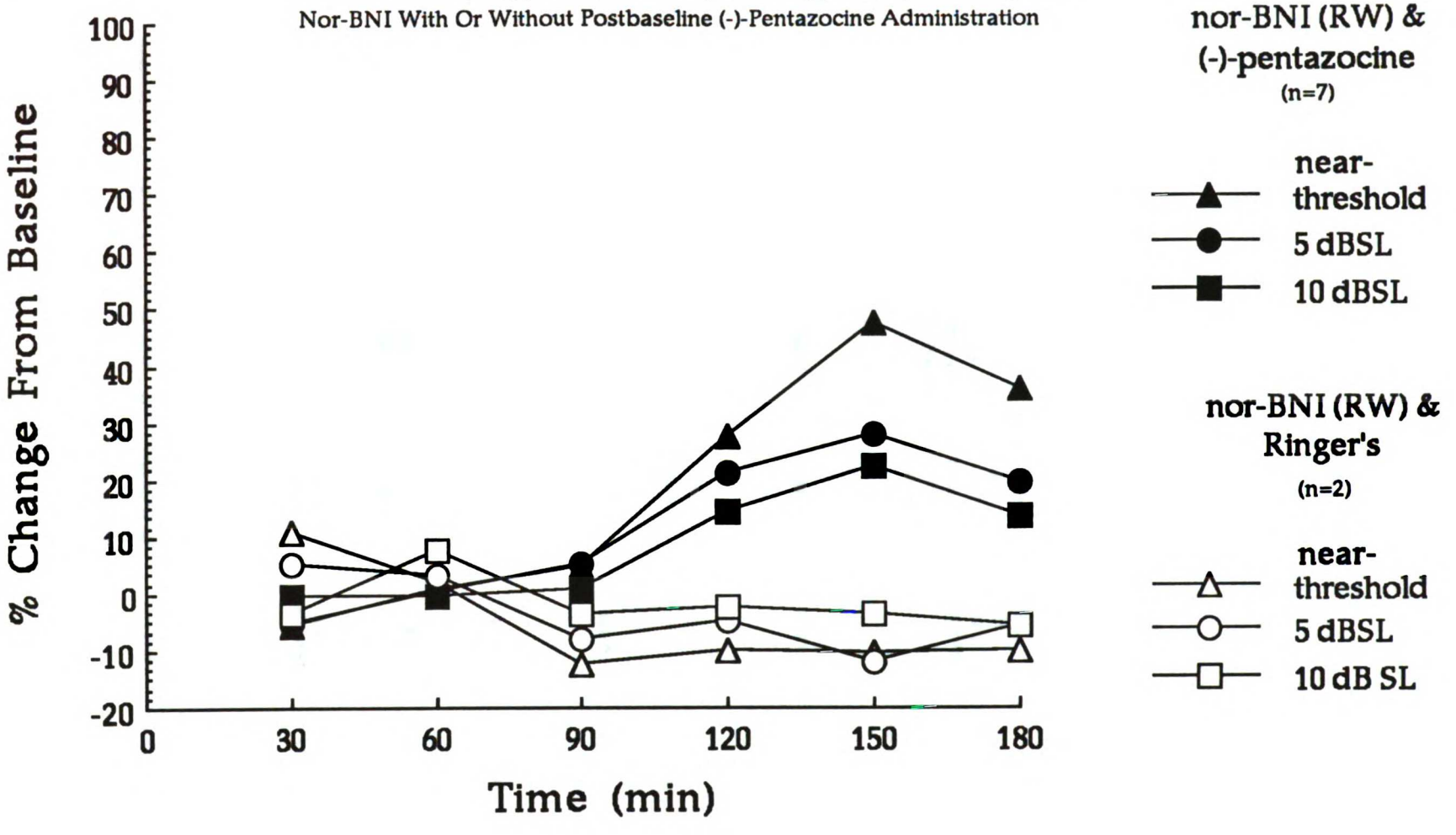


TABLE 9

Experiment 3

Data Matrix Of Mean CAP Latencies (In Millivolts) In Response To Near- Threshold
Stimulus Levels After Round Window Treatment With 1ul Perilymph Followed By
A Single (-)-Pentazocine (8 mg/kg; iv) Administration

N1 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 (1 ul) RW PERI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
19 (5/13)	3.30	3.26	3.26	3.12	3.13	3.13
20 (5/17)	3.23	3.19	3.17	3.11	3.21	3.18
23 (6/27)	3.08	3.12	3.08	3.08	3.08	3.11
24 (8/07)	2.90	2.92	2.95	2.89	2.90	2.91
25 (8/08)	3.25	3.22	3.25	3.33	3.26	3.24
26 (8/09)	3.01	3.02	2.99	3.01	2.94	2.96
MEAN=	3.13	3.12	3.12	3.09	3.09	3.09
SD=	0.16	0.13	0.13	0.15	0.14	0.13
BASELINE GM= 3.12 SD= 0.13						
% Change=	0.20%	-0.02%	-0.18%	-1.03%	-1.14%	-1.09%
w/o # 19						
MEAN=	3.09	3.09	3.09	3.08	3.08	3.08
SD=	0.15	0.12	0.12	0.16	0.16	0.14
BASELINE GM= 3.09 SD= 0.12						
% Change=	0.06%	0.06%	-0.13%	-0.26%	-0.45%	-0.39%

N2 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline 3 (1 ul) RW PERI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
19 (5/13)	4.46	4.42	4.30	4.25	4.16	4.17
20 (5/17)	4.28	4.33	4.37	4.35	4.24	4.29
23 (6/27)	4.16	4.11	4.10	4.11	4.13	4.11
24 (8/07)	4.02	3.96	4.00	4.00	4.01	4.07
25 (8/08)	4.42	4.51	4.50	4.56	4.46	4.48
26 (8/09)	4.23	4.20	4.30	4.25	4.19	4.22
MEAN=	4.26	4.26	4.26	4.25	4.20	4.22
SD=	0.16	0.20	0.18	0.19	0.15	0.15
BASELINE GM= 4.26 SD=0.17						
% Change=	0.03%	-0.05%	0.03%	-0.17%	-1.46%	-0.87%
w/o # 19						
MEAN=	4.22	4.23	4.25	4.25	4.21	4.23
BASELINE GM= 4.23 SD=0.17						
% Change=	-0.28%	-0.19%	0.47%	0.47%	-0.66%	0.00%

TABLE 10

Experiment 3

Data Matrix Of Mean CAP Latencies (in Millivolts) In Response To Near- Threshold
Stimulus Levels After Round Window Treatment With 1-2mM Nor-BNI (1ul) Followed By
A Single (-)-Pentazocine (8 mg/kg; iv) Administration

N1 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 1-2mM@1ul RW BNI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
9 (4/2)	3.02	3.01	3.02	3.05	3.04	3.02
10 (4/4)	3.02	3.06	3.01	3.11	3.06	3.09
11 (4/9)	3.31	3.36	3.36	3.36	3.34	3.35
12 (4/10)	3.17	3.20	3.19	3.17	3.19	3.19
13 (4/12)	3.05	3.04	3.06	3.04	3.03	3.04
17 (5/15)	3.11	3.10	3.09	3.11	3.07	3.09
18 (5/16)	3.13	3.14	3.12	3.14	3.10	3.16
MEAN=	3.12	3.13	3.12	3.14	3.12	3.13
SD=	0.10	0.12	0.12	0.11	0.11	0.11
BASELINE GM= 3.12						
% Change=	-0.21%	0.24%	-0.03%	0.56%	-0.12%	0.38%

N2 Latencies

ANIMAL #	Baseline1	Baseline2	Baseline3 1-2mM@1ul RW BNI	Postbaseline PNTZ obs 1	Postbaseline PNTZ obs 2	Postbaseline PNTZ obs 3
9 (4/2)	4.03	4.02	4.01	4.06	4.04	4.01
10 (4/4)	4.20	4.20	4.28	4.35	4.30	4.27
11 (4/9)	4.43	4.36	4.25	4.45	4.43	4.42
12 (4/10)	4.26	4.35	4.37	4.33	4.34	4.37
13 (4/12)	4.18	4.16	4.17	4.14	4.11	4.17
17 (5/15)	4.19	4.32	4.19	4.28	4.17	4.19
18 (5/16)	4.12	4.08	4.09	4.11	4.07	4.12
MEAN=	4.20	4.21	4.19	4.25	4.21	4.22
SD=	0.12	0.14	0.12	0.14	0.15	0.14
BASELINE GM= 4.20						
% Change=	-0.03%	0.24%	-0.20%	1.02%	0.14%	0.44%

nor-BNI. Similarly, no statistically significant between-group differences were observed postbaseline as a function of stimulus intensity for either the N1 [F(1,11)=1.46; p(ns)] or N2 amplitudes [F(1,11)=1.65; p(ns)]. Such evidence tends to further support a possible partial, but statistically non-significant blockade of (-)-pentazocine effects by nor-BNI in this limited number of animals.

t-tests: The possible blockade afforded by RW administration of nor-BNI was further evaluated by additional t-tests. Summary percent-change-from-baseline mean values obtained for each control (perilymph on RW; n=6) and experimental animal (nor-BNI on RW; n=7) over the period 0-30 and 30-60 minutes after (-)-pentazocine injection, were used. The t-test comparisons between the experimental and control groups were therefore performed on the average magnitudes of postbaseline amplitude changes determined animal by animal, following (-)-pentazocine.

Differences in postbaseline amplitude changes of N1 between the two groups observed in the first 0-30 minutes after (-)-pentazocine injection were not statistically significant using an alpha level of 0.05, at near-threshold stimulus intensities [t(11)=1.36; p=0.1], at +5dB SL [t(11)=1.64; p=0.06], or at +10dB SL [t(11)=1.45; p=0.08]. Similarly, postbaseline N1 amplitude changes between the two groups, observed 30-60 minutes after (-)-pentazocine were again not statistically significant at near-threshold [t(11)=1.26; p=0.11], at +5dB SL [t(11)=1.49; p=0.08], or at

+10dB SL [t(11)=1.45; p=0.08]. Differences in postbaseline N2 amplitude changes between the two groups were also not statistically significant. Taken collectively, these probability values indicate a trend for the nor-BNI versus (-)-pentazocine-treated experimental animals that suggests, but does not unequivocally demonstrate a partial block of the (-)-pentazocine amplification of CAPs by this specific K-receptor antagonist.

There were no obvious baseline or postbaseline changes in the cochlear microphonic amplitudes at any stimulus intensity following the round window or iv administration of K-receptor ligands given in any combination, in these experiments (Figures 12A and B below). Figure 12A contrasts postbaseline neural (N1) and microphonic effects of (-)-pentazocine recorded simultaneously at three stimulus intensities from the same control animals (n=6). As in Experiment 2, there was no evident (-)-pentazocine effect on CM amplitudes. Figure 12A also includes CAP and CM data from animal #19. Even the very large amplitude changes observed in the CAPs in this animal were not paralleled by changes in the cochlear microphonic. Such data again indicates that postbaseline amplitude effects of (-)-pentazocine are not due to factors that might simply alter the overall levels of stimuli reaching the cochlear hair cells, or their transduced responses to such stimuli.

FIGURE 12A: Relative Changes In CAP (N1) And CM Response Magnitudes At Three Stimulus Levels Before And After (-)-Pentazocine Administration In The Same Animals

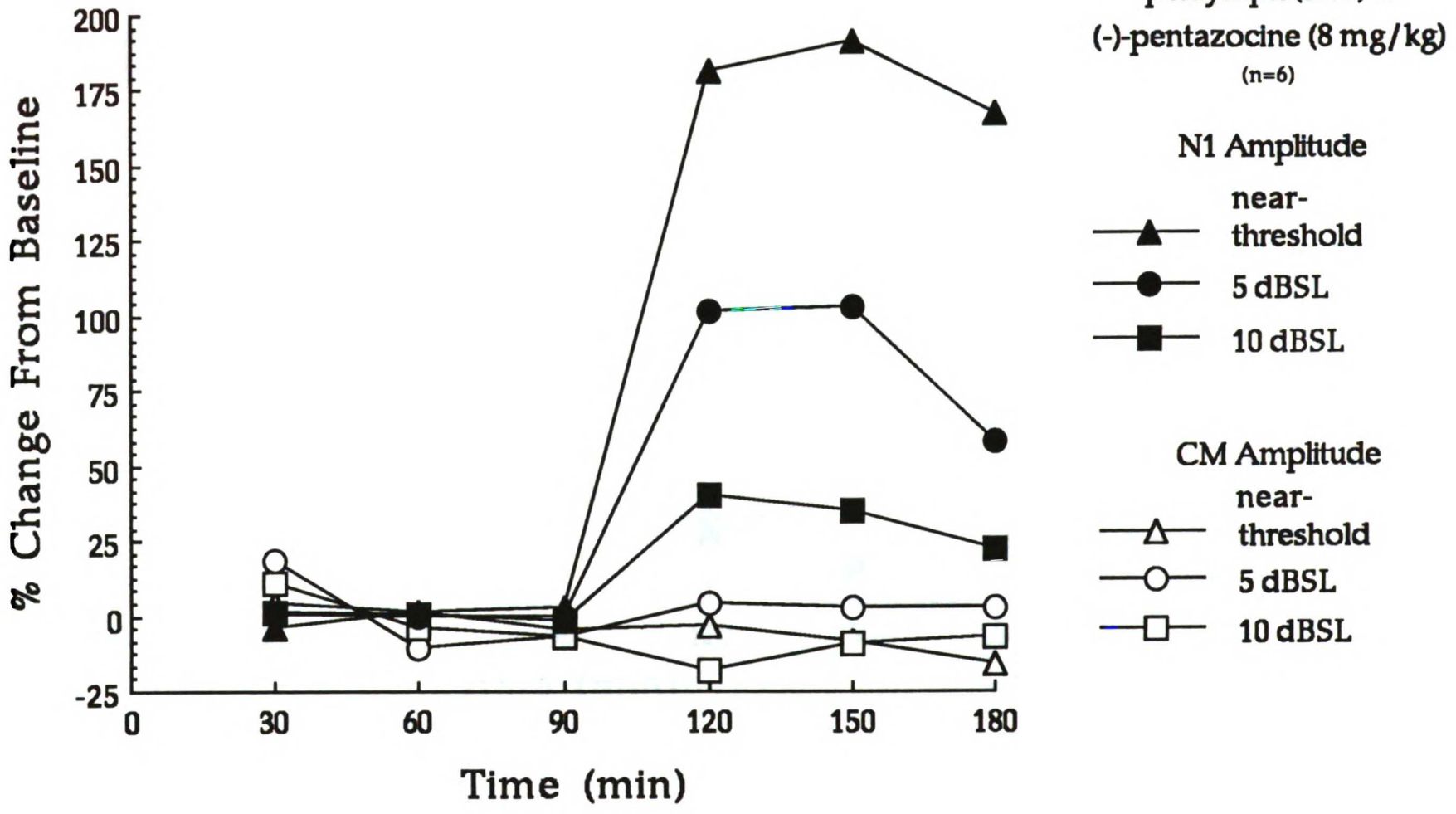
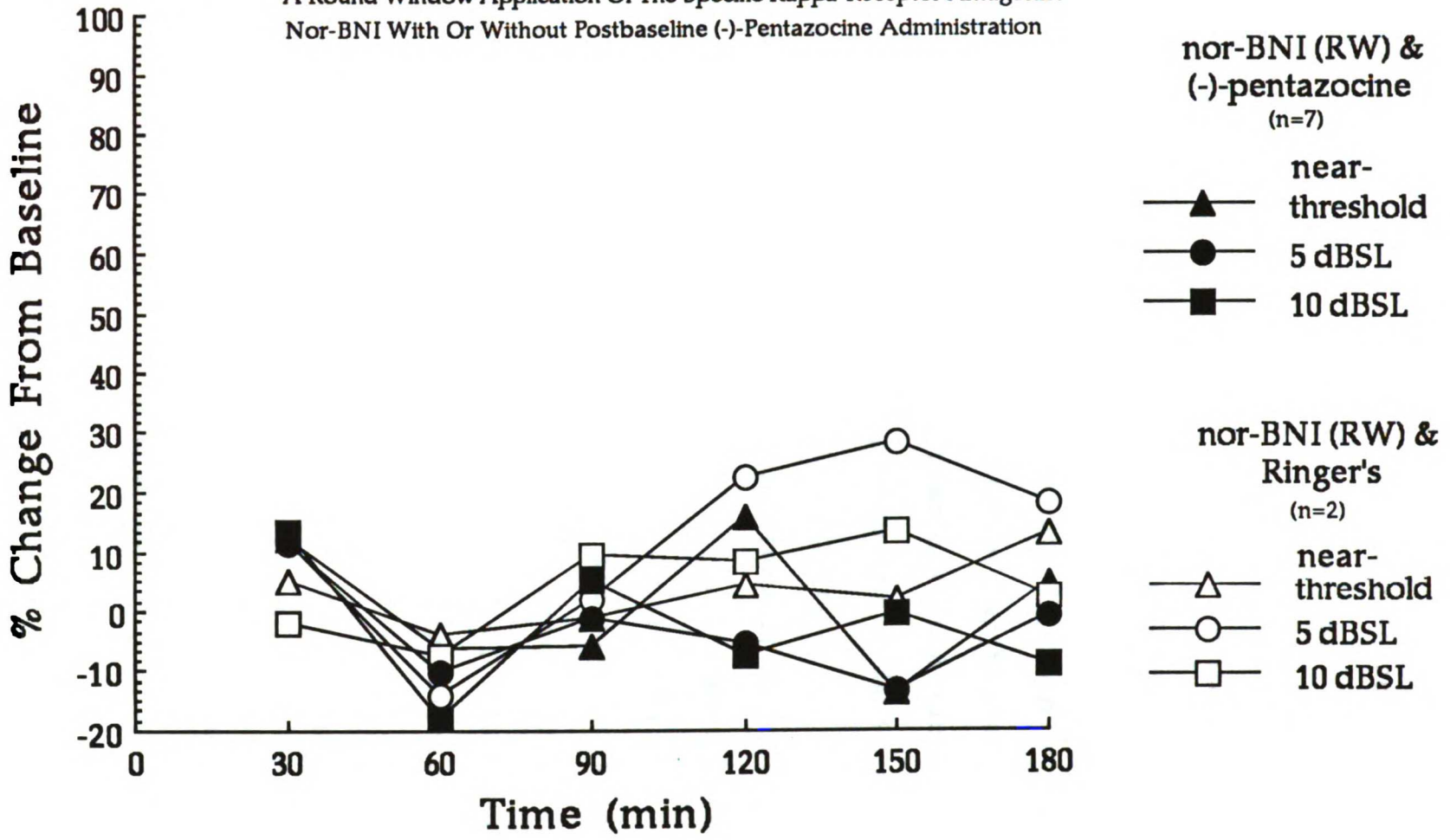


FIGURE 12B: Relative CM Response Magnitude Effects At Three Stimulus Levels Of A Round Window Application Of The Specific Kappa-Receptor Antagonist Nor-BNI With Or Without Postbaseline (-)-Pentazocine Administration



DISCUSSION

Little is presently known about the synaptic relationships between the neuroactive substances co-contained within the descending lateral efferent olivocochlear fibers, just as little is known about the operating characteristics and functional role of the lateral efferent neurons. It is well established that ACh, and derivatives of proenkephalin and prodynorphin coexist within lateral efferent neurons (e.g. Abou-Madi et al., 1987; Altschuler and Fex, 1986; Altschuler et al., 1984a; 1988; Fex and Altschuler, 1985; 1986). Endogenous neuroactive opioid ligands in lateral efferent neurons may function presynaptically by modulating the release of ACh, or they may cross the synapse and bind d- and/or K-opioid receptors on Type I auditory ganglion cell dendrites. Either a pre- or postsynaptic mechanism would likely result in complex changes in the overall firing rates of individual Type I auditory fibers. These changes would be expected to be manifested by changes in the magnitudes of the first and second negative peaks (N1 and N2) of the auditory nerve compound action potential (CAP), and in the magnitudes of the auditory brain stem response components.

Products of prodynorphin biosynthesis are found within the cochlea, and these products are known to bind K-receptors (Chavkin et al., 1982; Corbett et al., 1982; James et al., 1982). Results of all the experimental series

presented herein have demonstrated positive K-opioid receptor-mediated changes in waves N1 and N2 of the CAP, concomitant with changes in threshold sensitivity. These results cannot be attributed to actions at the μ-opioid or non-opioid haloperidol sensitive σ-receptor. Furthermore, such effects appear to be inversely dependent upon the intensity of the stimulus, and cannot be attributed to changes in middle ear mechanisms, temperature, electrode impedance, or the medial efferent system. Taken together with the probable partial (though statistically non-significant) blockade of these effects in the cochlea, these results suggest a K-opioid receptor mediation of auditory neural activity at the lateral efferent synapse.

Opioid Mediated Auditory Neural Disinhibition

In the present set of experiments, both the K/σ-receptor agonist (±)-pentazocine (16mg/kg) and the K-receptor agonist (-)-pentazocine (8mg/kg) produced significant and positive postbaseline amplitude changes in the N1 and N2 potentials of the CAP. Threshold sensitivity was improved an average of 5 to 7dB SPL. Since the amplitude of the CAP reflects overall (whole nerve) Type I activity and synchronization in response to auditory stimuli (e.g. Dallos, 1973; Davis, 1976; Glattke, 1983; Moller, 1983a; Salvi et al., 1983), the observed pentazocine effects would appear to be neural in origin.

Variance Due To Electrode Position: There is little possibility that the observed amplitude effects on near-

field recorded potentials were due to slight alterations in the position of the round window electrodes, as similar amplitude effects on far-field-recorded waves (I-IV) were observed following (±)-pentazocine administration in Experiment 1. Recall that an independent set of electrodes was employed to record these far-field potentials. Furthermore, the impedances were monitored and the positions of the electrodes were maintained at the round window before, during and after every recording session via the stereo dissecting microscope, which remained in a fixed position over the surgical field. Furthermore, electrode application in experimental and control groups was identical.

Variance Due To Changes In Stimulus Intensity: It is also unlikely that the observed pentazocine effects originated from changes within the organ of Corti (basilar membrane), or from changes effecting the overall sound pressure level (SPL) of the stimulus reaching the inner ear. Indeed, both CAP peak latencies and microphonic (CM) amplitudes were unaffected by pentazocine administration. As indicated earlier, the travel time of the peak response on the basilar membrane (from base to apex) contributes to the total latency of N1 and N2 components of the CAP (Eggermont, 1983; Glattke, 1983; Jacobson, 1985; Moller, 1985; Salvi et al., 1983), and the latencies of CAP peaks in the normal ear are a function of stimulus intensity (e.g. Dallos, 1973; Davis, 1976; Glasscock et al., 1981; Jacobson, 1985; Moller, 1985;

Moore, 1983; Sohmer, 1989; Stockard and Stockard, 1983). Furthermore, CAP latencies were unaffected by (-)-pentazocine, and as reviewed earlier, the CM amplitude is proportional to the transduction current passing through the individual outer (and inner) hair cells and would be directly effected by sound intensity changes (Corey and Hudspeth, 1979a; 1983a; Dallos, 1973; Honrubia and Ward, 1969; 1970; Hudspeth, 1982; Patuzzi and Thompson, 1991; Patuzzi et al., 1989; Sohmer, 1989; Yates, et al., 1989). Indeed, the CM was consistently stable in (-)-pentazocine-treated animals, concomitant with substantial CAP response amplification.

Variance Due To Middle Ear Mechanisms: It is also improbable that the observed pentazocine-induced positive changes in CAP amplitude reflected a relaxation in the tonic activity of the middle ear system. This is unlikely since ketamine/pentobarbital anesthetised animals generally exhibit an absence of tonic EMG activity within both the stapedius and tensor tympani muscles (Guinan and McCue, 1987; McCue and Guinan, 1988). In addition, the intact middle ear system has no influence on auditory responses at or near threshold intensities (Guinan and McCue, 1987). Furthermore, reductions in middle ear impedance would have been reflected as positive changes in CM amplitudes (Gerhardt et al., 1979), and by intensity-dependent changes in the latencies of the CAP peaks.

The possible assertion that the observed pentazocine-induced changes in the CAP reflected dynamic changes in the middle ear mechanism must also be ruled out, since: (a) the EMG thresholds for both muscles in ketamine/pentobarbital anesthetised animals are reported to be 90dB SPL for 1kHz tone bursts, and even higher for frequencies up to 5kHz (Guinan and McCue, 1987; McCue and Guinan, 1988); (b) transient broadband stimuli such as clicks (having durations <200msec) fail to elicit a middle ear reflex, independent of stimulus intensity (Djupesland, 1980); and (c) the acoustic reflex thresholds obtained in three animals in Experiment 1 were 82 to 90dB SPL at the frequencies 500 Hz and 1kHz, respectively. While generally consistent with reflex measures obtained in a previous study in this species (Gerhardt, Melnick and Ferraro, 1979), these values are very far above the levels at which maximum pentazocine effects were observed in these studies.

Variance Due To Alterations In Temperature: The potential effects of hyper- or hypothermia were also not a contributing factor to the results obtained in the present set of investigations. Indeed, the effects of both on the latencies and amplitudes of the far-field recorded ABR are well documented. Fluctuations in core temperature typically lead to a 2% to 5.3% progressive and cumulative prolongation in wave I-IV latency, such that earlier waves are much less effected than later waves. As a general rule, fluctuations in core temperature fail to produce consistent or reliable

amplitude changes in ABR waves (Kileny and McIntyre, 1985; Markland, Lee, Warren, Stoelting, King, Brown and Mahomed, 1987; Marsh, Yamane and Potsic, 1984; Sohmer, 1989; Sohmer, Gold, Cahani and Attias, 1989; Stockard, Sharbrough and Tinker, 1978; Williston and Jewett, 1982), or in the amplitudes of the near-field recorded CAP waves (Inamura, Kusakari and Takasaka, 1987). In one report, N1 latencies increased by about 0.04ms for every 1^{o} C drop in intracranial temperature (from 35 to 30^{o}C) in pentobarbital anesthetised guinea pigs (Inamura et al., 1987). In the present investigation, no latency changes were discerned at any intensity that could be attributed to the reasonable 0.5^{o} C core temperature variability, nor were there any obvious latency changes during baseline following the round window application of a 1μl solution ($\approx 36^{o}$ C) in Experiment 3.

Variance Due To The Medial Efferents: Finally, it is unlikely that the observed CAP amplitude effects of pentazocine reflected changes in the tonic activity of medial efferent (basal or circumnuclear) fibers. Indeed, as discussed earlier: (a) the medial efferent system plays no tonic role in determining auditory threshold sensitivity (Borg, 1971; Capps and Ades, 1968; Carlier and Pujol, 1982; Cody and Johnstone, 1982; Dewson, 1968; Handrock and Zeisberg, 1982; Igarashi, Alford, Nakai and Gordon, 1972; Igarashi, Cranford, Allen and Alford, 1979a; Igarashi, Cranford, Nakai and Alford, 1979b; Igarashi, Mauldin and

Jerger, 1979c; Liberman, 1988a; Rajan et al., 1990; Trahiotis and Elliott, 1970); (b) most medial efferent fibers (86 to 89%) lack spontaneous activity (Liberman and Brown, 1986; Robertson & Gummer, 1985); (c) many monaural responding medial efferent fibers exhibit very high (95dB SPL) thresholds (Cody and Johnstone, 1982a; Fex, 1962; 1965; Liberman, 1988b; Liberman and Brown, 1985; 1986; Robertson & Gummer, 1985); and (d) medial efferent fibers are not activated by short-duration stimuli like 100μsec clicks, independent of their stimulus intensity. Indeed, medial efferent discharge rates decline sharply if tone burst duration falls much below 25msec (Liberman and Brown, 1986). Therefore, the results obtained herein suggest an opioid-receptor mediated interaction at the lateral efferent synapse.

Likelihood Of Cochlear Penetration: Pentazocine has been reported to be highly lipophilic relative to other benzomorphans (Walker et al., 1990), is well absorbed from parenteral sites, and is rapidly metabolized (Brogden et al., 1973; Jaffe and Martin, 1990; Payne, 1973). Substances exhibiting relatively lower molecular weights (i.e. <1000 Daltons), such as pentazocine (285.44 Daltons), as well as each of the other substances investigated, will pass more readily across the blood-labyrinthine barrier than those exhibiting higher molecular weights (Juhn and Rybak, 1981; Juhn, Rybak and Prado, 1981; Salt and Konishi, 1986).

Peak amplitude changes following pentazocine were always observed within 60 minutes after administration. This time course is relatively consistent with the peak antinociceptive effects reported in humans, which occur within 2 to 15 minutes after iv, and 15 to 60 minutes after im or sc administration (Angel, 1983; Brogden et al., 1973; Jaffe and Martin, 1990; Payne, 1973). Indeed, in each of the three experiments, the effective pentazocine doses were administered at or above the effective parenteral antinociceptive doses previously reported in rodents (2mg/kg to 10mg/kg sc; Harkness and Wagner, 1983), and canines (5mg/kg iv; Vaupel et al., 1989), and were above the antinociceptive dose (30-60mg/70kg or 0.43 to 0.86mg/kg) previously reported in humans (Angel, 1983; Brogden et al., 1973; Bromm et al., 1987). Additional studies are required however, to more carefully document the time course of effects of both iv or intracochlear administered pentazocine under various stimulus parameters.

Opioid-Receptor Specificity Of Effects: The overall positive amplitude changes seen following the administration of racemic and levorotatory isomers of pentazocine and the general lack of effects of fentanyl and naloxone on near-field amplitudes and latencies suggest a K-, rather than a μ-opioid receptor-mediated effect on auditory potentials. As reviewed earlier, fentanyl exhibits a 67X greater affinity for μ-over K-receptors (Magnan et al., 1982; Paterson et al., 1983), is highly lipophilic (Bovill, 1987; Magnan et

al., 1982), and therefore enters the CNS with an affinity 156X greater than morphine (Bovill, 1987). The fentanyl dose (1mg/kg X2) employed in Experiment 1 was well above the effective antinociceptive doses previously reported in rodents (0.02 to 0.17 mg/kg sc; Millan, 1989; Shaw et al., 1988; Upton et al., 1982), rabbits (0.02mg/kg; Herz et al., 1970), and in humans (0.1 mg/70kg, or 1.43μg/kg; Jaffe and Martin, 1990; Marshall and Longnecker, 1990).

Recall that naloxone is about 10X and 30X less active at d-and K-receptors, respectively, compared to its μ-receptor affinity (e.g. Akil et al., 1984; Barnard and Demoliou-Mason, 1983; Chang, 1984; Chang and Cuatrecasas, 1979; Kosterlitz, 1985; Lahti et al., 1985; Paterson et al., 1983; 1984; Robson et al., 1983; Tam, 1985; Tiberi and Magnan, 1990). The cumulative iv dose (1.1mg/kg) employed in Experiment 1 was well above the effective parenteral dose (0.05 to 0.30mg/kg) required to antagonize the antinociceptive μ-receptor effects of morphine previously reported in animal studies (e.g. Duggan and North, 1984; Martin, 1984). Indeed, even lower iv doses (0.4 to 0.8mg or 5.7 to 11.4 μg/kg) of naloxone can reverse the effects of most μ-opioid agonists in humans (Bovill, 1987; Jaffe and Martin, 1990).

The overall absence of μ-mediated amplitude effects on auditory potentials corroborates previous research which also failed to identify μ-mediated alterations (i.e. using morphine, fentanyl and naloxone) in the latencies and

amplitudes of far-field (ABR) recorded potentials (Samra, Krutak-Krol, Pohorecki and Domino, 1985; Samra, Lilly, Rush and Kirsh, 1984; Velasco, Velasco, Castenada and Sanchez, 1984). However, in none of those investigations were μ-opioid effects systematically examined at different stimulus intensities. Suprathreshold intensity levels of 65dB SPL or greater were typically employed. Furthermore, the previous investigations cited (i.e. Samra et al., 1984; 1985; Velasco et al., 1984) used lower doses of fentanyl and naloxone than those used in the present study.

That the observed amplitude changes reflect actions at a K-opioid receptor is further suggested by the relatively greater K- over μ-receptor selectivity of pentazocine. Indeed, the antinociceptive K-receptor properties of pentazocine reside within its (-)-isomer (e.g. Brogden et al., 1973). Pentazocine exhibits the lowest affinity for the morphine (μ) receptor of all the known benzomorphans (e.g. Lahti et al., 1985), is 522X less effective than naloxone in precipitating morphine withdrawal in dogs (Martin, 1984), and is virtually devoid of δ-receptor activity (Lahti et al., 1985; Tam, 1985). Pentazocine only appears to interact with 'μ' at subcutaneous doses (in rats) of 32mg/kg (Brogden et al., 1973; Holtzman and Jewett 1972). That dose is about 4X the effective dose used in the present experiments. Therefore, although it is classified as a benzomorphan with mixed agonist/antagonist properties, the opioid properties of pentazocine reflect activity primarily at the K-receptor.

As reviewed earlier, (±)-pentazocine exhibits about an equal affinity for K-opioid and non-opioid σ-receptors (Walker et al., 1990). Therefore, in the present set of experiments, the strongest support for a K-receptor-mediated effect by (±)- and (-)-pentazocine is given by the failure of the dextrorotatory isomer (+)-pentazocine to produce similar postbaseline amplitude changes (Experiment 2). Recall that (-)-pentazocine fails to bind σ-receptors (e.g. Bowen et al., 1990a; de Costa et al., 1989; Largent et al., 1987), and (+)-pentazocine is devoid of opioid receptor-binding activity (e.g. Lahti et al., 1985; Tam, 1985; Tiberi and Magnan, 1990; Walker et al., 1990). Indeed, (+)-pentazocine is both a potent and selective ligand at the σ-receptor (Tam, 1985; Tam and Cook, 1984; Walker et al., 1990). As reviewed earlier, the σ-receptor is not an opioid (e.g. Bowen et al., 1990a; de Costa et al., 1989; Largent et al., 1987; Su, 1981; Tam, 1983; 1985; Tam and Cook, 1984), nor a dopamine (Bowen et al., 1990a; 1990b; de Costa et al., 1989; Gundlach et al., 1985; 1986; Largent et al., 1986a; 1986b; 1987; Matsumoto et al., 1990; Walker et al., 1990), nor a PCP-receptor (e.g. Quirion et al., 1987; Walker et al., 1990). Instead, the σ-receptor is often referred to as a 'naloxone- or etorphine-inaccessible'-'haloperidol-sensitive' receptor (e.g. Su, 1981; Tam, 1983, 1985; Walker et al., 1990).

Likelihood Of PCP Receptor Involvement: It is also important to consider the possibility of additional non-

opioid receptor involvement in these observed response amplification effects. Recall that the potent K/σ-receptor ligand (±)-pentazocine (Gundlach et al., 1985; Largent et al., 1986a; Zukin and Zukin, 1988) and the highly selective and potent σ-receptor ligand (+)-pentazocine (which produced no effects) are both devoid of phencyclidine (PCP) receptor activity (Rothman et al., 1988; Steinfels et al., 1988; Tam and Zhang, 1988; Walker et al., 1990). However, the dissociative anesthetic, ketamine which was employed for preanesthesia in these investigations, is related to PCP.

PCP-sensitive receptor sites are associated, though not exclusively (Jaffe, 1990; Quirion et al., 1987), with one of the most extensively characterized excitatory amino acid receptor subtypes, the N-methyl-d-aspartate (NMDA) receptor (Monaghan, Bridges and Cotman, 1989). NMDA receptors potently bind: (a) l-glutamate; (b) N-methyl-d-aspartate (NMDA), and less potently bind; (c) l-aspartate (Fagg, 1985; Fagg, Foster and Ganong, 1986; Monaghan et al., 1989). Since l-glutamate binds with high affinity to the NMDA (AA1), the quisqualate (AA2), and the kainate-preferring (AA3) amino acid receptor sites, it is generally believed to function as the neurotransmitter agonist at all excitatory amino acid synapses (Bloom, 1990; Cooper et al., 1986; Fagg, 1985; Fagg et al., 1986; McCormick, 1990; Monaghan et al., 1989; Nistri, 1985).

PCP and related dissociative arylcyclohexylamine anesthetic derivatives like ketamine selectively block

postsynaptic excitatory activity at the NMDA receptor (Anis, Berry, Burton and Lodge, 1983). Such actions at the NMDA receptor are not mediated by competitive inhibition of l-glutamate, however. Instead, PCP-sites on NMDA-receptors are likely to be allosterically coupled with an NMDA/l-glutamate macromolecular receptor complex, providing noncompetitive antagonism of the voltage-dependent cation channels (principally Ca^{++}) initiated by glutamate (Bloom, 1990; Fagg et al., 1986; Jaffe, 1990; Monaghan et al., 1989; Zukin and Zukin, 1988).

While ABR latencies and amplitudes are extremely resistant to high levels of barbiturates (e.g. Bobbin, May and Lemoine, 1979; Glasscock et al., 1981; Hall, Mackey-Hargadine and Allen, 1985; Kileny and McIntyre, 1985; Sohmer, 1989), ketamine administration (200mg/kg) in rodents can produce dose and stimulus intensity-dependent changes in both the latencies and amplitudes of the major components of the ABR (Church and Gritzke, 1987). Such effects have been observed as positive amplitude changes in ABR waves I-IV with increasing stimulus intensities. These reported effects are similar to those reported earlier in this investigation (Figure 1) with the use of Telazol, which consists partly of tiletamine, an anesthetic agent similar to ketamine (Hrapkiewicz et al., 1989). A lower 100mg/kg dose of ketamine, however, fails to alter latencies and amplitudes of the early wave (I-IV) components of the rodent ABR (Bobbin et al., 1979). An even lower 50mg/kg dose of

ketamine also fails to alter latencies and amplitudes of the ABR (Smith and Mills, 1989), which was the dose level employed in all investigations reported herein. Moreover, the plasma half life (t ½) of ketamine is 2.3 hours (Marshall and Longnecker, 1990), and ketamine administration preceded data collection by 3 to 4 hours in these studies. It should also be added that in spite of its reported advantages as an anesthetic agent (Hrapkiewicz et al., 1989), Telazol appears to have limited utility in long-term electrophysiological studies of the auditory system.

Kappa-Receptor Blockade With Nor-BNI: Results of the present investigation suggested that at least some of the (0.74 to 1.5μg/μl) nor-BNI diffused through the round window membrane. The feasibility of this assertion is supported by results from a number of round window membrane permeability studies. A number of factors are involved in determining the passage of substances through this membrane, and one significant factor is molecular weight (Juhn et al., 1988; Lundman et al., 1988). While the round window membrane is permeable to a wide variety of substances having a wide range of molecular weights (Anniko, Hellstrom, Schmidt and Spandow, 1988; Juhn, Hamaguchi and Goycoolea, 1988; Goycoolea, Muchow, Martinez, Aguila, Goycoolea, Goycoolea, Schachern and Knight, 1988; Lundman, Bagger-Sjoback, Holmquist and Juhn, 1988), generally however, low molecular weight-substances (<1000 Daltons) are incorporated into the cochlea via the round window at a faster rate than are

substances with higher molecular weights (Juhn et al., 1988). For example, NaCl (58.45 Daltons) and KCl (74.55 Daltons) are transported through the round window into the perilymph within 1 to 5 minutes, while substances with higher molecular weights (e.g. 12k to 415k Daltons) may be incorporated into the cochlea within 3 to 60 minutes (respectively; see Goycoolea et al., 1988; Juhn et al., 1988). Recall that the molecular weight of nor-BNI is only 734.73 Daltons.

While not statistically significant, the apparent partial blockade of (-)-pentazocine effects by the cochlear application of nor-BNI further supports a K-opioid receptor mediated modulation of the near-field recorded auditory potentials. Recall that nor-BNI exhibits a K-receptor binding affinity 77.5X greater than U-50488H (Takemori et al., 1988), and 20X (Tiberi and Magnan, 1990) to 44X greater than U-69593 (Smith et al., 1990).

Inability To Thoroughly Investigate U-50488H and U-69593: Finally, in these investigations, the auditory properties of the K-opioid receptor agonists U-50488H and U-69593 could not be evaluated at the respective doses of 10mg/kg and 2mg/kg. This was an unfortunate outcome since U-50488H exhibits up to a 120-1300X greater binding affinity for K- over μ-receptors, and is devoid of δ-receptor activity (Goldstein, 1984; Kosterlitz, 1985; Lahti et al., 1985; Lever et al., 1983; North, 1986; Paterson et al., 1984). U-69593, a still more potent analog of U-50488H,

exhibits a 484X greater binding selectivity for K-, relative to μ- and δ-opioid receptors (Lahti et al., 1985), and its affinity for K has been reported to be 1.3X greater than U-50488H (Lahti et al., 1985; Tiberi et al., 1988).

The reason for the observed lethal respiratory effects of these two K-receptor agonists in this species is not clear. As indicated earlier, U-50488H has been investigated in rodents at subcutaneous doses as high as 20 to 40mg/kg (Millan, 1989; Millan et al., 1989; Shaw et al., 1988; Von Voigtlander et al., 1983; Von Voigtlander and Lewis, 1988), and at iv doses as high as 10mg/kg administered over a 30 second period (Leighton et al., 1988). U-69593 has been administered to rats at subcutaneous doses as high as 5mg/kg (Millan, 1989; Millan and Colpaert, 1990), or iv within 30 seconds at 10mg/kg (Leighton et al., 1988).

Perhaps chinchillas, like guinea pigs, have higher overall concentrations of K-receptors. Indeed, relative forebrain concentrations of the three major opioid-receptor types vary considerably across species (Besse, Lombard, Zajac, Roques and Besson, 1990; Chang et al., 1981; Goodman et al., 1980; Hewlett, Akil, Carlini and Barchas, 1982; Mansour et al., 1987; Paterson et al., 1983; Tiberi and Magnan, 1990; Walker et al., 1988). Guinea pigs exhibit much higher forebrain concentrations (44-50%) of K-receptors relative to their lower μ-(24-25%) and δ-(25-32%) receptor concentrations (Mansour et al., 1988; Maurer, 1982; Paterson et al., 1984). Also, the relative proportions of K-, μ- and

d-opioid receptors in guinea pig spinal cord are 63%, 28%, and 9%, respectively (Tiberi and Magnan, 1990). Perhaps again, the additional K-receptors in chinchilla are concentrated within pontine and medullary brainstem centers involved in ventilatory control.

The distribution of μ-receptors within the CNS (Mansour et al., 1988) corresponds well their known respiratory and cardiovascular effects (e.g. McQueen, 1983; Pfeiffer, Feurstein, Faden and Kopin, 1982). It has been suggested (i.e. Pasternak, 1980; 1981; 1982; 1988a; 1988b) that μ_1-receptors responsible for supraspinal analgesia, differ functionally from those (μ_2-) producing opioid-induced respiratory depression. Indeed, there is some evidence that morphine's analgesic actions may be dissociated from its lethal, respiratory depressive actions (Ling, Spiegel, Nishimura and Pasternak, 1983; Ling, Spiegel, Lockhart and Pasternak, 1985; Pasternak, 1981). The 'classic' μ_2-, vs the d-receptor (e.g. Florez and Pazos, 1982) has been implicated in mediating the lethal respiratory depressive effects of morphine (Goodman et al., 1988; Ling et al., 1985 Pasternak, 1981; 1982; 1988b; Pasternak and Wood, 1986; Wood and Iyengar, 1988). Recall that U-50488H exhibits a 120-1300X greater binding affinity for K- over μ-receptors, and is devoid of d-receptor activity (Goldstein, 1984; Kosterlitz, 1985; Lahti et al., 1985; Lever et al., 1983; North, 1986; Paterson et al., 1984). However, in addition to its lack of d-receptor activity, U-50488H is also devoid of μ_2-receptor

activity, and exhibits a very poor affinity at μ_1-receptors as well (Clark and Pasternak, 1988). Therefore, further experiments are needed to more systematically investigate the properties of potent K-receptor ligands, and to determine the reason for their lethal K-mediated effects in this species.

Proposed Synaptic Mechanisms Of Action

Some Properties Of Opioid Receptor Actions: It should be noted that while the net hyperpolarizing effect of opioid-receptor binding is similar, μ- (or d-) and K-receptors appear to have little in common with respect to their coupling to ion channel conductances within neurons (Chavkin, 1988; Cherubini and North, 1985; Cox, 1988; Gross and MacDonald, 1987; North, 1986; North and Egan, 1983; Shen and Crain, 1989; 1990a; 1990b; Werz and MacDonald, 1985). For example, opioid binding with μ-receptor (e.g. DAGO; morphine; morphiceptin) or d-receptor ligands (DPDPE; DPLPE; DADLE; [Met^5]- and [Leu^5]-enkephalin) both in the CNS and PNS, produces pre- or postsynaptic neural hyperpolarization by activating voltage- and/or Ca^{++}-dependent K^+-channel conductances (Kandel, 1985a; Lefkowitz et al., 1990; Yoshimura and North, 1983). The increased K^+-channel conductance is in turn indirectly followed by a voltage-gated reduction in Ca^{++} conductance (Chavkin, 1988; Cherubini and North, 1985; Cooper et al., 1986; Dingledine, 1985; Duggan and North, 1984; Gross and MacDonald, 1987;

Henderson, 1983; Kandel, 1985b; North, 1986; North and Egan, 1983; North and Williams, 1983; 1985; Werz and MacDonald, 1985; West and Miller, 1983).

The voltage-dependent Ca^{++} influx at the presynaptic terminal is known to be essential for the binding of neurotransmitter vesicles to their release sites. This Ca^{++} influx also promotes exocytosis and neurotransmitter release (Cooper et al., 1986; Hille, 1984b; Kandel, 1985b; Lefkowitz et al., 1990; Zucker, 1987). The properties of the intracellular Ca^{++}-dependent repolarizing species of K^{+} channel described above are well understood (Chavkin, 1988; Ewald and Levitan, 1987; Henderson, 1983; Jackisch et al., 1986; Koester, 1985c; Levitan and Kaczmarek, 1987; North, 1986; North and Egan, 1983; North and Williams, 1985).

Likelihood Of Postsynaptic Inhibition: Neuroactive opioid peptides in the cochlea could very well act alone as inhibitory postsynaptic neurotransmitters, just as proenkephalins (Pepper and Henderson, 1980; Ruda, 1982; Yoshimura and North, 1983), GABA, and glycine function in the CNS (e.g. Cooper et al., 1986; Kandel, 1985a; Krnjevic, 1984; Schwartz, 1985a). Indeed, as a general rule, the net result of μ-, d- or K-opioid receptor binding is a reduction in neuronal discharge rate and/or a reduction in the amount of neurotransmitter release (i.e. inhibition) by subsequent action potentials in the neuron bearing the receptor (e.g. Cherubini and North, 1985; Cooper, Bloom and Roth, 1986; Cox, 1988; Duggan and North, 1984; Gross and MacDonald,

1987; Millan, 1986; North, 1986; North and Egan, 1983; Simonds, 1988). Postsynaptic hyperpolarization may occur via an increased Cl^- conductance (influx); or by an increased K^+ channel (efflux) conductance (Cooper et al., 1986; Kandel, 1985a; 1985b; Koester, 1985a; 1985b; Krnjevic, 1984; Lefkowitz, et al., 1990; North, 1986; Phillis, 1984; Zucker, 1987). Kappa-opioid receptor ligands can produce postsynaptic hyperpolarization via direct suppression of a voltage-dependent N-type Ca^{++} current (Gross and MacDonald, 1987; North, 1986; Shen and Crain, 1990a; 1990b; Werz and MacDonald, 1985). This current normally contributes both to the peak and duration of the postsynaptic action potential (McCormick, 1990; Tsien, 1987).

In the present investigation, however, the administration of the K-receptor agonist pentazocine led to an <u>increase</u> in auditory sensitivity and/or a facilitation in neural responses to low stimulus levels. It is therefore unlikely that the observed auditory effects were due to postsynaptic inhibition of auditory activity at the lateral efferent/Type I synapse. Another consideration is that K-receptors are allosterically coupled with a macromolecular cholinergic receptor complex, providing noncompetitive postsynaptic antagonism of ACh actions (Henderson, 1983).

<u>Likelihood Of Presynaptic Facilitation</u>: Over the past several years, investigations conducted both within the CNS and periphery (PNS) and especially on vertebrate autonomic sympathetic ganglia, have provided convincing evidence that

opioid peptides can modulate the release of other neuroactive substances (e.g. Chavkin, 1988; Cherubini and North, 1985; Cox, 1988; Dingledine, 1985; Duggan and North, 1984; Jackisch, Geppart and Illes, 1986; Konishi, 1985; Martin, 1983; North, 1986; North and Egan, 1983; West and Miller, 1983; Wood and Iyengar, 1988). For instance, several studies have shown that μ-, δ-, k- and ξ-receptors decrease ACh turnover, and modulate catecholamine, monoamine, and neuropeptide turnover in many regions of the CNS (e.g. Cedarbaum and Schleifer, 1990; Gauchy, Desban, Krebs, Glowinski and Kemel, 1991; Thompson, Matsumoto, Hohmann and Walker, 1990; Thompson and Walker, 1990; Walker, Thompson, Frascella and Friederich, 1987b; Werling, Frattali, Portoghese, Takemori, and Cox, 1988; West and Miller, 1983; Wood and Iyengar, 1988).

Presynaptic facilitation generally occurs by reduction in a K^+ channel conductance, which acts to broaden the duration of the action potential and prolong the opening of voltage-gated Ca^{++} channels, and thereby Ca^{++} influx. This leads in turn to the subsequent release of more neurotransmitter (Cooper et al., 1986; Kandel, 1985a; 1985b; Kosterlitz 1984; Krnjevic, 1984; Lefkowitz et al., 1990; North, 1986; Phillis, 1984; Zucker, 1987). If it is generally assumed that ACh neurotransmitter release at the lateral efferent-Type I ganglion cell synapse is hyperpolarizing, then it is unlikely that the observed changes in near-field amplitudes reflected a presynaptic K-

receptor facilitation of ACh release following pentazocine administration, in the present investigation.

A presynaptic facilitating mechanism via opioid receptors is an unlikely explanation for other reasons as well. Ontological evidence in mammalian species has suggested a lateral efferent system that functions early in development to hyperpolarize IHCs (Carlier and Pujol, 1976; Ginzberg and Morest, 1983; 1984; Pujol, 1985; Pujol et al., 1978; 1979; 1980; Pujol and Lenoir, 1986; Schwartz, 1986; Whitehead, 1986). Furthermore, anatomical evidence reviewed earlier (Liberman, 1980b) suggests that tonic input from lateral efferent neurons might be required to establish or maintain the distribution of spontaneous activity and sensitivity of Type I auditory fibers (especially in those fibers exhibiting low SDRs) via hyperpolarization (Liberman, 1988b; 1990).

It should be added at this juncture that complete transection of the lateral efferents near Oorts anastomosis fails to affect ABR thresholds (e.g. Littman et al., 1991). Interestingly, complete unilateral midline transection of both the lateral and medial efferent fibers actually fails to affect either thresholds or rate level functions of Type I auditory fibers. To the contrary, the efferent transection procedure leads to a decline in spontaneous discharge rates for high-spontaneous discharge rate (SDR)-fibers (Liberman, 1990).

Likelihood Of Presynaptic Inhibition: Many CNS actions of opioid peptides appear to be presynaptic (Araujo and Collier, 1987; Atweh and Kuhar, 1983; Chavkin, 1988; Cuello, 1978; Dingledine, 1985; Duggan, 1983; Duggan and North, 1984; Glazer and Basbaum, 1980; 1981; Hokfelt et al., 1980; 1984; Iverson, Iverson and Bloom, 1980; Jackisch et al., 1986; Kennedy and Krier, 1987; Kinouchi, Maeda, Saito, Inoki, Fukumitsu and Yoshiya, 1989; Konishi, 1985; Konishi, Tsunoo and Otsuka, 1979; 1981; Leander, 1983; Martin, 1983; Miller and Pickel, 1980; Mudge et al., 1979; Mulder, Wardeh, Hogenboom and Frankhuyzen, 1984). A major action of neuroactive opioid peptides in the PNS seems to involve presynaptic inhibition of neural activity and neurotransmitter release (Cherubini, Morita and North, 1985; Cherubini and North, 1985; Gross and MacDonald, 1987; Konishi, 1985; North, 1986; North and Egan, 1983; Shen and Crain, 1989; 1990a; 1990b; Waterfield, Smokcum, Hughes, Kosterlitz and Henderson, 1977; Werz and MacDonald, 1985).

A presynaptic, K-opioid receptor inhibition of ACh release from the lateral efferent terminals is a more parsimonious explanation for the observed pentazocine effects. This hypothesis is strengthened by evidence cited earlier supporting the coexistence of neuroactive peptides with ACh in lateral efferent neurons. Indeed, the coexistence and corelease of multiply active peptide cores from within the same neuron has proven to be a common occurrence (see: Akil and Watson, 1983; Araujo and Collier,

1987; Chan-Palay and Palay, 1984b; Cooper et al., 1986; Hokfelt, Johansson, Ljungdahl, Lundberg, and Schultzberg, 1980; Larsson, 1984; Lewis et al., 1987; Miller, 1983; Panula, Yang and Costa, 1984; Schwartz, 1985a; Vincent, 1984; Walker et al., 1987a). The coexistence of opioid and non-opioid neuroactive peptides together with small molecule neurotransmitters within the same neuron, probably represents the rule rather than the exception (Chan-Palay and Palay, 1984c; Cooper et al., 1986; Gilbert and Emson, 1983; Hokfelt, Johansson and Goldstein, 1984; Jan and Jan, 1985; Konishi, 1985; Kosterlitz, 1984; Miller, 1983; Schultzberg, 1984; Schultzberg, Hokfelt, Nilsson, Terenius, Rehfeld, Brown, Elde, Goldstein and Said, 1980; Schwartz, 1985a; Walker et al., 1987a).

In general, presynaptic inhibition of neurotransmitter release can occur: (a) by a direct reduction of a voltage-gated Ca^{++} channel conductance; (b) by an increased Cl^{-} conductance; or (c) by an increased K^{+} channel conductance. Presynaptically, both mechanisms in (b) and (c) will reduce the likelihood of activating a voltage-gated Ca^{++} channel by short-circuiting (hyperpolarizing) the presynaptic action potential. Regardless of the ion channel involved, in all three cases there is a reduction in presynaptic Ca^{++} influx, and a subsequent reduction in the release of neurotransmitter. Indeed, the general response to opioid substances, at least in the periphery, is a reduction in the

cellular uptake of Ca^{++} (Gross and MacDonald, 1987; Simonds, 1988; Werz and MacDonald, 1985; West and Miller, 1983).

Kappa Receptor Channel Properties: Perhaps K-receptor ligands (natural or synthetic) presynaptically inhibit the release of ACh from lateral efferent terminals by a direct action on a voltage-dependent Ca^{++} conductance. Indeed, many K-opioid receptor ligands (dynorphin A; dynorphin A (1-8) and (1-9); dynorphin B; α- and ß-neoendorphin; U-50488H) produce presynaptic hyperpolarization in the PNS by direct reduction of a voltage-dependent Ca^{++} conductance (Cherubini and North, 1985; North, 1986). That K-opioid receptors modulate activity in peripheral cholinergic neurons is supported by the demonstration of K-mediated inhibition of ACh release onto postsynaptic parikarya of neurons within the guinea pig myenteric plexus. This action occurs of course, via a direct reduction of Ca^{++} influx into the presynaptic terminal (Cherubini and North, 1985).

Likelihood Of Postsynaptic Excitation: Finally, there is the additional though remote possibility that K-receptor actions following pentazocine administration were the result of postsynaptic excitation at the lateral efferent synapse. In addition to confirming the hyperpolarizing ionic properties of μ- δ- and K-opioid ligands (described above) one lab also reported excitatory effects of μ- δ,- and K-receptor activation (Shen and Crain, 1989; 1990a; 1990b). Such effects were observed in a subpopulation of mouse dorsal root ganglia cell extracts grown in culture,

employing much smaller (nM vs μM) opioid ligand concentrations. In some neurons, low doses of μ- and d-receptor ligands, and the K-receptor ligand Dynorphin B (1-13), were found to produce excitatory effects (i.e. prolonged action potentials) via a reduction in a voltage-sensitive K^+ channel conductance. However, the excitatory properties observed with low doses of U-50488H were linked to the activation of voltage-dependent Ca^{++} channel conductances (Shen and Crain, 1989; 1990a; 1990b).

Summary and Conclusion: The exact role played by the (medial and lateral) olivocochlear system in peripheral auditory processing is not well defined at this time. It has been suggested that medial efferent fibers provide a gain control (Geisler, 1974a; Kim, 1984; 1986) to the operating range of single units (Gifford and Guinan, 1983; Guinan and Gifford, 1988b; Wiederhold, 1970; 1986; Wiederhold and Kiang, 1970). More convincing however, is the evidence in support of a medial efferent system that functions to improve the discriminability of signals in backgrounds of noise (Dewson, 1967; Nieder and Nieder 1970a; 1970b; Winslow, 1988; Winslow and Sachs, 1984; 1985; 1987).

It has been consistently argued that all of the observed effects following electrical stimulation of the efferent pathway are due to the effects at medial efferent synapses (e.g. Gifford and Guinan, 1987). Indeed, there are presently no published reports on either the spontaneous or driven response properties of unmyelinated lateral efferent

fibers, and therefore nothing is known of their physiology. Inconclusive attempts have been made to elucidate their function (Comis, 1970; Desmedt and LaGrutta, 1963; Fex, 1965; 1967; Liberman, 1990; 1991). The general failure to determine the role played by the lateral efferents in audition (e.g. Wiederhold, 1986) has resulted in the virtual exclusion of this major fiber projection from any theoretical consideration, with perhaps one or two exceptions where neural inhibition has been suggested (Fex, 1967; Kim, 1984; 1986; Liberman, 1988b).

Previous investigations have shown that medial efferent activation generally exerts greater effects on neural responses to lower stimulus levels, typically those which are less than 40dB SL (Dewson, 1967; Fex, 1962; Gifford and Guinan, 1983; Guinan, 1986; Guinan and Gifford, 1988a; 1988b; Nieder and Nieder 1970a; 1970b; Wiederhold, 1970; 1986; Wiederhold and Kiang, 1970; Winslow and Sachs, 1987). Results obtained in these investigations suggest that this property may also be true of the lateral efferent system.

Future Directions And Possible Clinical Relevance

Future Studies: Additional investigations are needed to determine the dose response properties of iv administered K-receptor agonists such as (-)-pentazocine on electrophysiological indices of peripheral auditory sensitivity. The exact duration of such effects on CAP response amplitudes and threshold sensitivity is also of interest. Experiment 3, of course, needs to be replicated using higher round window-applied doses of the specific K-receptor antagonist nor-BNI, combined with a range of (-)-pentazocine doses given iv. Additional dose effect relationships need to be carefully determined for round window application of K-receptor agonists, which would include U-50488H and U-69593.

It is also of interest to understand whether the observed K-receptor effects are pre- or postsynaptically (allosterically) linked to ACh, or whether a postsynaptic K-receptor functions independently of ACh. To this end, these pharmacological experiments could again be conducted following the transection and degeneration (≈6months) of the efferent olivocochlear bundle (Liberman, 1990). The adequacy of efferent transection could be evaluated using light microscopy of sectioned cochleas stained for acetylcholinesterase (Liberman, 1990). The ability to replicate these amplitude effects under such conditions would suggest a postsynaptic mechanism independent of ACh. It is also predicted that amplitude effects (disinhibition)

similar to those observed in the present investigation would be observed following systemic or cochlear administration of cholinergic (muscarinic or nicotinic) receptor blocking drugs.

Endogenous proenkephalin derivatives which coexist within lateral efferent neurons may play a similar or different role in the lateral efferent modulation of auditory function via δ-receptors. Therefore, δ-receptor ligands may produce similar or different effects on CAP response amplitudes and threshold sensitivity. To answer these general questions, similar investigations could be conducted to determine the auditory changes in response to opioid δ-receptor ligands administered iv (e.g. DPDPE, DPLPE, DPDCE) or diffused through the round window of the cochlea (e.g. [Met^5]- and [Leu^5]-enkephalin).

It is anticipated, however, that a clearer understanding of the apparent opioid-induced disinhibition will be gleaned most from further experiments employing K- or δ-preferring ligands, and click or frequency specific tone burst stimuli which are embedded in backgrounds of noise. These procedures could then be combined with additional experiments using electrical stimulation of the medial efferent fibers. Indeed, it may be possible to demonstrate that the mammalian auditory system has evolved a bandpass filtering mechanism, with perhaps two distinct components: a broad spectrum-filtering (noise suppressive) medial and a narrow spectrum-filtering (disinhibitory)

lateral efferent system of fibers. Indeed, medial efferent fibers exhibit a much more diffuse cochleotopic innervation pattern than does the lateral bundle (Guinan et al., 1984; Warr et al., 1986). The innervation range of a single medial efferent fiber (in cat) can span from 0.55 to 2.8mm, or roughly 2.2 to 11.2% of the total cochlear length, with the majority spanning more than 1.5mm or 6% of the total (Ginzberg & Morest,1983; Liberman & Brown, 1985; 1986). In the guinea pig, efferent fibers span OHC distances of 0.22 to 1.5mm, and the majority range from 0.20 to 0.79, or roughly 1 to 4.1% of the total cochlear length (Brown, 1985b; 1987b; Robertson, 1984). Therefore, available data suggests that a comparatively greater range of CFs may correspond to single medial efferent OC neurons. The lateral efferent fibers on the other hand, are strictly cochleotopic in their projections to the auditory periphery (Guinan et al., 1984; Warr et al., 1986), indicating that their innervation of primary afferents is rather frequency specific.

Potential Clinical Relevance: It may be possible that a greater tonic suppression directed by the lateral efferents on the low SDR-afferents (i.e. Liberman, 1980b) enables these fibers to maintain a prominent discharge rate peak at 1.5kHz over a wide range of stimulus intensities (Shofner & Sachs, 1986). The stable low frequency rate-place representation by the low SDR-fibers could act to facilitate the encoding (by rate) of the lower frequencies (<2kHz)

important for vowel recognition, especially in backgrounds of noise (i.e. Delgutte & Kiang, 1984).

Endogenous opioids may play an important neuromodulatory role in the normal function of the efferent olivocochlear system. Indeed, they may be sporadically released from lateral efferent terminals under certain non-stressful environmental conditions. Conceivably, factors which would disrupt the tonic activity of lateral efferent fibers would permit certain populations of primary afferents to be driven into saturation. The endogenous opioid peptides could conceivably modulate lateral efferent activity by diminishing the effectiveness of ACh as a hyperpolarizing neurotransmitter. For instance, under non-stressful conditions, the release of endogenous opioid peptides from the lateral efferent system might act in concert with the medial efferents (i.e. Winslow, 1988) to improve the detection of signals in noise backgrounds, in order to maximize the outcome of various forms of foraging behavior.

Perhaps a more prolonged release of endogenous opioid peptides in the cochlea might be triggered by stress-related events, as is thought to occur within the CNS. A stress-related release of these peptides within the cochlea would be perceived as counterproductive to the overall function of the efferent system. When confronted with a potentially stressful (fight or flight) environmental predicament, an organism conceivably has little need for a peripheral sensory mechanism that normally contributes to the

deliberation of fine-tuned perceptions. Rather, the relatively short term exposure to aversive environmental conditions might cause the physiology of the organism to adaptively shift into an operating mode whereby response selection to the environment might advantageously become limited or narrowed. Hypothetically, peripheral sensory systems may then begin to function under a state of 'vigilance' whereby all environmental stimuli temporarily acquire equal saliency (i.e. neural disinhibition) and 'figure-ground' discrimination is therefore the poorest. Much experimentation is needed however, in order to validate these assertions.

A Cochlear Model

A model is offered (Illustration 5) which summarizes much of what is presently known regarding lateral and medial olivocochlear efferent physiology, and general cochlear physiology. For the sake of simplicity, the model only presents the probable actions of ACh within medial efferent (B_{ME} and C_{ME}), and lateral efferent fibers.

Inner Hair Cell Physiology: The initiation of auditory signalling in the mammalian periphery is highly dependent upon the release of excitatory neurotransmitter from within the IHCs (e.g. Ashmore, 1991; Pickles, 1988), which are the primary sensory cells of the auditory nerve (e.g. Santos-Sacchi, 1988; Spoendlin, 1988). Indeed, practically all that is known regarding the properties of the auditory nerve

stems from investigations of the Type I fibers (e.g. Harrison, 1988; Javel, 1986; Pickles, 1988).

Transduction of the mechanical traveling wave into neural activity by the IHCs is characterized by the initial deflection of IHC stereocilia, followed by an inward flow of (endolymphatic) transduction current across the apical IHC membrane (e.g. Ashmore, 1991; Brownell et al., 1986; Dallos, 1973; 1981; Gitter et al., 1986; Gitter and Zenner, 1988; Pickles, 1988; Santos-Sacchi, 1988). Physiological events occurring at the IHC level following auditory stimulation, are likely to proceed in a manner previously described based upon results obtained from extensive investigations of ion channel conductances within the saccular hair cells of the American bullfrog (i.e. Corey and Hudspeth, 1979a; 1979b; 1983a; 1983b; Holton and Hudspeth, 1986; Hudspeth, 1982; 1983; 1985; 1986; Hudspeth and Corey, 1977; Lewis and Hudspeth, 1983a; 1983b; Roberts, Jacobs and Hudspeth, 1990). What follows, therefore is a brief summary of the nonmammalian transduction events likely to occur within mammalian IHCs during normal auditory stimulation. Available data from mammalian IHCs will also be included when appropriate.

During the resting state, the hair cell is exposed to a constant thermal buffeting by surrounding molecules, and each transduction channel may fluctuate from an open to a closed state permitting a small steady flow of positively charged ions to cross into the hair cell (Hudspeth, 1983;

1985). In mammalian species, only about 15% of the IHC transducer channels are open at rest (i.e. Dallos, 1985a; 1985b). The apical transduction channel, as in mammalian IHCs (Kros and Crawford, 1989), prefers cations over anions (Corey and Hudspeth, 1979a; Hudspeth and Corey, 1977), and the random opening of appropriate channels allows cations to flow in, lowering slightly, the hair cell membrane potential.

A positive deflection of 1-100nm, of the stiff actin-filled (2-8 μm long) nonmammalian stereocilia bundle (consisting of about 100 stereocilia) is sufficient to gate an inward flow of current through the few hundred transducer channels associated with the bundle, about 4 channels per stereocilium (Holton and Hudspeth, 1986). The flask-shaped mammalian IHCs support about 60 (apically) to 77 (basally) stereocilia, having lengths of from 1μm (cochlear base) to greater than 8μm in the cochlear apex (i.e. Harrison and Hunter-Duvar, 1988; Santi, 1988). With the delivery of an auditory stimulus, an active stereociliar deflection in a positive direction further increases the apical membrane conductance for positively charged ions (Corey and Hudspeth, 1979a; Santos-Sacchi, 1988), and the membrane potential of the (nonmammalian) hair cell depolarizes to about -40mV (Corey and Hudspeth, 1979a; Hudspeth, 1982; 1983; 1986).

This mechanosensitive transduction current traverses the apical hair cell membrane (Corey and Hudspeth, 1979a; 1983a; Hudspeth and Corey, 1977; Hudspeth, 1982) via

elastically gated (e.g. Hudspeth, Roberts and Howard, 1989) transduction channels located at or near the distal tips of the stereocilia (Hudspeth, 1982; 1983; 1985). The principal cation within the amphibian saccular endolymph is K^+ (Corey and Hudspeth, 1979a; Hudspeth, 1982; 1983; 1985; 1986) which also appears to be the major carrier of the depolarizing current across the mammalian apical IHC membrane (Ashmore, 1991; Brownell et al., 1986; Dallos, 1973; 1981; Gitter et al., 1986; Gitter and Zenner, 1988; Pickles, 1988; Russell, 1983; Salt and Konishi, 1986; Salt and Thalmann, 1988; Smith et al., 1954; Tasaki and Spyropoulos, 1959).

The lateral membrane of the amphibian saccular hair cell also bears a voltage-sensitive Ca^{++} conductance that is activated at membrane potentials more positive than -60mV, and is therefore partially activated during the resting state (Hudspeth and Corey, 1977; Lewis and Hudspeth, 1983a; 1983b). Indeed, the random, resting level influx of Ca^{++} into the hair cell has been offered as an explanation for the spontaneous discharge of afferent fibers (Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a). With the delivery of an auditory stimulus, hair cell depolarization by K^+ influx (from the hair cell apex) opens these voltage-sensitive Ca^{++} channels, resulting in a Ca^{++} influx. As Ca^{++} enters, the additional positivity further depolarizes the hair cell, augmenting the K^+-induced depolarization (Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a). Since the inward Ca^{++} conductance is not appreciably

inactivated during prolonged depolarization, it is estimated to be involved in the endogenous release of afferent neurotransmitter from the hair cells (Hudspeth, 1983; 1986; Lewis and Hudspeth, 1983a; 1983b). Indeed, the voltage-dependent influx of Ca^{++} is known to be essential for coupling presynaptic action potentials to the release of neurotransmitter, by promoting the presynaptic membrane fusion of neurotransmitter vesicles with their release sites, and by promoting the exocytosis and release of the prepackaged neurotransmitter vesicles (Cooper et al., 1986; Hille, 1984b; Kandel, 1985b; Lefkowitz et al., 1990; Zucker, 1987).

The rising intracellular concentration of Ca^{++} then activates basolaterally located Ca^{++}-dependent K^{+} channels, at potentials above approximately -60 to -45mV (Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a; 1983b; Roberts et al., 1990). Activation of this intracellular Ca^{++}-dependent K^{+} conductance allows the inward apical depolarizing K^{+} current to exit the hair cell through channels located within the basolateral hair cell surface (Corey and Hudspeth, 1979a; 1979b; 1983a; 1983b; Hudspeth, 1982; 1983; 1985; 1986; Roberts et al., 1990). The ensuing K^{+} efflux, traveling down the cytoplasmic-perilymphatic ion concentration gradient then begins to repolarize the hair cell membrane. These Ca^{++} -activated K^{+} channels not only repolarize the cell, but also produce the electrical resonance that tunes each cell. The inward Ca^{++} also

facilitates the release of neurotransmitter (Roberts et al., 1990). As the intracellular potential begins to drop (following K^+ efflux), there is a temporarily reduction in the net inward current across the basolateral voltage-sensitive Ca^{++} channels (Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a; 1983b).

The K^+ efflux repolarizes the hair cell beyond the resting negativity until the excess Ca^{++} within the cytoplasm is buffered and rapidly extruded by metabolic pump or sequestration. The subsequent intracellular decline in Ca^{++} proceeds to close the basolateral Ca^{++}-dependent K^+ channels, and the hair cell returns to an approximation of its initial resting state. The continuing apical influx of depolarizing K^+ current then proceeds to initiate another cycle of electrical oscillation (Hudspeth, 1983; 1985; 1986; Lewis and Hudspeth, 1983a; 1983b). Hyperpolarization of the hair cell by antagonistic (negative) stereociliary displacement will close the voltage-dependent Ca^{++} channels (Hudspeth, 1983; Hudspeth and Corey, 1977), and is thought to reduce the amount of released neurotransmitter under stimulus-driven conditions. The essential features of this ionic and mechanoelectric transduction model originally proposed by Hudspeth (1983; 1985; 1986), are applicable to an understanding of IHC function in mammalian species, and are contained within the larger explanatory model presented below.

Outer Hair Cell Physiology: The cylindrically shaped mammalian OHCs, have base to apical lengths within the cochlea, from 20μM to 50μM, respectively (e.g. Harrison and Hunter-Duvar, 1988), and appear to play a dual role in the auditory system (i.e. LePage, 1989). They have a much poorer afferent innervation than the IHCs (e.g Spoendlin, 1988) and may serve as active, force-generating (Geisler, 1991; LePage, 1989) 'mechanical effectors' that provide a 'boost' (within about the first 40dB) for basilar membrane mechanics (Ashmore, 1991; Geisler, 1986; Kim, 1984; 1986; Mountain and Cody, 1989; Neely and Kim, 1986; Salvi et al., 1983; Santos-Sacchi, 1988; Zenner, 1986a; 1986b). This mechanical boost is then transferred to the IHC stereocilia as an increased electrochemical transduction current, in some orderly fashion (i.e. Ashmore, 1991; Mountain and Cody, 1989; Pickles, 1988).

The actin filled stereocilia of the OHCs (and IHCs) are interconnected by cross-links (Hackney and Furness, 1989; Harrison and Hunter-Duvar, 1988; Pickles, 1988; Pickles, Osborne, Comis, Koppl, Gleich, Brix and Manley, 1989; Santi, 1988), and OHC stereocilia pivot at their cuticular base (Lim, 1986; Nielsen and Slepecky, 1986; Zenner, 1986b). The OHC generally number from 50 to 150 per bundle, with slightly greater numbers in the base, and have (base to apex) lengths of from 0.5-1μm to 8μm or greater. Each OHC supports about three stereociliar rows, configured in a "W"-pattern. Each stereociliar row is progressively graded in

length as a function of its distance from the modiolus (e.g. Harrison and Hunter-Duvar, 1988; Santi, 1988).

Unlike the flattened distal tips and wider diameter (≈0.45μM) of the IHC stereocilia, OHC stereocilia are rounded at their extremes and are generally (≈0.20μM) thinner (Santi, 1988). While it appears that the tips of the tallest OHC stereocilia are embedded within the tectorial membrane, the IHC stereocilia by contrast, show no evidence of tectorial membrane embedding (Lim, 1980; Steel, 1986). Therefore, while the stereocilia of the OHCs are probably displaced directly by movements of the tectorial membrane, the IHC stereocilia are thought to be displaced by the viscous drag of fluid within the subtectorial space (Pickles, 1988).

OHCs Under Normal Operating Conditions: It is assumed that the three rows of OHC stereocilia bundles, the OHC sub-plasma membrane, and the overlying tectorial membrane form a resonant system consisting of stiff levers, elastic hair cell attachments (for a restorative force), and an inertial mass, in the tectorial membrane (Ashmore, 1991; Geisler, 1986; Holley and Ashmore, 1989; Mountain and Cody, 1989; Steel and Jen, 1988; Turner and Nielsen, 1983). During the resting state, it is further assumed that the OHCs are exposed to a constant thermal buffeting by surrounding molecules. A passive positive deflection of OHC stereocilia opens apical hair cell transduction channels, and cations (mostly K^+) rush into the OHCs, causing depolarization

(Ashmore, 1989; Dallos, 1985a; 1986; Zenner, 1986a; Zenner et al., 1985). The influx of K^+ may well open a voltage- or Ca^{++}-dependent (Ashmore, 1987; 1991) basolateral K^+ channel, allowing for the repolarization of the OHCs by K^+ efflux. Indeed, recall from an earlier discussion that a voltage-sensitive, intracellular Ca^{++}-sensitive, and stretch-sensitive hyperpolarizing K^+-channel conductance has indeed been found within the mammalian lateral OHC membrane (Gitter et al., 1986; Li et al., 1991; Zenner, 1986b).

The OHC depolarization normally produced by an inward K^+ transduction current (e.g. Zenner, 1986a; Zenner et al., 1985) is surmised to produce either a stiffening of the OHC stereocilia (Kim, 1984; 1986) or an inward retraction of the actin-rich (Lim, 1986; Lim et al., 1989; Nielsen and Slepecky, 1986) OHC cuticular plate (Zenner, 1986a; 1986b), micromechanically channeling the flow of endolymphatic K^+ transduction current toward the IHC stereocilia via the tectorial membrane (Liberman and Dodds, 1984a; 1984b; Mountain and Cody, 1989). It has been suggested that the OHC stereocilia may simply secure the tectorial membrane in a position suitable for IHC activation (Steel, 1986). Perhaps OHC-induced changes in IHC transduction are mediated mechanically by the tallest rows of stereocilia on both types of hair cells through the tectorial membrane (Liberman and Dodds, 1984a; 1984b; Liberman and Kiang, 1984). Of course the precise OHC-tectorial membrane-IHC mechanism is still a matter of controversial conjecture. As discussed

earlier, if the stimulus is intense enough, the influx of K^+ may also produce a shortening of the OHCs, an affect not mediated by a (medial efferent-type) Ca^{++}-dependent contractile protein mechanism, as described and below (Ashmore, 1987; Dulon et al., 1988; 1991; Ulfendahl, 1987; Zajic et al., 1991; Zenner et al., 1985; 1989).

Evidence For A Type Of OHC Motility: Brownell and associates (Brownell, Bader, Bertrand and DeRibaupierre, 1985) were among the first to demonstrate that OHCs are capable of electrically-induced mechanical shortening along their longitudinal axis. When isolated mammalian OHCs are intracellularly stimulated (200 msec duration; 100 to 200 pA), a bidirectional mechanical change in their length is observed. The delivery of intracellular depolarizing currents to the basal synaptic region produces a decrease in OHC length and an increase in width, while hyperpolarizing currents cause an increase in cell length and a lessening in cell width (Ashmore, 1987; Brownell et al., 1985; Zenner et al., 1989).

A variety of forms of OHC motility have since been demonstrated, having time constants ranging from μsec to msec to sec (Ashmore, 1987; Zenner, 1986a; 1986b). For instance, an electrically elicited contractile response of about 2 μM (4%) can develop within 120-255 μsec (Ashmore, 1987). Electrically-induced contractile properties are never observed in supporting cells or IHCs (Brownell et al., 1985;

Flock et al., 1986). Transcellular stimulation with sinusoidal alternating current delivered along the long axis of the hair cell is also effective in evoking motile responses from OHCs (Brownell et al., 1985; Zenner, 1986b).

Exposure of intact OHCs to a high K^+ solution also results in slower (msec to sec) hair cell shortening, in a range from 1-8μM or from 2.4-11% (Dulon, Aran and Schacht, 1988; Dulon, Zajic and Schacht, 1991; Ulfendahl, 1987; Zenner et al., 1989; Zenner, Zimmermann and Schmitt, 1985; Zajic, Dulon and Schacht, 1991), effects thought to be due to osmotic factors. Investigations of OHC physiology have indicated that the presence of extracellular Ca^{++} is not required for OHC contraction. Extracellular Ca^{++} is required, however, for OHC relaxation in the presence of a bathing medium containing the anion Cl- (Dulon et al., 1988; Zenner 1986a; Zenner et al., 1985). Intracellular Ca^{++} is, on the other hand, necessary for OHC contraction and shortening (Dulon et al., 1988; 1991; Flock et al., 1986; Schacht and Zenner, 1986; 1987; Slepecky, Ulfendahl and Flock, 1988; Ulfendahl, 1987; Zenner, 1986a; Zenner et al., 1989).

A Lipid Second Messenger System: Polyphosphoinositides are common lipids found within the plasma membrane of cells in several types of neural, endocrine, connective and muscle tissue (Berridge, 1984; Berridge and Irvine, 1984). These lipids are messengers in a fundamental transmembrane signalling system for neuromodulators and hormones known to

elevate intracellular Ca^{++} levels (Berridge, 1984; Berridge and Irvine, 1984; Hesketh, 1983; Huganir, 1987; Ross, 1990; Tsien, 1987). Indeed, several neurotransmitters including ACh, and several important postsynaptic receptors including the muscarinic cholinergic type (M_1 and M_3) are known to stimulate the enzymatic hydrolysis of the membrane-bound lipid, phosphatidylinositol bisphosphate (PPD), thereby liberating diacylglycerol and inositol trisphosphatate (IP_3) as transmembrane second messengers (Berridge, 1984; Berridge and Irvine, 1984; Brown, 1990; Cooper et al., 1986; Huganir, 1987; Lefkowitz et al., 1990; Taylor, 1990a; Tsien, 1987). The second messenger IP_3 then mobilizes Ca^{++} from internal stores (i.e. endoplasmic reticulum), thereby raising the concentration of free Ca^{++} within the cell (Berridge, 1984; Berridge and Irvine, 1984; Hesketh, 1983; Huganir, 1987; Ross, 1990; Tsien, 1987). The unbound intracellular Ca^{++} can trigger the contraction of actinomyosin filaments, as in smooth, skeletal or cardiac muscle (Alberts, Bray, Lewis, Raff, Roberts and Watson, 1983; Huganir, 1987; Tsien, 1987), and/or may act by itself as a second messenger activating phosphorylase kinase which enhances the breakdown of glycogen to supply ATP for the contractile process (Hille, 1984b). The action of IP_3 is regulated by an inositol trisphosphatase (see: Berridge, 1984; Berridge and Irvine, 1984).

Lipids Within OHCs: High metabolic levels of PPD and related lipids have been found within mammalian OHCs (Schacht and

Zenner, 1986; 1987; Zenner et al., 1989), providing direct evidence for the existence of these phosphoinositide mechanisms within the mammalian auditory system. Muscarinic agonists have been shown to stimulate the formation of IP_3 in the guinea pig organ of Corti (Niedzielski and Schacht, 1991) or developing rat cochlea, via a muscarinic M_3 receptor (Guiramand et al., 1990a; 1990b). Muscarinic receptor-induced IP_3 turnover is greater (1.8X) in OHCs from the basal, compared to the apical organ of Corti (Niedzielski and Schacht, 1991). Nicotinic receptors in the basal pole of the mammalian OHC have also been shown to elicit OHC contraction by opening Ca^{++} channels (Plinkert et al., 1991).

In addition, isolated OHCs exhibit a slow contractile response (within 50 to 200msec) in a Ca^{++} -free medium in the presence of very low concentrations of IP_3 (Schacht and Zenner, 1986; 1987; Zenner et al., 1989), or by increasing the intracellular levels of unbound Ca^{++} (Schacht and Zenner, 1986; 1987; Zajic et al., 1991; Zenner et al., 1989). No such contractile change in shape or tonicity is observed, however, following elevation of unbound intracellular Ca^{++} within IHCs (Dulon et al., 1991; Zajic et al., 1991).

Such evidence has generally suggested that the more basally vs apically (relative to the round window) concentrated medial efferent terminals innervating the organ of Corti, which utilize ACh as a neurotransmitter and

synapse near the OHC base, alter cochlear micromechanics by activating a cascade of intracellular second messengers (including Ca^{++}). Such action then produces a depolarizing actomyosin-mediated OHC contraction (e.g. Dallos et al., 1991; Niedzielski and Schacht, 1991; Schacht, 1986; Zajic et al., 1991; Zenner et al., 1989).

Investigations of the membrane properties of isolated mammalian OHCs have also revealed the existence of a number of other potentially important ion channels within the lateral OHC wall (Gitter, Fromter and Zenner, 1986; Li, Jia, Iwasa and Kachar, 1991; Zenner, 1986b). Indeed, there is also a graded, hyperpolarizing potassium (K^{+}) channel conductance having an open probability that increases with: (a) OHC membrane depolarization (i.e. voltage sensitive); (b) intracellular Ca^{++} ion concentration; or (c) OHC stretch (Gitter et al., 1986; Li et al., 1991). There is also evidence for a nongraded chloride (Cl^{-}) conductance on the lateral wall of the mammalian OHCs (Gitter et al., 1986).

Medial Efferent Mechanism Of Action

Currently, the most parsimonious explanation offered for the observed medial efferent olivocochlear-induced suppression of auditory single unit, and whole nerve (N1 and N2) response in mammalian species has been attributed to a medial efferent-modulated biomechanic alteration in the basilar membrane/tectorial membrane coupling between the outer and inner hair cells (Mountain, 1980; 1986; Mountain and Cody, 1989; Rhode, 1984; Siegel and Kim, 1982). Indeed,

both anatomic (Benson and Ryugo, 1987; Brown, 1987a) and physiologic evidence (Dallos, Evans and Hallworth, 1991; Guinan, 1986; Mountain, 1980; 1986; Nuttall, 1986; Siegel and Kim, 1982; Zenner, 1986a; 1986b) have suggested that the OHCs are actively involved (perhaps primarily) as peripheral 'effectors' of cochlear nonlinear biomechanics in the control of basilar membrane damping characteristics (Geisler, 1986; Kim, 1984; 1986; LePage, 1989; Mountain and Cody, 1989; Zenner, 1986a; 1986b).

This view is also supported by the observed medial efferent-induced reduction (e.g. Mountain, 1980; 1986; Nuttall, 1986) in the purely mechanical second order ($f_2 - f_1$) and cubic difference tone ($2f_1 - f_2$) intermodulation distortion components (Buunen and Rhode, 1978; Gibian and Kim, 1982; Javel, 1986; Kim et al., 1980; Siegel et al., 1982), and otoacoustic emissions (Glattke and Kujawa, 1991; Mott, Norton, Neely and Warr, 1989; Norton, Mott and Champlin, 1989). This view is additionally supported by the discovery of the contractile protein actin, and actin related molecules (such as myosin, α-actinin, fimbrin and tropomyosin) within the stereocilia, cuticular plate, and synaptic cell membrane regions of the OHCs (Flock, 1983; Flock, Flock and Ulfendahl, 1986; Lim, 1986; Lim et al., 1989; Pickles, 1988; Tilney, DeRosier and Mulroy, 1980; Zenner, 1986a; 1986b).

Additional corroborating evidence indicates that activation of the medial efferent system postsynaptically

mobilizes the intracellular release of endoplasmic reticular-bound Ca^{++} by liberating the lipid transmembrane second messenger IP_3. This triggers an actin-dependent OHC motile response (Dulon et al., 1988; Slepecky et al., 1988; Zenner, 1986a) in a manner similar to the phosphatidylinositol activity observed in smooth muscle (Cooper et al., 1986; Huganir, 1987; Schwartz, 1985b; Tsien, 1987). Presented below are some of the likely mechanical and ionic mechanisms involved in the medial efferent modulation of neural and transduction potentials, which were summarized above in Table 2.

Mechanism of Effects: By modulating the length, tension and stiffness of the OHCs along their longitudinal axis, an active biomechanical explanation has been singularly adequate to elucidate many actions of the descending medial efferent fibers. Perhaps medial efferent-induced changes in OHC shape damp the micromechanical activity of the OHCs and alter the coupling between outer and inner hair cells (i.e. Geisler, 1991; Mountain and Cody, 1989; Neely and Kim, 1986; Rhode, 1984; Zenner et al., 1989).

It has been suggested that by decoupling the OHC-tectorial membrane from the motion of the basilar membrane, the IHC stereocilia would move less in proportion to the movement of the basilar membrane (i.e. Guinan, 1986). The OHCs are securely attached at their perinuclear region, to the Dieter's cells (e.g. Santi, 1988), which are capable of stretching (LePage, 1989), and to the reticular lamina at

the OHC apex. A change in OHC length would also change the separation between the reticular lamina and the basilar membrane (Neely, 1989). Indeed, it has been demonstrated with optic probe investigations, that these OHC motor units can alter the shape of the basilar membrane independent of the traveling wave, changing its relative position to the tectorial membrane along select regions, which may serve to facilitate or damp sensitivity at select frequency bands (LePage, 1989).

Alterations in the mechanical properties of the organ of Corti following medial efferent activation would therefore result in a reduction of mechanical stimulation reaching the IHC stereocilia, subsequently leading to the observed reduction in the IHC depolarizing DC potential (e.g. Brown and Nuttall, 1984). Following from this, would be the additional reduction in the net quantity of released excitatory neurotransmitter at IHC-Type I afferent synaptic junctions (Brown and Nuttall, 1984; Brown et al., 1983a; Gifford and Guinan, 1983; Guinan, 1986; Guinan and Gifford, 1988a; 1988b; Kim, 1986). Such events would explain the observed amplitude decline in the gross discharge (N1 and N2) of auditory neurons, and the observed threshold elevations in single auditory fibers following medial efferent activation (e.g. Brown and Nuttall, 1984; Dewson, 1967; Fex, 1959; 1962; 1967; Galambos, 1956; Kiang, 1984; Konishi and Slepian, 1971a 1971b; Gifford and Guinan, 1983; 1987; Guinan and Gifford, 1988a; 1988b; 1988c; Wiederhold,

1970; 1986; Wiederhold and Kiang, 1970; Wiederhold and Peake, 1966; Winslow, 1988).

The damping of micromechanical activity by OHC contraction could also alter (by alterations in transduction currents) the sensitivity of the IHCs (Guinan, 1986; Kim, 1984; 1986; Nuttall, 1986; Siegel and Kim, 1982; Zenner, 1986a; 1986b) producing the observed reductions in both IHC tuning (Brown and Nuttall, 1984; Brown et al., 1983a; Nuttall, 1986), and conceivably in single auditory fiber tuning (Guinan and Gifford, 1988d; Wiederhold, 1970; 1986). Indeed, it is likely that contractile factors which reduce or dampen the resonating properties of OHCs, also dampen the resonances of other structures within the organ of Corti, including the IHCs (Pickles, 1988; Turner and Nielsen, 1983). The OHCs are also critical for the generation of single auditory fiber tuning curves (Liberman and Dodds, 1984b). More specifically the first OHC row, known to receive considerably more medial efferent terminals (i.e. Ginzberg and Morest, 1984; Liberman and Brown, 1986) contributes more to the generation of single unit tuning-curve tips than does the second or third OHC rows (Liberman and Dodds, 1984b). A medial efferent-induced reduction (positive shift) in the voltage amplitude of the negative (SP^-) summating potential (Fex, 1959; Konishi and Slepian, 1971b) is also consistent with a reduced transduction current shunt through the IHCs, and a damping of basilar membrane displacement (Johnstone and Johnstone, 1966).

It has never been apparent how an active micromechanical alteration in the organ of Corti could be called upon to explain the observed medial efferent-induced: (a) amplitude augmentation of the CM (e.g. Fex, 1959; 1962; 1967; Gifford and Guinan, 1987; Wiederhold, 1986; Wiederhold and Peake, 1966); (b) decline in the EP (Brown and Nuttall, 1984; Desmedt and Robertson, 1975; Fex, 1967; Gifford and Guinan, 1987; Konishi and Slepian, 1971a; 1971b); or (c) decline in single fiber spontaneous discharge (Guinan and Gifford, 1988a; 1988c; 1988d; Wiederhold and Kiang, 1970). Indeed, it has been suggested that the mechanical coupling of OHCs with IHCs via the OHC stereocilia and tectorial membrane is alone insufficient to explain all of the medial efferent effects observed in mammalian species (Guinan and Gifford, 1988a; 1988c; Wiederhold, 1986). The possibility of an additional medial efferent action which employs a passive ionic shunting mechanism, is discussed below.

Recall from an earlier review, that while most medial efferent fibers synapse with OHCs at their base, a smaller number also terminate higher, at the lateral circumnuclear regions of the OHCs (Altschuler and Fex, 1986; Engstrom and Ades, 1972; Iurato, 1974; Liberman and Brown, 1986; Lim, 1986; Spoendlin, 1966; 1969; Spoendlin and Gacek, 1963; Wright and Preston, 1973; Pujol and Lenoir, 1986; Smith and Rasmussen, 1965; Spoendlin, 1966). The iontophoretic application of the medial efferent neurotransmitter (ACh; see discussion below) to the basal synaptic region of the

OHCs produces a shortening of the cell, similar to the effects of a depolarizing current (Brownell et al., 1985), while ACh fails to produce a response if administered at locations distal to the basal region (Brownell et al., 1985). Such evidence has supported the view that the basal (not the lateral) portions of the OHC membrane are most important in effecting cochlear mechanics via initiating OHC contractions (Brownell et al., 1985; Mountain, 1980). These results also suggest that the more numerous, basally innervating medial efferent fibers, rather than the less numerous circumnuclear medial efferent fibers, may be the population of fibers primarily responsible for initiating the cascade (e.g. Niedzielski and Schacht, 1991) of intracellular second messengers, which produce depolarizing OHC contractions.

Hypothetically, a postsynaptic change in the membrane resistance of OHCs (i.e. Brownell, 1982; Mountain et al., 1980) or in Type I afferents, leading to either a K^+ efflux (e.g. Li et al., 1991) or CL^- influx (Hille, 1984a; Kandel, 1985a; 1985b; Lefkowitz et al., 1990), would result in cellular or neural hyperpolarization, respectively. In an early investigation of efferent function (Desmedt and Robertson, 1975), cochlear perilymphatic perfusions with low Cl^- solutions during midline efferent activation were reported to reduce both the medial efferent-induced suppression of the N1 and EP, as well as the CM potentiation. Desmedt and Robertson (1975) concluded that

medial efferent activation results in an increased OHC conductance to perilymphatic Cl^-, and other small anions, and that a Cl^- influx carries a medial efferent-OHC hyperpolarizing current. Recall as well, that OHC relaxation following contractile depolarization requires extracellular Ca^{++} in the presence of a bathing medium containing Cl^- (Dulon et al., 1988; Zenner 1986a; Zenner et al., 1985). Taken together with the demonstrated lateral OHC membrane, Cl^- conductance discussed earlier (Gitter et al., 1986; Zenner, 1986b), such evidence tends to suggest the existence of a hyperpolarizing Cl^- channel on the lateral surface of the OHCs. As discussed below, the hyperpolarizing influx of Cl^- may well play an important role in medial efferent-OHC physiology.

Possible Functional Segregation Of Medial Efferents: A Ca^{++}-dependent circumnuclear medial efferent (CME) induced Cl^- influx could act to stabilize and repolarize (Hille, 1984a) the OHC from expected net depolarizing effects following the contractile, IP_3-induced intracellular release of Ca^{++} by basal medial efferent (BME) activation. The possibility of a repolarizing inward Cl^- conductance (Gitter et al., 1986; Zenner, 1986b) is viewed as one component of a self-regulating medial efferent system. The membrane-associated cisternae of the OHCs are structurally similar to the sarcoplasmic reticulum of smooth muscle (Flock et al., 1986), which serve to internalize unbound intracellular Ca^{++} ions via a metabolic pump (Berridge and Irvine, 1984; Tsien,

1987). Perhaps a similar Ca^{++} pump within the OHC endoplasmic reticulum prevents excess depolarization caused by the release of cations, by regulating intracellular Ca^{++} levels.

A Passive OHC Shunting Mechanism: Fex (1962; 1967) was the first to suggest that the medial efferent system produces a drop in the EP by a shunting of transduction current through the hair cells. Indeed, an efferent-induced drop in the EP and an augmentation in the CM would be expected outcomes of an increased endolymphatic current shunt through the OHCs (Geisler, 1974b; Wiederhold, 1967; 1986; Pickles, 1988).

The hypothetical CME-induced Cl^- influx described above could alter the depolarizing OHC transduction (K^+) current, resulting in a current shunt directed away from the IHC transducer. For instance, activation of CME fibers, and producing an increase in intracellular anions (Cl^-), may increase the intracellular electrochemical gradient for cations. The effect of this might be to augment the apical K^+ inward conductance during positive OHC stereociliar displacement. The hypothetically augmented inward K^+ conductance might then lead to an even greater intracellular concentration gradient for K^+, producing an increased outward Ca^{++}-dependent conductance for K^+. Recall from an earlier discussion that a potassium channel indeed exists within the lateral membrane of the mammalian OHC (Gitter et al., 1986; Zenner, 1986b). Therefore, the combined increase

in inward and outward current brought about by a circumnuclear medial efferent-induced repolarizing Cl^- influx, may be the shunting mechanism responsible for the observed medial efferent-induced potentiation of the CM.

A CME-initiated shunting of K^+ transduction current through the rows of OHCs as described above, may also be the mechanism that leads to the observed decline in the scala media-generated EP. As discussed earlier, factors which reduce the EP, also reduce spontaneous discharge rates in single auditory fibers (Liberman and Dodds, 1984a; Sewell, 1984b), elevate auditory nerve threshold (Sewell, 1984a), reduce the IHC depolarizing DC current, and reduce IHC tuning (Brown et al., 1983b; Nuttall, 1984; 1985). Extracellular Ca^{++} is also essential for medial efferent-induced changes in the N1, CM and EP in the mammalian cochlea (Konishi and Kelsey, 1970). Indeed, it is highly likely that the presynaptic release of neurotransmitter from both BME and CME terminals synapsing upon OHCs is Ca^{++}-dependent.

Cholinergic receptors on OHCs may be nicotinic (e.g. Plinkert et al., 1991) or slower acting (Taylor, 1990b; Lefkowitz et al., 1990) muscarinic (Niedzielski and Schacht, 1991). Finally, ACh release from lateral efferent terminals may effect hyperpolarization in Type I fibers, by producing alterations in a Cl^- or a K^+ conductance. A summary of the effects of medial efferent activation, together with the possible mechanisms described within the text are summarized

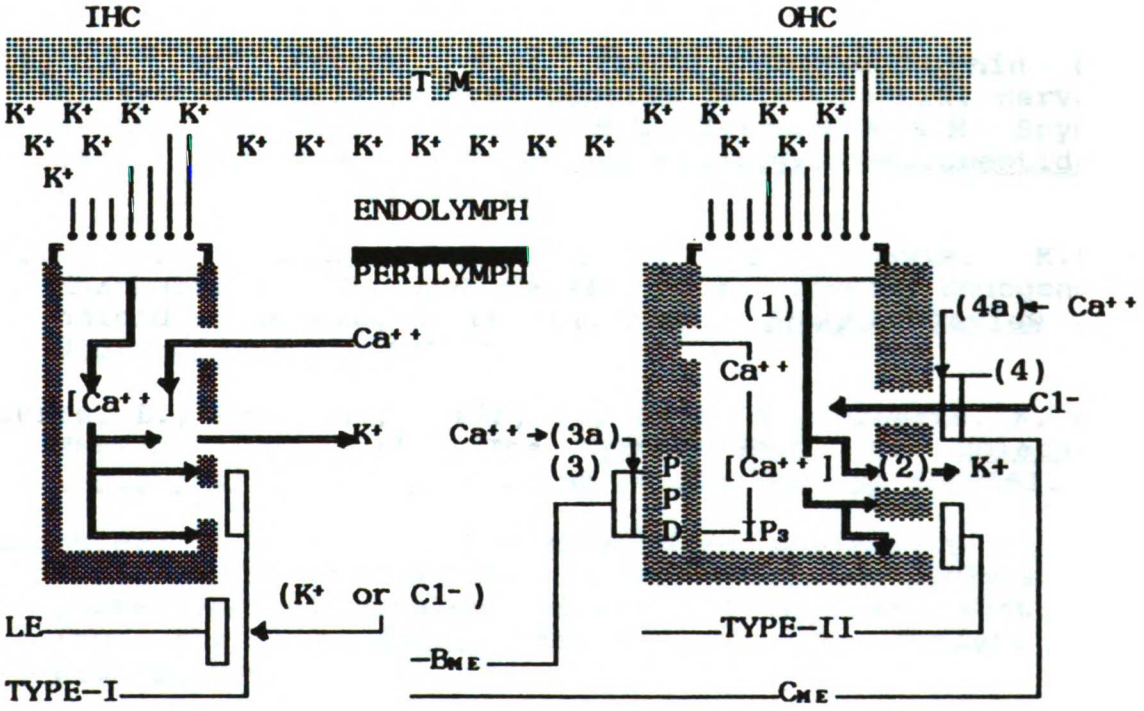
TABLE 11

Summary Table of Medial Efferent effects and possible mechanisms. There is as yet, no direct physiological evidence for the hypothesized basal medial efferent (B_{ME}) and circumnuclear medial efferent (C_{ME}) subsystems. Possible lateral efferent effects are not included here.

MEDIAL EFFERENT EFFECT	POSSIBLE MECHANISM(S)
↓ Distortion Products	BME-Induced OHC contraction mediated via IP_3 second messenger
↓ SP^-	same as above
↓ IHC DC Potential	OHC contraction or ↓ in EP
↓ IHC Tuning	same as above
↓ N1 and N2 Amplitude	same as above
↓ single unit driven discharge	same as above
↑ single unit threshold	same as above
↓ single unit tuning	same as above
↑ CM	CME-Induced Cl^- Influx Producing an OHC K^+ current shunt
↓ EP	same as above
↓ single unit spontaneous discharge	↓ in EP

Illustration 5

An integrated model of mammalian efferent physiology. As part of an active mechanical system, ACh is released from the medial efferent fibers. A Ca^{++}-dependent activation (3a) of the basal medial efferent (B_{ME}) fibers results in a postsynaptic hydrolysis of PPD, liberating diacylglycerol (not shown) and IP_3 as transmembrane second messengers. The second messenger IP_3 mobilizes Ca^{++} from internal stores and produces OHC depolarization. The unbound Ca^{++} triggers the contraction of actinomyosin filaments within the hair cell cytoskeleton. Alterations in the OHC shape are mechanically conveyed to the IHC by the stereocilia-tectorial membrane (TM) attachment. In the model, the IHC functions in a manner identical to nonmammalian hair cells described in the text. As part of a passive resistive system, a Ca^{++}-dependent (4a) circumnuclear-medial efferent (C_{ME})-induced Cl^- influx at resistance (4) repolarizes the Ca^{++}-induced OHC depolarization and also increases the intracellular electrochemical gradient for K^+, resulting in an increased K^+ influx at (1). The greater concentration of intracellular K^+ produces an increased current flow of K^+ through the resistance at (2). Lateral efferent (LE) release of ACh results in hyperpolarization of Type I fibers by activating either an inward Cl^- conductance, or an outward K^+ conductance.



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APPENDIX A

PERILYMPH

CHEMICAL	MOLARITY	M.W.	GMS
NaCl	130.00 mM	58.44	7.6
KCL	4.00 mM	74.56	0.3
$CaCl_2$	4.00 mM	147.00	0.59
$MgCl_2$	2.00 mM	203.30	0.41
HEPES	12.50 mM	238.30	2.98
Glucose	10.00 mM	180.16	1.80

add 650 uL of 10 N NaOH to adjust pH to 7.4. (This changes the NaCl to 136.00 mM)
Add everything to DI H_2O and bring up to a final volume of 1000 mL.

APPENDIX B

Modified Program; Experiments 2 and 3

```
options ls=78;

data a;
infile DATA MATRIX;
input wave $ level $ id trt r1 r2 r3 r4 r5 r6;
proc sort;
by wave level;


proc glm;
class trt;
title ' TIME EFFECTS ';
model r1--r6 = trt / nouni;
  manova h= intercept  m= -.50*r1-.50*r2+r3,
    -.33*r1-.33*r2-.33*r3+.33*r4+.33*r5+.33*r6
   mnames=baseline drug  /  short summary;
by wave level;


proc glm;
class trt;
title ' TIME x DRUG EFFECTS ';
model r1--r6 = trt / nouni;
  manova h= trt  m=  r1+r2+r3+r4+r5+r6,
   -.50*r1-.50*r2+r3,
     -.33*r1-.33*r2-.33*r3+.33*r4+.33*r5+.33*r6
   mnames=overall baseline drug  /  short summary;
by wave level;
```

Sample Test For Trends; Experiments 2 and 3

```
options ls=78;

data a;
infile DATA MATRIX;
input wave $ level $ id trt r1 r2 r3 r4 r5 r6;
meanint=mean(r4,r5,r6);
meanbase=mean(r1,r2,r3);
meanch=(meanint-meanbase)/meanbase;
proc sort;
by wave trt id level;

data foo;
        do i=1 to 4;
        set a;
        by wave trt id;
        if level='thresh' then int0=meanch;
        if level='fiveSL' then int5=meanch;
        if level='tenSL' then int10=meanch;
        if level='thirtySL' then int30=meanch;
        if last.id then do;
                output;
                return;
                end;
        end;
keep wave trt id int0 int5 int10 int30;

*proc print;

proc glm;
model int0 int5 int10=trt/nouni;
repeated intensity 3 ( 0 5 10 ) polynomial/summary;
by wave;
```