Modulation of Cochlear Afferent Response by the Lateral Olivocochlear System: Activation via Electrical Stimulation of the Inferior Colliculus

By

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ABSTRACT

The olivocochlear (OC) efferent innervation of the mammalian inner ear consists of two subdivisions, medial (MOC) and lateral (LOC), with peripheral terminations on outer hair cells and cochlear afferent terminals, respectively. The cochlear effects of electrically activating MOC efferents are well known: the MOC efferents suppress cochlear responses by reducing outer hair cells' contribution to cochlear amplification. In contrast, LOC peripheral effects are unknown, because their unmyelinated axons are difficult to stimulate.

To overcome the difficulty of directly activating the LOC system, stimulating electrodes were placed in the inferior colliculus (IC) to activate the LOC system indirectly, while recording cochlear responses bilaterally from anesthetized guinea pigs. Neural potentials and outer hair cell based potentials were recorded before and after IC stimulation for tens of minutes. Stimulation at some IC sites produced novel cochlear effects attributable to activation of the LOC system: long-lasting (1-20 min) enhancement or suppression of cochlear neural responses (compound action potentials (CAP) and round window noise), without changes in cochlear responses dominated by outer hair cells (otoacoustic emissions and cochlear microphonics). These novel effects also differed from classic MOC effects in their laterality and their dependence on level and frequency of the acoustic stimulus. The changes in CAP are well described as "constant %" increases or decreases. These effects disappeared upon sectioning the entire OC bundle, but not after selective lesioning of the MOC tracts or the cochlea's autonomic innervation.

Based on this evidence, I conclude that the LOC pathway comprises two functional subdivisions capable of inducing slow increases or decreases in response magnitudes in the auditory nerve. The LOC system originates in the lateral superior olive (LSO), which is the ascending nucleus where sound localization is computed. Such a system may be useful in maintaining accurate binaural comparisons necessary for sound localization in the face of slow changes in interaural sensitivity.

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BACKGROUND

MOC and LOC Neuroanatomy

First discovered by Rasmussen in 1946, the olivocochlear (OC) efferents are a collection of nerve fibers with somata in the superior olivary complex (SOC) in the brainstem, and axons that project to the organ of Corti (see Figure 1). Perfusion of the retrograde tracer, HRP, through the cochlea revealed labeled somata in the SOC that appeared to be spatially segregated (Warr 1975). Injecting tracer into the medial regions of the SOC labeled thick (0.5-2.75μm) myelinated axons to the outer hair cells (OHCs), while injections into the lateral regions labeled thin unmyelinated fibers to the afferent dendrites on the inner hair cells (IHCs) (Warr and Guinan 1979; Brown 2001). In guinea pig, the lateral (L)OC efferents were shown to be approximately 2000 in number and to project almost wholly (99%) to the ipsilateral cochlea, while the medial (M)OC efferents, about 1200 in number, project mostly (70%) to the contralateral cochlea (Robertson 1985). Figure 1 illustrates this anatomy in addition to demonstrating where crossing OC fibers (VanNoort 1969) run superficially, ventral to the floor of the fourth ventricle (4thV).

Centrally, the LOC system in rodents can be morphologically subdivided into small neurons "intrinsic" to the lateral superior olive (LSO), and larger neurons that lie around the "shell" of the LSO (Vetter and Mugnaini 1992). Peripherally, IHC efferents have thin axons that can be divided into two subclasses. The most numerous subclass enters the inner spiral bundle and turns to spiral in a "unidirectional" fashion for less than 1 mm and then forms many en passant and terminal swellings that are within both

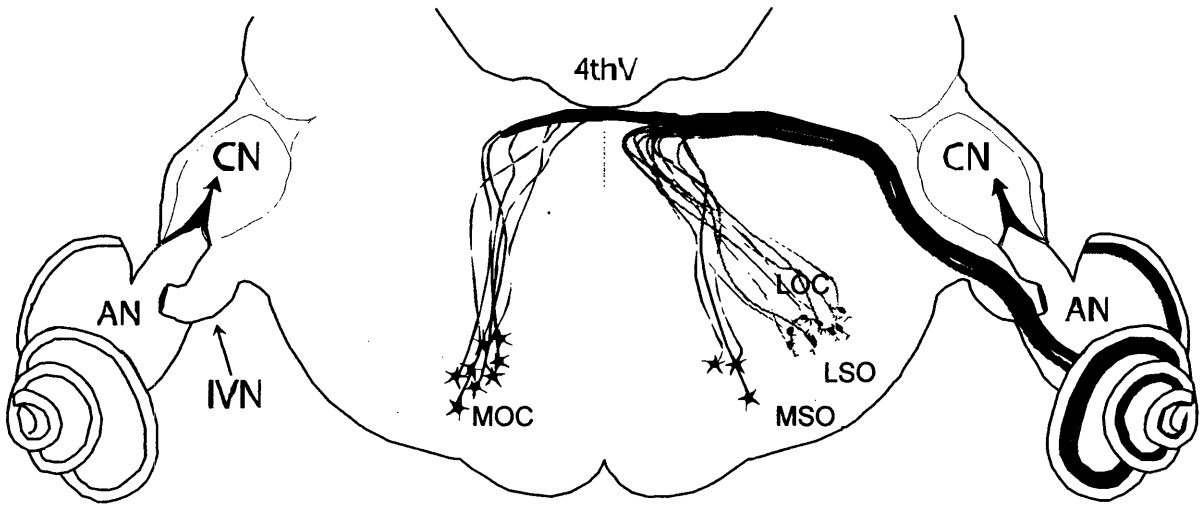


Figure 1: Schematic of transverse section through the brainstem at the level of SOC, illustrating the origins and course of the olivocochlear efferents to one cochlea. ~70% of MOC efferents originate contralaterally while ~99% of LOC efferents originate ipsilaterally. MSO: medial superior olive; LSO: lateral superior olive. (Adapted from Liberman 1990)

the inner and tunnel spiral bundles (Brown 1987). A less common subclass enters into the inner spiral bundle and bifurcates sending "bidirectional" branches both apically and basally that often course in the tunnel spiral bundle for a large portion of the cochlear spiral, forming terminals throughout their extended spiral course (Brown 1987). In guinea pig, focal injections of anterograde tracer that were within the LSO labeled "unidirectional" IHC efferents, while injections at the shell of LSO labeled "bidirectional" IHC efferents (Warr et al. 1997). Both of these LOC system subgroups projected almost wholly to the ipsilateral cochlea (Warr et al. 1997).

The terminals of the MOC and LOC systems have characteristic distributions along the full base to apex length of the cochlea. The number of MOC terminals per outer hair

cell peaks near the base of the cochlea around the 10 kHz CF region (Liberman et al. 1990). The bias of terminal endings toward the base is most pronounced in the cochlea contralateral to the MOC somata; as a result, the contralateral to ipsilateral ratio of terminal endings is roughly 3 at the base and about 1.5 at the apex (Guinan et al. 1984). The numbers of LOC efferent synapses per radial fiber, in contrast, are similar along all cochlear locations ipsilateral to the somata (Liberman et al. 1990). The high-threshold radial afferents on modiolar side of the hair cell receive significantly more efferent synapses than low-threshold radial fibers contacting the pillar side (Liberman et al. 1990).

The LOC system putatively contains many classic neurotransmitters (e.g., acetylcholine, GABA, and catecholamines; Eybalin 1993) and peptides (e.g., CGRP, opoiods, and urocortin; Eybalin 1993; Vetter, Li et al. 2002). It appears that peptides co-localize with classic neurotransmitters in the LOC system, for review see (Eybalin 1993). The extent to which the classic neurotransmitters co-localize or form distinct cytochemical groups is still a matter of debate. Some studies suggest that acetylcholine and GABA are mutually exclusive in the LOC system (Vetter et al. 1991), and others suggest that they are mutually inclusive (Safieddine et al. 1997; Maison et al. 2003a). The little evidence that LOC neurotransmitters correspond to anatomical subgroups appears contradictory, perhaps because the studies have been done across different species. The MOC system is primarily cholinergic (Sewell 1996), and GABA and CGRP appear to be extensively colocalized with acetylcholine in mouse and guinea pig (Maison et al. 2003a).

Peripheral Effects of 4thV Shocks

In 1956, Galambos placed stimulating electrodes near the OC bundle in the floor of the fourth ventricle (4thV) (see Figure 1) and demonstrated that 4thV shocks reduced the click-evoked compound action potential (CAP) recorded at the round window (RW). The CAP is the sum of synchronized action potentials of the auditory afferents innervating the IHCs (Kiang et al. 1976). It was soon noted that, in addition to reducing the CAP, 4thV shocks increased the cochlear microphonic (CM) (Fex 1962). 4thV shocks were later shown to shift basilar membrane displacement – sound level functions along the sound level axis by as much as 27dB (Murugasu and Russell 1996). Similar shifts were observed in the IHC receptor potential - level functions (Brown et al. 1983), and in firing rates of auditory afferent measured as a function of sound level (Wiederhold and Kiang 1970). Lastly, 4thV shocks affect otoacoustic emissions, and almost always decrease them (Mountain 1980; Siegel and Kim 1982). This suppression is typically greatest for low sound level primary tones (Moulin et al. 1993). The simplest interpretation of all these data is that MOC activation decreases basilar membrane motion and all downstream responses are suppressed (Narayan et al. 1998). For a review of the effects of 4thV shocks with additional acoustic stimuli, see Guinan 1996.

CAP suppression

Activation of the MOC system produces qualitatively similar reductions in both CAP (Gifford and Guinan 1987), and basilar membrane motion (Murugasu and Russell 1996). Indeed, recording both basilar membrane motion and auditory afferent firing rates together in the same animal revealed that only minor signal transformations intervene between mechanical vibration and auditory nerve excitation (Narayan et al. 1998).

Mechanical amplification, thought to be generated by OHCs, increases frequency-dependent hearing sensitivity by more than 40 dB (Dallos and Harris 1978; Dallos 1992). Amplification is driven by OHC electromechanical transduction, whereby trans-membrane voltage drives cellular length changes at audio frequencies (Brownell et al. 1985; Ashmore 1987) through conformational changes in a membrane protein, called prestin (Zheng et al. 2000). This transduction provides positive feedback essential for mechanical amplification (Robles and Ruggero 2001; Liberman et al. 2002).

Acetylcholine, which is released by the MOC system, increases basolateral OHC conductance (Erostegui et al. 1994), a change that has been shown to reduce the voltage driven OHC motility (Santos-Sacchi et al. 1998). When the MOC efferents release acetylcholine, it binds to OHC nicotinic receptors containing the alpha-9 subunit (Elgoyhen et al. 1994; Liberman et al. 1999), and probably an alpha 9 / alpha 10 heteromer (Oliver et al. 2001). The effects of activation of the MOC system on cochlear potentials are eliminated in mice carrying a null mutation for the nACh alpha-9 gene (Vetter et al. 1999). The nACh alpha-9 receptor has a nonspecific cation channel through which Ca^{2+} enters and triggers activation of nearby Ca^{2+}-dependent K+ channels thereby increasing basolateral K+ conductance (Housley and Ashmore 1991; Fuchs and Murrow 1992; Erostegui et al. 1994; Blanchet et al. 1996; Evans 1996). Increasing this basolateral OHC conductance (G_{MOC}, Figure 2) increases the current flow through stereocilia, the OHC, and consequently through the scala media and scala typani. It follows that sound-driven intracellular receptor potentials, which correspond to current flow through transmembrane resistance (R_r), decrease simultaneously with synaptic conductance G_{MOC} increases. The decrease in voltage across R_r decreases cochlear motility and thus cochlear amplification is reduced and afferent fiber thresholds go up.

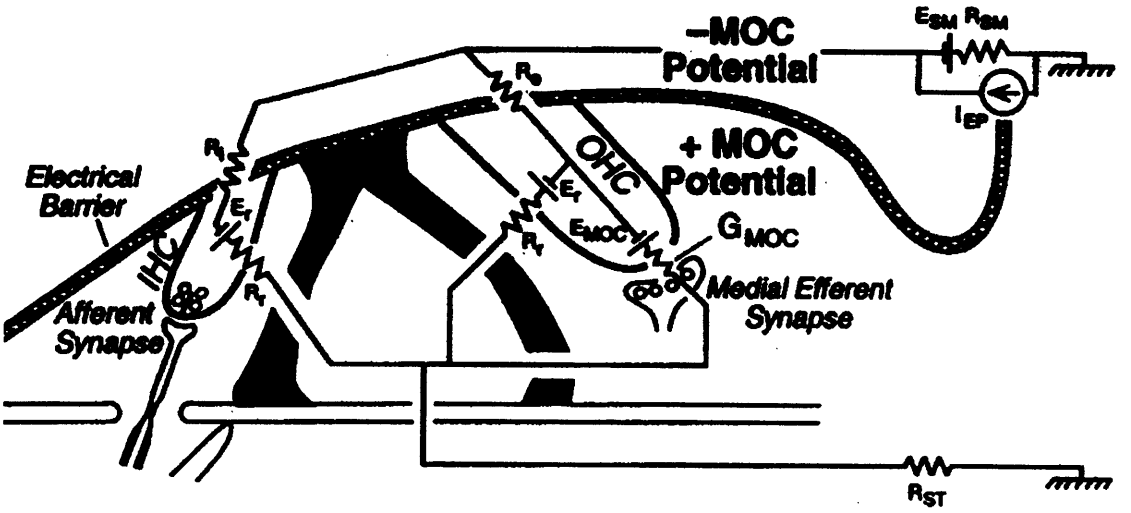


Figure 2: A discretized model of the main components affecting current flow through the cochlea. Release of acetylcholine by the MOC efferents increases the OHC conductance G_{MOC}, resulting in greater current from the scala media through the OHC to scala tympani, with a return path through distant tissue modeled by R_{ST} and R_{SM}. R_i and R_o represent the apical resistances of the hair cells that are modulated by sound driven motion of the organ of corti. E_r and R_r represent the equilibrium potential and resistance of the hair cells. E_{MOC} represents the equilibrium potential of the OHC efferent synapse. E_{SM} represents the equilibrium scala media potential. I_{EP} represents the stria vascularis current source driving the large endocochlear potential. Adapted from (Guinan 1996).

CM enhancement

4thV shocks reduce CAP while at the same time enhance CM: this can be explained by the electrical properties of the cochlea and the fact that CM is an extracellularly measured potential. The CM is produced by sound-driven opening and closing of ion channels in hair cell stereocilia (R_o and R_i in Figure 2), which create a sound driven variation of current through the hair cells (Dallos and Santos-Sacchi 1983) – primarily through the OHC (Dallos and Cheatham 1976) – and consequently through the entire cochlear partition. The current through the hair cells is driven by active current sources

(I_{EP} in Figure 2) in the stria vascularis (Prazma 1975), which are electrically connected to the hair cells by fluid and tissue, modeled here as R_{SM} and R_{ST}. The current through this distant tissue produce voltage potential changes, called cochlear microphonic (CM), that can be measured with an electrode on the round window and a reference electrode in the neck muscles. Increasing this basolateral OHC conductance (G_{MOC}, Figure 2; see previous section for description) increases the current flow through stereocilia, the OHC, and consequently through the scala media and scala typani. This increase in current increases the voltage potential across distant tissue causing an increase in the CM recorded from the round window (RW).

Modulation of DPOAEs

Distortion product otoacoustic emissions (DPOAEs) are distortion tones produced in the cochlea in response to two exogenous primary tones, f_1 and f_2 ($f_2 > f_1$), which are transmitted back through the middle ear to the external ear where they can be measured. These distortion tones appear to arise from cochlear nonlinearities probably originating in the saturating non-linearity of the transduction process, and coherent reflection off pre-existing micromechanical impedance perturbations in the cochlea (Kalluri and Shera 2001). Reducing mechanical amplification by activating the MOC efferents both reduces the amplification of distortion tones, and reduces the generation of distortion by the OHC non-linearity at the f_2 place of the basilar membrane (Kim et al. 1980).

Because of the complicated mechanisms that give rise to distortion products, it is difficult, if not impossible to translate changes in DPOAEs to changes in basilar membrane motion. The suppression of $2f_1$-f_2 DPOAEs is often less than the suppression of the CAP evoked by tone pips at the f_2 frequency (Puria et al. 1996), which is closely

matched to the suppression of basilar membrane motion (Dolan and Nuttal 1994; Murugasu and Russell 1996; Narayan et al. 1998). Although efferent stimulation usually decreases DPOAEs, enhancements are also occasionally observed (Mountain 1980; Siegel and Kim 1982). One possible explanation for the enhancement is that a two tone suppression paradigm is set up whereby reducing the suppressor tone with MOC stimulation causes an increase at the $2f_1$-f_2 frequency.

Effects on RW noise and spontaneous afferent firing

In addition to reducing sound-evoked auditory afferent firing, 4thV shocks can reduce "spontaneous" firing in the absence of exogenous sound (Wiederhold and Kiang 1970; Guinan and Gifford 1988b). Electrical coupling of OHC conductance changes to IHC transmembrane potential via changes in endocochlear potential (EP) appear to be a primary mechanism underlying this phenomenon. First, measurement of EP and spontaneous firing rates with and with out 4thV stimulation revealed highly correlated reductions in both measures (Guinan and Stankovic 1995). Second, 4thV shocks reduce EP by 2-4 mV (Konishi and Slepian 1971), and spontaneous firing is reduced by 1-7% for each 1 mV reduction in EP (Sewell 1984). A second hypothesis is that spontaneous rates are reduced by reductions in mechanical amplification of internal biologically generated noise (Guinan and Gifford 1988b), however, while this could be true, it does not provide a sufficient explanation given the observation that the best frequency tone-evoked responses of afferent fibers with very high thresholds (greater than biological noise levels in normal animals) are significantly reduced by activation of the MOC system (Guinan and Gifford 1988b).

A gross-potential measure of spontaneous auditory afferent firing has been proposed based upon the observation that, in the absence of acoustic stimuli, the electrical potential recorded at the round window demonstrates a spectrum with a peak around 800-1000 Hz that resembles that of the contribution of a single auditory afferent action potential to the RW potential (Kiang et al. 1976). Simultaneous recording (and cross-correlation) of spontaneous afferent firing and the RW potential revealed that each diphasic action potential contributed a single-unit voltage component to the RW potential independent of the afferent's characteristic frequency (Kiang et al. 1976; Prijs 1986). Kainic acid applied to the intact RW membrane eliminates CAP, spontaneous activity (Bledsoe et al. 1981), and the 800-1000 Hz peak in the RW Noise spectrum (Dolan et al. 1990). Consistent with selective destruction of auditory afferents, CM was not significantly affected in these experiments. The 800-1000 Hz peak in the RW Noise spectrum is also eliminated with tetrodotoxin, but not by ablating the cochlear nucleus, providing further evidence that it arises from auditory afferent firing (McMahon and Patuzzi 2002).

Contralateral noise stimulation (which also activates the MOC system, see Guinan 1996 for review) has been shown to suppress spectral components of RW Noise around 800-1000 Hz (Cazals and Huang 1996; da Costa et al. 1997; Lima da Costa et al. 1997). This result is consistent with the observation that activation of the MOC system with 4thV shocks suppresses the spontanteous activity of some auditory afferents (Wiederhold and Kiang 1970; Guinan and Gifford 1988b). Care must be taken in interpreting the change in RW noise: exogenous acoustic stimulation with continuous tones can suppress or enhance the spectral components around 800-1000 Hz depending upon the frequency, intensity, and bandwidth of the stimulus (Dolan et al. 1990; Cazals and Huang 1996). Single-tone suppression of spontaneous firing has not been observed

in mammals, thus it appears that enhancing firing in one region of the cochlea may potentially reduce the RW noise.

Effects on maximum firing rates

4thV shocks reduce the maximum firing of auditory afferents (Guinan and Gifford 1988a). Mechanically coupled changes do not explain this result since increasing the sound level should compensate for a reduction in gain. This result is better explained by EP-mediated electrical coupling. 4thV shocks reduce EP by 2-4 mV (Konishi and Slepian 1971), and spontanteous firing is reduced by 1-7% for each 1 mV reduction in EP (Sewell 1984). Furthermore, the time-course of the reduction in maximal firing is more highly correlated with the reduction in EP than with reductions in DPOAEs (Guinan and Stankovic 1995). It has also been suggested that the LOC system could play a role since changes at the level of the afferent dendrites could affect maximum firing. However, this hypothesis is inconsistent with the observation that mice carrying a null mutation for the nACh alpha9 gene, which is expressed in the OHCs but not in the spiral ganglion cells, failed to show CAP or DPOAE suppression during MOC stimulation (Vetter et al. 1999). Although only moderate sound levels were measured, it is likely that an LOC effect would be presented at moderate, as well as high sound levels. Finally, it is unlikely that the 4thV shocks activate the unmyelinated and uncrossed LOC fibers since moderate shock levels do not activate small fibers from a distance (Ranck 1975).

The MOC System Mediates the Effects of 4thV Shocks

As outlined above, CM enhancement coupled with CAP and DPOAE suppression from 4thV shocks is well explained as arising from an OHC-based effect. Given that it

is not unequivocal that OHCs only receive projections from the MOC system (Vetter et al. 1991), it is worth exploring several additional observations that reinforce the conclusion that this classic constellation of effects of 4thV shocks is mediated by the MOC system.

Stimulation in the medial and lateral regions of the SOC, both produced bilateral CM enhancement and CAP suppression qualitatively similar to that seen with 4thV stimulation, although higher shock levels were required when stimulating the lateral regions (Gifford and Guinan 1987). For both locations the greatest suppression of CAP was seen with shocks between 200-400 Hz (Gifford and Guinan 1987), a rate that is consistent with activation of myelinated neurons (Ranck 1975). Unmyelinated fibers, in contrast, are best activated by shocks between 10-100 Hz (Ranck 1975).

Stimulation in the medial zone of the superior olive produced CM and CAP effects 2.6 times as large in the contralateral ear than in the ipsilateral ear (Gifford and Guinan 1987), consistent with the fact that over 2/3 of MOC neurons project contralaterally (Guinan et al. 1984). The summation of the ipsilateral and contralateral CAP and CM changes was approximately equal to those produced by 4thV shocks (Gifford and Guinan 1987), suggesting that 4thV shocks activate both crossed and uncrossed MOC fibers.

4thV shocks and shocks in the region of MOC somata both produced CM enhancement and CAP suppression that was blocked by strychnine (Desmedt and Monaco 1962; Gifford and Guinan 1987), suggesting that these effects were mediated by a common peripheral receptor. Finally, mice carrying a null mutation for the nACh alpha9 gene, which appears to have restricted expression in the cochlea that includes the OHCs but not the afferent dedrites (Elgoyhen et al. 1994), failed to show suppression of

cochlear responses (CAP, DPOAEs) (Vetter et al. 1999) when shocks were delivered to the 4thV.

The MOC System Mediates Both Fast and Slow Effects of 4thV Shocks

In addition to MOC fast effects, 4thV shocks have been shown to produce slow onset and offset suppression of auditory afferent potentials in guinea pig (Sridhar et al. 1995). The magnitude of this suppression builds up to a maximum in about 1 minute, and then begins to diminish through the end of the shocks, after which the remaining effect decays exponentially with a time constant of roughly 0.6 minutes (Sridhar et al. 1995).

The CAP suppression of both slow and fast effects is mirrored by increases in CM (Sridhar et al. 1995). Both effects are blocked by similar concentrations of nicotinic blockers (curare, decamethonium, hexamethonium), muscarinic blockers (atropine, 4-DAMP), glycinergic (and α9 nAChR) antagonist strychnine, and bicuculline, which is a GABA antagonist shown to block acetylcholine effects on OHCs (Kujawa et al. 1994; Sridhar et al. 1995). Both exhibit suppression that is greater when measured in response to low sound level acoustic stimuli (Sridhar et al. 1995). Lastly, both have a similar dependence on 4thV shock rate and are extinguished by lateral 4thV incision, which should sever both the LOC and MOC systems (Sridhar et al. 1995).

Predicting Effects of LOC System Activation from First Principles

Hypotheses of LOC efferent function can be derived from LOC neuroanatomy, pharmacology, LOC lesions, and from analogies to other hair cell systems. Given that the LOC system synapses on the afferent dendrite / IHC complex (Smith 1961; Liberman 1980), their effect is expected to include changes neural potentials (i.e., CAP and RW noise), but not OHC-based potentials (i.e., CM or DPOAE). Such an effect

would be consistent with observations that exogenous application (Sahley and Nodar 1994; d'Aldin et al. 1995) and genetic elimination (Maison et al. 2003c) of putative LOC neurotransmitters affect neural but not OHC-based potentials. Other less likely possibilities exist, including distant LOC effects on OHCs as has been suggested for urocortin (Vetter et al. 2002), or even direct LOC efferents projections to the OHCs as suggested by Vetter and colleagues (Vetter et al. 1991), or effects on afferent firing might change the sound driven MOC reflex feedback loop and thereby indirectly affect OHC function (Liberman and Brown 1986).

Putative LOC neurotransmitters, when perfused through the cochlear scalae, produce effects on neural potentials: 1) Putative LOC neurotransmitters both enhance (e.g., acetylcholine, dynorphins, and CGRP (Felix and Ehrenberger 1992; Sahley and Nodar 1994)), and suppress (e.g., GABA, enkephalin, and dopamine, (Felix and Ehrenberger 1992; Burki et al. 1993; d'Aldin et al. 1995)) acoustically or neurochemically driven neural potentials; 2) Putative LOC neurotransmitters both enhance (Felix and Ehrenberger 1992) and suppress (Burki et al. 1993) spontaneous neural potentials, i.e., neural firing in the absence of exogenous stimulation; 3) Putative LOC neurotransmitters suppress and enhance CAP by nearly a constant % (or dB) change independent of the sound level of the acoustic stimulus (d'Aldin et al. 1995; Maison et al. 2003a), whereas effects on the OHCs (e.g., from MOC stimulation or furosemide application) reduce CAP and basilar membrane responses to low level acoustic stimuli at least an order of magnitude more than to high level acoustic stimuli (Desmedt 1962; Ruggero and Rich 1991; Murugasu and Russell 1996). This suppression and enhancement are qualitatively complementary, suggesting that the LOC system may have complementary subsystems that could cancel one another if simultaneously activated.

Cochlear de-efferentation of cats (Liberman 1990) and chinchillas (Zheng et al. 1999) resulted in a decrease in spontaneous activity of cochlear afferent fibers, suggesting that the LOCs may play a role in setting spontaneous rates, perhaps through tonic excitatory input, or via effects on expression levels of receptors for afferent transmitter. The lack of significant change to the neural thresholds or sharpness of tuning (Liberman 1990; Zheng et al. 1999) suggests that neither the MOC or LOC system provides tonic input to the OHC system or affect the diversity of auditory afferent thresholds, which have been shown to correlate with morphological differences in the caliber and location of afferent dendrite terminals on the body of the inner hair cell (Liberman 1982). Neither LOC lesions nor application of putative LOC neurotransmitters shifted cochlear response curves along the sound level axis. Both lesions and putative neurotransmitters can change spontaneous cochlear potentials as well as those evoked with high level acoustic stimuli (d'Aldin et al. 1995; Zheng et al. 1999; LePrell et al. 2003); activation of the LOC system might produce similar effects.

Analogy to other hair cells systems suggests that the LOC system may enhance cochlear neural potentials (i.e., driven rates, spontaneous rates, CAP, and RW noise) and that its effects may be long-lasting (minutes). Hair cells and sensory neurons in the vestibular system and lateral line system also receive an efferent innervation. In contrast to the mammalian cochlea, in these other systems the myelinated efferent fiber component contacts both hair cells and nerve fibers. Thus, we may infer something from these systems about the effects of axo-dendritic efferent synapses. Stimulation of toadfish vestibular efferents, which terminate on primary vestibular afferents (Lindeman 1973), increased both spontaneous and rotationally-driven primary afferent responses (Highstein and Baker 1985). The largest effects were on low spontaneous rate fibers (Highstein and Baker 1985), which is interesting in light of the observation that low

spontaneous auditory afferents receive the greatest number of LOC fibers (Liberman et al. 1990). Electrical and acoustic activation of the myelinated vestibular efferents terminating on vestibular primary afferents excited afferent firing (McCue and Guinan 1994). CGRP, which is present in at least one class of efferent fibers in every hair cell system studied (Adams et al. 1987; Takeda et al. 1987; Sliwinska-Kowalska et al. 1989; Wackym et al. 1991), has been shown to enhance spontaneous firing by increasing neurotransmitter release from Xenopus lateral line hair cells (Sewell and Starr 1991). Additionally, CGRP application reduces driven firing in the Xenopus lateral line organ for tens of minutes after the washout of CGRP (Bailey and Sewell 2000).[1]

[1] Most putative LOC neurotransmitters use 7TM receptors and G-proteins, which commonly set up cascades that last seconds to minutes. It should be noted that the anesthesia in the current experiments could potentially affect the time-course of action of the LOC system.

INTRODUCTION

The peripheral effects of activating the LOC system are unknown. All reported effects of stimulating the OC system appear to be mediated by the relatively fewer fibers constituting the MOC system (Guinan 1996). En route from the olivary complex to the cochlea, OC fibers coalesce into a bundle that arcs dorsally to run superficially beneath the floor of the 4th ventricle (4thV). Since the 1950's, it has been known that electrical stimulation at midline of the 4thV leads to suppression of cochlear responses, i.e., CAP (Galambos 1956), cochlear afferent firing (Wiederhold and Kiang 1970), intracellular responses from hair cells (Brown and Nuttall 1984), otoacoustic emissions (Mountain 1980; Siegel and Kim 1982) and basilar membrane motion (Murugasu and Russell 1996). All of these cochlear responses showed OC-mediated suppression of responses to low-level sounds that are consistent with well-studied MOC effects on the outer hair cell based "cochlear amplifier" (Guinan 1996). The effects of the LOC system, in contrast, are not well studied because no strategy has been uncovered to activate them.

A new approach was taken to activate the LOC system in the current study. Thin unmyelinated fibers, like those of the LOC system, are difficult to activate directly with electrical shocks (Ranck 1975; Gifford and Guinan 1987). We reasoned that the LOC system might be more easily activated indirectly. The inferior colliculus is a way station for ascending and descending auditory pathways (Nieuwenhuys 1984; Huffman and Henson 1990). The bilateral degeneration of axons projecting into the LSO seen following IC ablation (VanNoort 1969) suggests that the LOC system may also be a target of IC projections.

Building on the hypothesis that IC stimulation could activate the LOC system, we sought to identify novel changes in cochlear responses following IC stimulation, i.e., changes fundamentally different from those produced by the MOC system. IC stimulation, like 4thV stimulation, can activate that MOC system (Mulders and Robertson 2000). Indeed, in the present study stimulation at some IC sites produced MOC fast effects, and also MOC slow effects (see Sridhar at al. 1995 for description). But additionally, two other classes of changes in cochlear responses were identified in the present study. One class enhanced neural responses (i.e., CAP, and round window noise, a putative measure of spontaneous neural activity), without changing potentials arising presynaptic to the inner hair cell synapse (i.e., CM and DPOAEs). The second class suppressed neural responses, but not presynaptic, OHC based, responses. Both classes were characterized by long-lasting (i.e., minutes) changes in CAP that were largest when evoked with high sound level tones. Plotting this change in CAP on a logarithmic scale revealed a "constant % change" for all sound levels.

The major efferent pathways to the cochlea were surgically sectioned to test the hypothesis that these two novel effects are mediated by the LOC system. Both classes of novel cochlear changes remained following lesions that selectively interrupted the bulk of the MOC system but left the LOC system intact. Lesions that unilaterally interrupted both the LOC and MOC systems eliminated the novel effects on the cut side; the uncut side was not affected. Lastly, elimination of the source(s) of autonomic fibers to the cochlea failed to reduce novel effects. Based on these lesion data, it is concluded that IC stimulation can be used to activate the LOC system, and that the LOC system can both suppress and enhance peripheral neural potentials.

The idea that the LOC system can produce dual and complementary effects in the periphery, i.e., long-lasting suppression or enhancement of neural responses, fits well with other observations. LOC system is heterogeneous with respect to neurotransmitters. Application of putative transmitters to hair cell systems can have excitatory (e.g., opioids (Sahley and Nodar 1994)), inhibitory (e.g., dopamine (Ruel et al. 2001), GABA (Arnold et al. 1998)), or mixed (e.g., CGRP (Bailey and Sewell 2000)) effects on cochlear neural responses. The idea of a dual LOC peripheral action also fits with anatomical evidence, both in the brainstem and in the periphery, that there may be at least two classes of LOC neurons, e.g., cholinergic versus GABAergic (Vetter et al. 1991), unidirectional versus bidirectional with respect to cochlear projection patterns (Brown 1987), or intrinsic versus shell with respect to soma location in the brainstem (Warr et al. 1997).

METHODS

Animals and Anesthesia: Albino guinea pigs (250-650 g) of either sex were anesthetized with Droperidol (10 mg/kg, i.m.), fentanyl (0.2 mg/kg, i.m.), and Nembutal (25 mg/kg, i.p.). Boosters were given every two hours, or upon elevation in heart rate: droperidol and fentanyl (1/3 original dose), and Nembutal (1/5 original dose).

Surgery: Following the administration of anesthesia, a tracheotomy was performed. The animal was intubated and connected to a mechanical respirator. To eliminate effects of middle ear muscles, paralysis was induced with turbocurarine (1.25 mg/kg, i.m.) in the majority of animal preparations, and maintained by re-injection every 2 hours. Respiration volume was adjusted to maintain CO_2 levels between 4-5% during the experiment. The temperature in the experimental chamber was maintained near 33°C, as needed to maintain the animal's rectal temperature at 36-39°C. The animal was placed in a custom head-holder adapted to a stereotaxic apparatus. The posterior cranium and bullas were exposed, and the cartilaginous portion of the external auditory canals were cut. Small holes were shaved in the posterior aspect of the bullas to gain access to the round windows bilaterally. Silver-ball electrodes were centered on the round window (RW) membrane, and held in place by wax that covered but did not seal the bullar cavities. A posterior craniotomy was made extending anteriorly to the tentorium cerebelli, posteriorly near the foramen magnum, and laterally about 4mm. The cerebellum was aspirated to expose the floor of the 4thV, and the inferior colliculi.

Acoustic Stimulation and Response Measurement: Etymotics Research ER10c microphone / dual sound-source assemblies were bilaterally sealed in the ear canal. The ER10c microphone was calibrated in a coupler to a reference microphone (Bruel & Kjaer 1/4" condenser microphone). Digital stimulus generation and response digitization were synchronously and simultaneously sampled at 100 kHz using I-O boards (PCI-6052E, National Instruments) in a PC-driven data acquisition system under LabVIEW Control. Acoustic stimuli included tone pips (5-ms duration with 0.5 ms rise-fall times ($\cos^2$ shaping), clicks (100 μs duration) and continuous tones to elicit distortion product otoacoustic emissions (DPOAEs).

Simultaneous response measures were always obtained in both cochleae. Responses extracted from the round-window signal, including the compound action potential (CAP), the cochlear microphonic (CM) and the round-window noise, were amplified 10^4X by Grass amplifiers, filtered (0.3 - 30 kHz) before digitization and averaging. Ear canal sound pressure (for measurement of distortion product otoacoustic emissions and monitoring of the levels of the primaries) was amplified 100X by the Etymotics pre-amp. Averaged round-window responses and ear canal sound-pressure waveforms were streamed to the disk for offline analysis using a dual processor PC running custom LabVIEW software.

To facilitate comparisons of CAP, CM, 800-1000 Hz RW noise, and DPOAE data, response amplitudes were converted to a decibel scale and normalized to a five-minute pre-shocks window. Many trials exhibited slow baseline drift. This was removed by fitting a straight line to 1-minute data windows ~20 minutes before and after the shock epoch, and then subtracting out that fitted line. The slope of this line was maximally 0.03 dB / min.

Stimulating Electrodes and Shock Trains: All shock trains were generated with National Instrument's PCI-6111 16-bit D/A converter. Before paralysis, a bipolar stimulating electrode fashioned from two fine platinum wires (0.005") with a 1 mm tip separation was placed on the OCB at the floor of the 4thV, and the facial twitch threshold determined. For 4thV stimulation, shock trains consisted of 0.15 or 0.3 ms monophasic pulses delivered at 300-333 Hz, 10 dB above the twitch threshold determined at the 4thV (no facial twitch was observed when stimulating the IC at this levels).

Two types of bipolar electrodes were used to stimulate the IC. Early experiments, including most level-function experiments, used a bipolar electrode made of two fine platinum wires (0.005") with a tip separation of 1 mm. Later experiments, including all time-function experiments, used a Microprobe® 5 kΩ platinum bipolar electrode with a tip exposure of 125μm and tip separation of 500μm. Both types of electrode were inserted to a depth of 0.5 - 1.0 mm. The locations of IC stimulation sites were marked, based upon visual inspection, on a diagram of the caudal surface of the IC. The IC shock trains consisted of 0.2 - 1.0 ms (0.8 ms typical) alternating polarity pulses, delivered at 50 - 200 Hz (100 Hz typical), at a level 5-15 dB (10 dB typical) above twitch threshold determined at the 4thV.

In one experiment, electric shocks were delivered to the LSO with a monopolar 15kΩ tungsten electrode with tip exposure of 125 μm, and with a 1 μl Hamilton syringe that was insulated except for 0.5 mm at the tip. In both cases, the reference cathode was in the neck muscles. The tungsten electrode was placed into the LSO stereotaxically while recording gross responses to ipsi, contra, and bilateral stimuli in a manner described elsewhere (Guinan et al. 1972). The Hamilton syringe was placed along the

same path as the tungsten electrode. LSO shock trains were 40Hz, pulses were 1 ms duration alternating polarity, delivered at levels of ~ 1 volt. Electrode location was verified in Nissl-stained brainstem sections (see below).

Measurement Paradigms: Two paradigms were used. All measures were made simultaneously in the two ears.

For the first ("CAP-level function") paradigm, only tone-pip-evoked CAP was measured, as a function of tone-pip level, with and without shocks. One pip was presented every 500 ms; the shock trains were 350 ms in duration, with the last pulse ending 10 - 20 ms before the tone pip. Responses to 16 pips were averaged at each level, and three level functions were measured in succession: without, with, and then again without the shocks.

For the second paradigm ("time-function paradigm"), a number of measures of cochlear response, including CAP, CM, DPOAEs, and 800-1000 Hz RW noise, were obtained every 13 seconds (Figure 3B). These measures we made before, during and after a shock epoch lasting from 1 - 5 minutes (Figure 3A). During the first 8 seconds of each 13 second repeating period, CAP and CM were measured: one of four acoustic stimuli was presented every 125 ms alternating among a) 13 kHz tone-pip @ 15dB above CAP threshold, b) 3 kHz tone-pip @ 35dB above CAP threshold, c) 13 kHz tone-pip @ 50 dB above threshold, and d) a 0.1 ms click @ 50dB above CAP threshold (Figure 3C). CAP threshold was defined as the stimulus level required to evoke a 20uV response. After one quartet of stimuli was presented, polarities were reversed and another quartet presented; this 1-s octet (Figure 3C) was repeated 8 times, and separate averages of the RW response to each stimulus computed. After each 8 s ensemble of CAP measurements, a tone pair was presented for 1 s and DPOAEs measured, followed

by 4 sec of silence in which RW noise was measured (Figure 3B). For DPOAEs, f2 was always at 13 kHz (f_2:f_1 = 1.2), and f_2 level was 10 dB < f_1 level. The levels of the primaries were 20 dB greater than that required to elicit a $2f_1$-f_2 DPOAE of -5dB SPL.

Figure 3

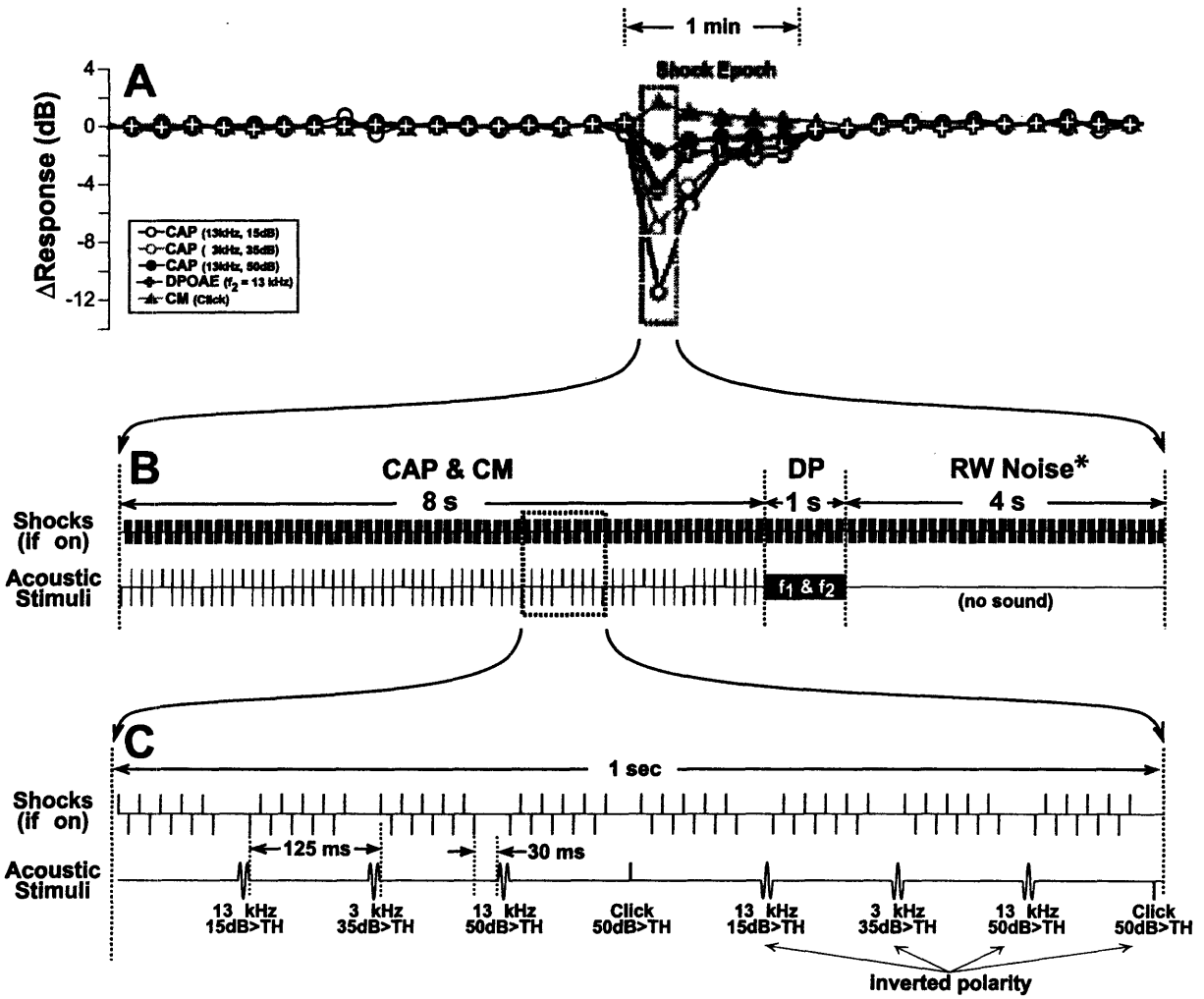


Figure 3: Schematic illustration of one measurement trial of the time-function paradigm **(Panel A)**, and the timing of stimulus presentation and shock trains during one 13-second repeating unit **(Panels B and C)**. See text for further details. *RW noise was recorded only before and after the shock epoch.

This 13-s interleaving series of acoustic stimuli was repeated *ad infinitum.* During shock epochs, the shock pulse train was presented at a 75% duty cycle: a 30 ms pause was inserted every 125 ms, with 25 ms preceding the tone pip or click (Figure 3C). The same shock pattern was maintained through the 1-s DPOAE measures and the 4-s silent period. RW noise was not measured during the shock epochs due to shock artifacts. Before and after the shock epochs, RW noise was measured at the time point indicated in Figure 3B.

To extract, from these time functions, the magnitude and time constant of long-lasting post-shocks effects on response measures, the normalized post-shock data for each measure, from each trial, were fit with an exponential function of the form

$$A*e^{(-t/T)}$$

where A is the initial post-shock magnitude of the change in response in dB, and T is the time constant characterizing the rate of return toward pre-shock baseline. A non-linear algorithm minimizing the mean squared error was used. This algorithm required initial parameters, set by first fitting the data with a straight line.

Pathway lesions: Three types of surgical lesions were performed, in various combinations, to elucidate the pathways mediating the shock-evoked effects under study: 1) transections of the crossed OCBs via a shallow cut at the midline on the floor of the 4thV; 2) unilateral transections of the entire OCB via a deeper cut positioned laterally at the sulcus limitans on the floor of the 4thV; and 3) unilateral surgical removal of the superior cervical ganglion (SCG).

OCB transections were performed with a surgical microknife using well-characterized surface landmarks on the floor of the 4thV (Kujawa and Liberman 1997). In selected cases, the bundle transections were verified histological in acetylcholinesterase stained brainstem sections of aldehyde fixed animals (Osen and Roth 1969).

To ablate the superior cervical ganglion (SCG), the ganglion was first dissected from underneath the bifurcation of the carotid artery, and then removed by cutting the sympathetic trunk, the branch joining the facial and vagus nerve through the auricular branch of the vagus, and the sympathetic branches to the base of the skull. The surgical approach was based on descriptions in the literature (Terayama et al. 1966; Spoendlin 1981; Hultcrantz et al. 1982).

RESULTS

Overview

Experimental objectives were twofold. First, we aimed to identify novel changes in cochlear responses produced by stimulation in the inferior colliculus (IC). We hypothesized that IC stimulation might activate the LOC system, and affect the cochlea differently than activation of the MOC system with 4thV stimulation. Our second aim was to use surgical lesions and bundle transections to determine the neural pathways mediating novel cochlear changes produced by IC stimulation.

IC stimulation produced two classes of novel cochlear effects, introduced in columns A and B of Table 1. In the discussion section it will be concluded that these two classes of effects are mediated by the LOC system, and that they are the same class of effects observed with stimulation in the LSO near LOC somata (columns D and E). The naming shorthand *CAP[+, -, or 0] CM[+, -, or 0]* used in these columns indicates the signs of the changes in CAP and CM. The sign of CAP and CM were sufficient to create robust classes of effects reinforced by all other measures (e.g., the sign of DPOAE and 800-1000 Hz RW noise).

In addition to the two classes of putative LOC effects, IC stimulation produced changes in cochlear function that resemble *MOC[slow]* effects (column C) and *MOC[fast]* effects (column G). *MOC[slow]* and *MOC[fast]* are MOC mediated effects, defined by Sridhar et al. using 4thV shocks, that have offset time-courses of 0.1 and 40 seconds, respectively. 4thV stimulation in the current experiments replicated these effects (columns F and H).

Table 1

		Slow. Post-Shock Effects						Fast. During-Shock Effects		
Shock Site		Inferior Colliculus			LSO		4thV	IC	4thV	2
Time Function Category		CAP[+]CM[0]	CAP[-]CM[0]	CAP[-]CM[+]	N A	N A	MOC[slow]	MOC[fast]	MOC[fast]	3
Level Function Category		CAP[+]	CAP[-]	N A	CAP[-]	CAP[0]	N A	MOC[fast]	MOC[fast]	4
Post Synaptic	ΔCAP	↑↑ / ↓↓	↓↓ / ↑↑	↓	↑	↓	↓	↓	↓	5
	ΔRW	↑	↓	↓	N.A.	N.A.	↓	N.A.	N.A.	6
Pre Synaptic	ΔCM	↔	↔	↑	N.A.	N.A.	↑	↑	↑	7
	ΔDP	↔	↔	↕	N.A.	N.A.	↓	↓	↕ *	8
ΔCAP Characteristics	Offset Time Constant (sec)	540 / > 9	60 / > 9	40	> 9	> 9	40	< 9	0.1*	9
	Laterality Contra:Ipsi	1.2	1.0	~2	Ipsi Only	Ipsi Only	1.0	~2	1.0	10
	Level Dependence Low SPL:High SPL	1.0 - 2.5 / -2 - 2	0.5 - 2.0 / 1 - 2	4 - 9	~ 1	~ 1	9 - 10	13.7	10.7	11
SCG Ablation		No Change	No Change	N.A.	N.A.	N.A.	N.A.	No Change	No Change	12
COCB (MOC) Lesion		No Change	No Change	N.A.	N.A.	N.A.	N.A.	Contralateral Elimination	N.A.	13
Unilateral OCB (MOC+LOC) Lesion		Unilateral Elimination	N.A.	Unilateral Elimination	N.A.	N.A.	Unilateral Elimination*	Unilateral Elimination	Unilateral Elimination	14
Putative Source		LOC	LOC	MOC	LOC	LOC	MOC	MOC	MOC	15
		A	B	C	D	E	F	G	H	

Table 1: A tabular summary of results, with post-shock effects (columns A-F) listed separately from during-shocks effects (columns G and H), as indicated by the labels across the top and the column labels at the bottom. Each column represents a different effect category: category names appear in the 3rd and 4th header rows (for time-function and level-function paradigms, respectively). Effect categories are listed twice if they were evoked with stimulating electrodes in different brainstem regions (regions indicated in the 2nd header row). For IC evoked effects (Columns A-C, G), corresponding time-function (black) and level-function (gray and right to diagonal) names are indicated. In the results cells beneath, data from the two paradigms (when available) are entered on opposing sides of the diagonal lines. In the top 4 rows of results, arrows indicate the direction of change of the cochlear metric indicated at the left of the row; magnitude of change is coded by the size of the arrow: three sizes are used. N.A. indicates data not available. Entries indicated by asterisks are derived from other studies: ΔDP and offset time constant values in column H are from (Siegel and Kim 1982) and (Warren and Liberman 1989), respectively; the results of unilateral OCB lesions in column F is from (Sridhar et al. 1995).

Table 1 provides a summary and organizing framework for the complex array of effects that are presented in the results section. To understand in detail the characteristics of each class of effects, one must keep in mind four important variables. First, consider the brainstem stimulation site: IC, LSO, or 4thV (see row 2). Second, consider whether effects were fast enough to classify during brainstem stimulation, or slow and therefore classified after brainstem stimulation (see row 1). Third, consider whether the "level function" paradigm or the "time function" paradigm was used to evaluate the effects of brainstem stimulation on cochlear responses; these two paradigms correspond to two separate experimental series. Fourth, consider collectively the sign, magnitude, laterality, time-constant, sound level dependence, and frequency dependence of changes in postsynaptic (i.e., neural) and presynaptic (i.e., OHC-based) responses produced by brainstem stimulation (see rows 5-11). Each effect class is defined by a unique set of changes along these dimensions. Focus is placed on columns A and B, which represent putative LOC effects evoked by IC stimulation.

First Experimental Series: CAP-Level Functions

Overview

Stimulating electrodes were placed at 41 different sites across the surface of the IC (Fig. 4G) in 14 guinea pigs. In nine of these experiments, stimulating electrodes were also placed on the OCB at the floor of the 4thV so that explicit comparisons of cochlear effects could be made. In one experiment, stimulating electrodes were advanced stereotaxically into the LSO, the site of origin of the LOC system.

The experimental paradigm in the first series bilaterally measured tone-pip evoked CAP amplitude-vs.-sound-level functions ("level functions") and compared the CAP amplitudes measured without shocks to those measured with shock trains delivered unilaterally to the IC, LSO, or 4thV.

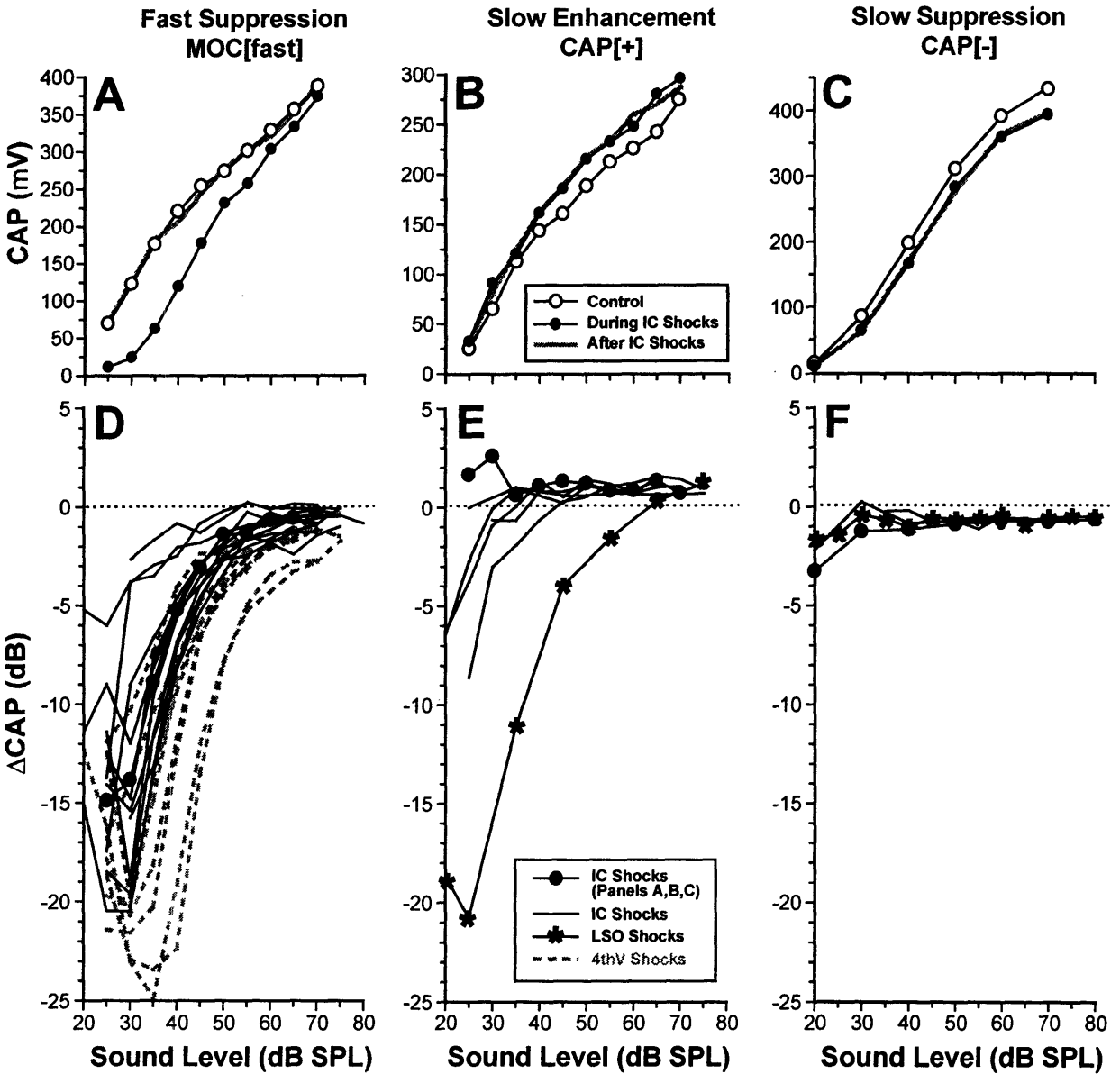
The first series of experiments achieved the principal aim of identifying brainstem stimulation sites that could produce changes in CAP-level functions different from those seen with 4thV stimulation. Several IC sites produced effects that were maximal at high sound levels with offset time-constants much greater than 100 ms; those enhancing CAP (n=6) were named *CAP[+]*, while those suppressing CAP (n=3) were named *CAP[-]*. LSO stimulation also produced *CAP[+]* and *CAP[-]* effects.

Three classes of cochlear effects elicited by IC stimulation sites

Stimulation at 12 of the 41 IC sites in 9 of 14 animals elicited CAP suppression indistinguishable from that seen with 4thV stimulation in the same animals. The location of the 12 IC sites evoking only MOC effects is illustrated in Panel G of Figure 4 (see key). As illustrated in Panel A, CAP suppression was greatest at low sound levels, and only fast effects were observed, i.e., CAP amplitudes returned to control levels within seconds after the termination of the shock train (gray line in panel A). These fast effects are referred to as *MOC[fast]*.

In Panel D of Figure 4, contralateral-ear data is superimposed from the 12 IC sites and from 4thV simulation in the 9 animals (see key). Panel D shows that for these sites, CAP suppression at low sound levels was much greater than at high sound levels, regardless of the absolute magnitude of the suppression. To illustrate the transformation from "CAP(uV)" to "ΔCAP(dB)", the data in panel A is replotted in panel D and

Figure 4


Fast Suppression
MOC[fast]
Slow Enhancement
CAP[+]
Slow Suppression
CAP[-]
A
B
C
D
E
F
CAP (mV)
ΔCAP (dB)
Sound Level (dB SPL)
Control
During IC Shocks
After IC Shocks
IC Shocks (Panels A,B,C)
IC Shocks
LSO Shocks
4thV Shocks

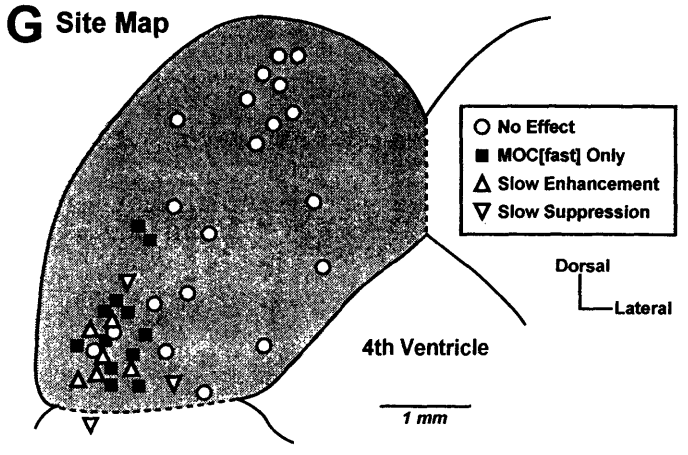

G Site Map
No Effect
MOC[fast] Only
Slow Enhancement
Slow Suppression
Dorsal
Lateral
4th Ventricle
1 mm

Figure 4: Three classes of effects on contralateral CAP seen via the CAP-level paradigm with IC shocks, compared to CAP effects elicited with 4thV shocks (dashed lines) or LSO shocks (stars). For all data, CAP was evoked by tone pips at frequencies between 11 and 13 kHz. **Panels A, B, and C**: Each panel shows sample results obtained with IC shocks at a different electrode site. In each case, three CAP amplitude-vs-level functions are superimposed: 1) a "control" run (open symbols) obtained without IC shocks, 2) a "during IC shocks" run (filled symbols) averaged from CAP waveforms recorded 20 msec after the termination of each shock burst, and 3) a "after IC shocks" run, without shocks, interleaved at each SPL (after a 1 sec delay) with the "with IC shocks" run. Given a) the stimulus repetition period (500 ms), b) the number of averages at each SPL (16), and c) the added inter-run delay (1 sec), the "after-IC shocks" data at each SPL are obtained 1 - 9 sec after the termination of the last shock burst. CAP amplitude is the peak-to-peak value. **Panels D, E, and F**: One CAP-level trial from each of the 21 IC stimulation sites (black lines), from each of 9 animals with OCB electrode placement (dashed gray lines), and from 2 sites with LSO stimulation (stars) have been selected, classified according to effect type, and superimposed in the appropriate panel in this row. One IC site in each panel (black circles) corresponds to the same data shown in the panel immediately above. The ΔCAP values in Panels D, E and F are defined, at each level, as

$$\Delta\text{Response} = 20 * \log_{10}(\text{During Shocks Response} / \text{Control Response}).$$

Panel G: Topographic organization of 41 electrode sites in the IC according to the type of effects evoked (see inset key). Sites with "No Effect" (n=20) are defined as those evoking an "effective attenuation" of the CAP (Gifford and Guinan, 1987) of less than 2 dB at all SPLs for all trials. Almost all sites with slow suppression or slow enhancement after the shocks, also showed fast suppression during the shocks. The schematic shows the appearance of the left IC from the caudal aspect, as revealed after aspiration of the cerebellum. See text for further details.

highlighted with filled circles. ΔCAP is the logarithm of CAP "with shocks" normalized to the pre-shocks CAP magnitude (see Fig. 4 caption).

Stimulation at 6 different IC sites (see Fig. 4G) enhanced CAP-level functions. This enhancement persisted for many seconds (at least) after termination of the shocks. For the case illustrated (Fig. 4B), CAP amplitudes remain elevated in the "After IC Shocks" period, which occurred ~5 seconds after the end of each shock train (Fig. 4B, gray line). These slow enhancements were typically observed bilaterally, and were never seen when shocking the 4thV in the same animal (included as dashed gray lines in panel D). These enhancing effects in CAP-level functions are referred to as "*CAP[+]*".

IC sites eliciting CAP enhancements often showed complex behavior. Four of the six IC sites (as well as LSO stimulation) showed level-dependent ΔCAP suppression at low levels coupled with ΔCAP enhancements at high levels (Fig. 4E). Lesions in two different experiments, that interrupted only MOC fibers, eliminated the low-level suppression, without changing the high-level enhancement (see the last section of the Results). Further evidence for different sources for the suppression and enhancement in such complex functions comes from the observation that only the enhancements persisted into the "after-shocks" period.

Stimulation at a third set of IC sites caused bilateral suppression of CAP-level functions (n=3, see Fig. 4G for IC location) that differed from *MOC[fast]* effects in that 1) suppression was greater (in absolute mV response shift) at high sound levels than low sound levels, and 2) suppression was sustained in the "After IC Shocks" measurements (Fig. 4C). Such a pattern of suppression was never seen with 4thV shocks in the same animals. As illustrated in Figure 4F, stimulation at all three sites produced ΔCAP functions with little level dependence (Fig. 4F); a constant ΔCAP function is indicative

of a CAP level function with a constant fractional change for all sound level measured. These novel suppressive effects in CAP-level functions are referred to as "*CAP[-]*".

In order to quantify level dependence, we take the ratio in ΔCAP measured at 15 dB versus 50 dB re threshold. This is named the level ratio. (The particular choice of sound levels is relevant for comparison to data obtained via the time-function paradigm (see next section), where only these two levels were assayed). The average level ratio was 13.7 versus 10.7 for IC- versus 4thV-evoked *MOC[fast]* effects, respectively. The average level ratio for the *CAP[-]* groups was closer to unity (1.5). The average level ratio for the *CAP[+]* group was -0.17. The negative number result from a mix of low sound level suppression and high sound level enhancement exhibited in many of the *CAP[+]* trials.

Effects of IC stimulation observed using the level function paradigm are summarized by the gray lettering in columns A, B, and G of Table 1. Three characteristics differentiated the *CAP[+]*, *CAP[-]*, and *MOC[fast]* classes: the sign, offset time-constant, and sound-level dependence of the change in CAP (rows 5, 9, and 11).

Tonotopy and Laterality of IC-evoked MOC[fast] Effects

Data in Figure 4 were obtained with tone pips between 11.0 to 13 kHz. In one experiment (Fig. 5B), the tonotopic organization of descending projections was investigated by measuring CAP suppression at 3, 6, and 13 kHz when shocking the IC at three stimulation sites spanning its dorsal-ventral axis (inset to Fig. 5B). The ventral stimulation sites maximally suppressed CAP at 13 kHz, while stimulation more centrally in the IC maximally suppressed at 6 kHz, and stimulation dorsally maximally suppressed CAP at 3 kHz (Fig. 5B). Thus, the preponderance of "No Effect" sites in central and

dorsal IC regions schematized in Figure 4G may be an artifact of the tone-pip frequency utilized: if lower frequency pips had been studied, many of these sites would likely have shown *MOC[fast]* effects as well.

Data summarized in Figure 4 were extracted from the cochlea contralateral to the IC electrode. Effects in the ipsilateral cochlea were qualitatively similar. Quantitative differences were evaluated only for *MOC[fast]* effects, as evoked by electrodes in the ventrolateral corner of the IC, where the density of stimulation sites was highest. As summarized in Figure 5A, the IC-evoked CAP suppression in the ipsilateral ear was always smaller in magnitude; and the inter-ear difference increased with increasing stimulus frequency.

CAP enhancement and suppression evoked by LSO shocks

In one experiment, stimulating electrodes were stereotaxically advanced into the LSO. On the first penetration, with a fine tungsten electrode, stimulation produced *CAP[-]* effects ipsilaterally (Fig. 4F, gray stars) with no effect contralaterally (data not shown: contralateral $|\Delta CAP|$ was < 0.5 dB between 30-70 dB SPL). On the second penetration, with a larger electrode, stimulation produced *MOC[fast]* effects at low SPLs, but *CAP[+]* effects at high sound level in the ipsilateral ear (Fig. 4E, gray stars). Contralaterally, only *MOC[fast]* effects were observed (ΔCAP was less than -1 dB between 20-70 dB SPL, similar to the gray dashed lines in Fig. 4D); activation of the distant MOC fibers with LSO stimulation has been previously reported (Gifford and Guinan 1987). Evaluation of Nissl-stained sections revealed that the electrode was centered rostrocaudally on the lateral border of the LSO. When stimulating electrodes were moved, in the same animal, to the 4thV, only *MOC[fast]* effects were observed

(one of the dashed gray lines in Fig. 4D). As summarized in Table 1, these effects of LSO stimulation were similar to those seen with IC stimulation (compare columns D and E to columns A and B).

Figure 5

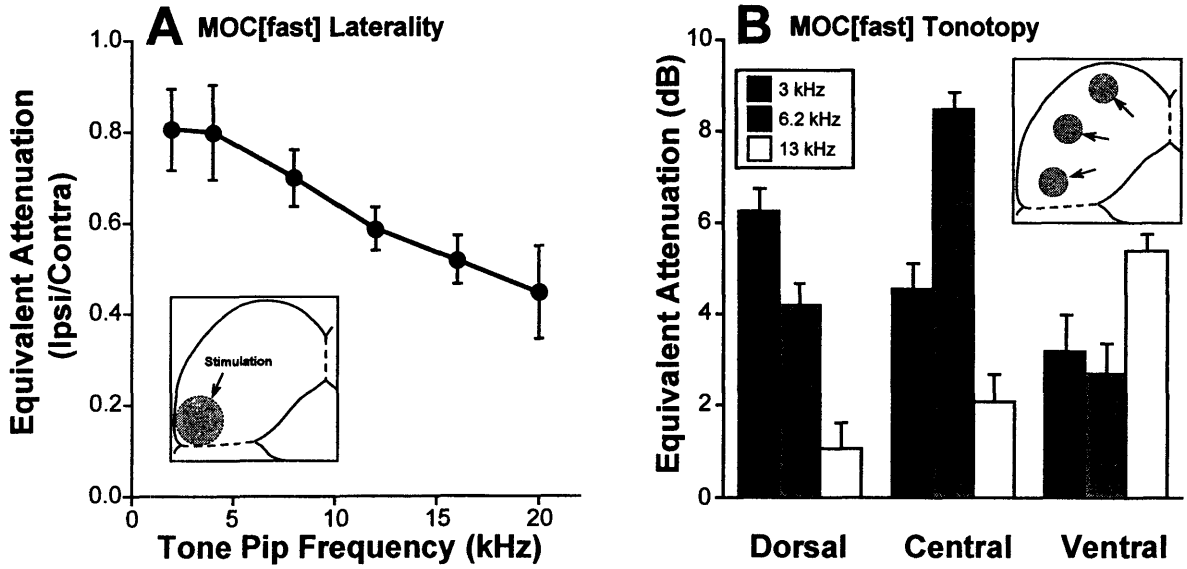


Figure 5: Laterality and tonotopic organization of MOC[fast] effects elicited by IC stimulation, as seen via the CAP-level paradigm. **Panel A:** CAP-level functions were run with tone pips at 2, 4, 8,12, 16, or 20 kHz with stimulation electrodes at 12 sites in the ventrolateral corner of the IC of 14 curarized animals. For each run, the magnitude of the CAP effect in each cochlea was computed as an effective attenuation: the rightward shift of the CAP-vs-level function seen at 20 dB re threshold when comparing data with and without IC shocks (See Guinan and Gifford, 1987). The laterality is then shown as the ratio of the shock-evoked dB shifts between the two ears (ipsi / contra). Means ± SEMs are shown. **Panel B:** In one experiment, IC-evoked effects on contralateral CAP were compared at three frequencies (see key), at three different locations along the dorso-ventral IC axis in one curarized guinea (see map insert). Magnitude of shock-evoked effects were defined as described for Panel A. Error bars represent SEMs across the four trials at each tone pip frequency per site. The tip of the electrode was placed at a depth of 1 mm from the surface of the IC.

Second Experimental Series: Time Functions

Strategy and Overview

Having identified brainstem sites that produced novel changes in CAP, the second paradigm was designed to characterize these effects along additional dimensions (e.g., CM, DPOAEs, and RW Noise), and to determine the pathway mediating them. Two classes of responses observed in the second series were similar to *CAP[+]* and *CAP[-]*, because they exhibited slow suppression or enhancement of the CAP similar in magnitude at high and low sound levels. Furthermore, these changes in CAP were without significant change in CM: these categories are referred to by the shorthand notation *CAP[+]CM[0]* and *CAP[-]CM[0]* (see rows A and B Table 1). A third effect class, *CAP[-]CM[+]*, may represent MOC "slow effects" defined by Sridhar et. al. (Sridhar et al. 1995), as both show long-lasting CAP suppression mirrored by CM enhancement (compare rows C and F of Table 1).

Long-lasting time-course, which is a hallmark of the putative LOC effects, was evaluated by making repeated measurements of cochlear responses to a few key acoustic stimuli *before, during,* and *after* a 1-2 minute epoch of shock trains delivered to one IC (See Figure 3A). Each set of pre-shock, during-shock, and post-shock data, normalized to the pre-shock baseline (e.g., Fig. 6A), is referred to as a "*trial*". The duration of trials ranged between 5 to 45 minutes. In this second series of experiments, 43 IC stimulation sites were studied in 21 animals, with a total of 200 trials. To differentiate effects occurring pre- versus post-synaptic to the IHC/afferent synapse, two "neural' measures (CAP and RW noise) and two "OHC-based" measures (CM and

Figure 6

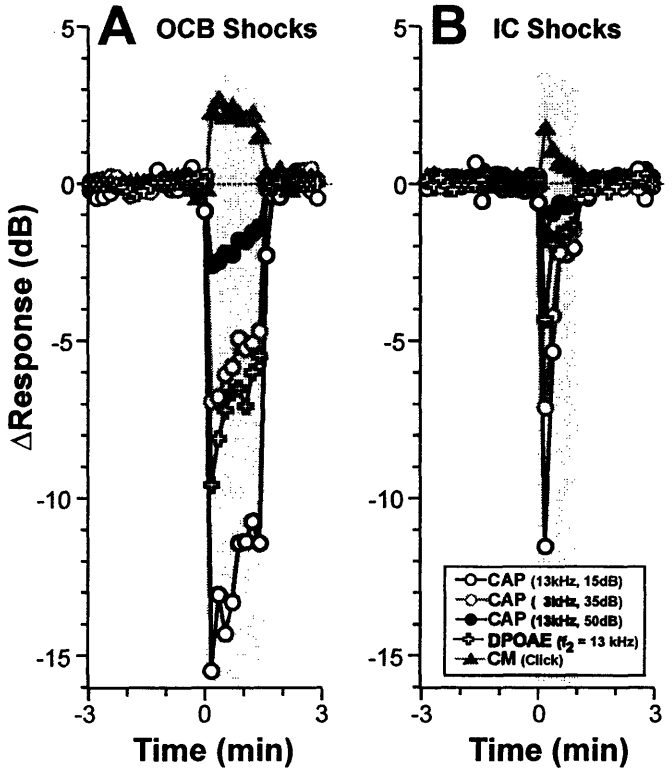


Figure 6: Comparison of MOC[fast] effects seen with the time-function paradigm for OCB shocks **(Panel A)** vs. IC shocks **(Panel B)**. For each electrode site, 5 simultaneously recorded cochlear responses are illustrated: one average for each measure is obtained by this paradigm (Figure 3) every 13 seconds, in interleaved fashion, before, during and after a shock epoch (gray box). In these, and subsequent, plots of time-function data, each response magnitude is expressed as a change (ΔResponse in dB) re its pre-shock mean value (during a 5-minute pre-shock time window):

$$\Delta\text{Response} = 20 * \log_{10}(\text{Response} / \text{Mean Pre-Shock Response}).$$

In these, and subsequent, plots of time-function data, the level of the stimuli is expressed in dB re CAP threshold at that tone pip frequency.

DPOAEs were obtained in each trial (Fig. 3). CAPs were measured at two frequencies (3 and 13 kHz) to allow comparison of IC-evoked effects on sound-evoked neural activity in basal and apical halves of the cochlea. These two frequencies were chosen to help differentiate MOC "slow effects" from those possibly due to LOC activation: MOC effects peak with 13 kHz tone pips and are smaller when measured with 3 kHz ton pips. At 13 kHz, two sound levels (15 dB and 50 dB re CAP threshold) were use to reveal any level dependence effects. At 3 kHz, the tone pips were always presented at an intermediate level: i.e., 35 dB re CAP threshold. Rows 5, 9, 10, and 11 of Table 1 summarize the effects on CAP for each effect class.

The second indicator of cochlear neural activity was RW noise: i.e., biological activity in the absence of exogenous sound, measured by an electrode placed on the RW (Dolan et al. 1990). Like the spectrum of extracellularly recorded action potentials, the RW spectrum exhibits a peak around 800-1000 Hz. The magnitude of the 800-1000 Hz components of the RW noise spectrum was extracted to assess levels of spontaneous activity in the auditory nerve. The sign of the RW noise is indicated in row 6 of Table 1 (the size the arrow, relative to the size of the CAP arrow, indicates the magnitude of the RW noise effect relative to the CAP effect).

The two measures of pre-synaptic, i.e., OHC-based, effects in the time-function paradigm were CM and DPOAEs. The CM was measured in response to moderate-level clicks, as has been done in previous studies of MOC-based effects to infer changes in OHC conductance (Sridhar et al. 1995). DPOAEs were measured with the higher frequency of the two-tone pair (f2) at 13 kHz, to match the tone-pip frequency used to evoke CAP. The level of the primaries were set 20 dB above the level required to evoke

a –5 dB SPL $2f_1$-f_2 DPOAE. The sign of the change in these pre-synaptic cochlear responses is shown in rows 7 and 8 of Table 1.

Fast versus slow effects: qualitative findings

As expected from the CAP-level results, IC shocks often produced effects during the shock epochs with fast onset and offset (Fig. 6B), similar to those seen with 4thV shocks (Fig. 6A), i.e., suppression of CAP and DPOAEs coupled with enhancement of CM. Given the time sampling of this paradigm (Fig. 3), the speed of effect onset/offset can only be specified as ≤13 s. As for IC-evoked *MOC[fast]* effects measured with CAP-level functions (Figs. 4A,D), the suppression of CAP to low-level tones is greater than for high-level tones (Black versus gray unfilled circles in Fig. 6B). For IC stimulation, the complementary effects on CAP and CM tended to decay more dramatically during the shock epoch than seen with 4thV stimulation. These fast, during-shocks effects on the contralateral CAP and CM disappeared when the crossing MOC pathways were sectioned at the midline (see the last section of the Results), thus they likely represent activation of the MOC system. In search of LOC-mediated effects, we concentrated attention on the post-shock (i.e., long-lasting) effects of IC stimulation.

4thV stimulation with the time-function paradigm elicited MOC "slow" effects like those described by Sridhar et al (1995): i.e., long-lasting CAP suppression coupled with long-lasting enhancement of click-evoked CM (data not shown). Such *MOC[slow]* effects were evoked in 2 out of 5 animals in which the time-function paradigm was performed with 4thV shocks. Similar effects were also evoked with the time-function paradigm at some IC sites..

Figure 7

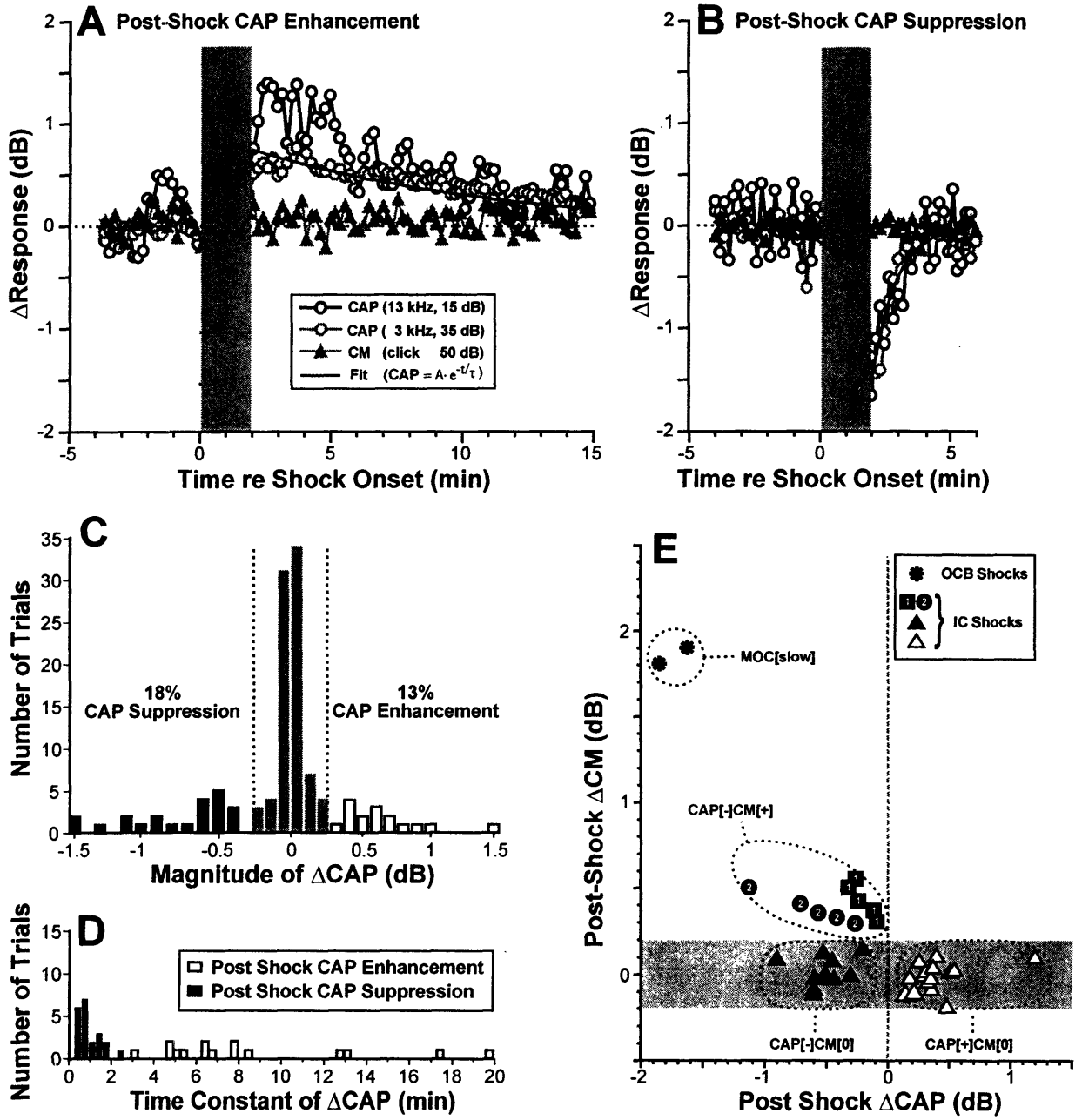

A Post-Shock CAP Enhancement
ΔResponse (dB)
Time re Shock Onset (min)
CAP (13 kHz, 15 dB)
CAP (3 kHz, 35 dB)
CM (click 50 dB)
Fit (CAP = A·e^-t/τ)
B Post-Shock CAP Suppression
ΔResponse (dB)
Time re Shock Onset (min)
C
Number of Trials
18%
CAP Suppression
13%
CAP Enhancement
Magnitude of ΔCAP (dB)
D
Number of Trials
Post Shock CAP Enhancement
Post Shock CAP Suppression
Time Constant of ΔCAP (min)
E
OCB Shocks
IC Shocks
MOC[slow]
CAP[-]CM[+]
CAP[-]CM[0]
CAP[+]CM[0]
Post-Shock ΔCM (dB)
Post Shock ΔCAP (dB)

Figure 7: Categorization of long-lasting IC-evoked effects on the cochlea based on the magnitude and sign of post-shock changes to CAP and CM. **Panels A and B:** These time-function data show example trials from two IC stimulation sites of long-lasting shock-evoked changes in CAP amplitude, one enhancing (A) and one suppressing (B), without accompanying changes in CM magnitude. Exponential curve fits used to extract the magnitude and time constant of these post-shock changes in CAP are shown by the solid lines. For other data display conventions, see Figure 6. The trial in Panel A was obtained after midline section of the OCB in an animal with the superior cervical ganglion removed ipsilateral to the IC electrode. All presented data is from the cochlea contralateral to IC stimulation. The trial in Panel B was obtained in another animal, after midline OCB section. **Panels C and D:** Summary plot of the magnitudes (C) and time constants (D) for all 119 well-characterized trials of the time-function paradigm. These data are extracted from the CAP evoked by 3 kHz tone pips, because the variance of these were typically lower than the variance of CAP evoked with 13 kHz tone pips. Multiple trials were often obtained at a single electrode site: the 21 suppressive trials in Panel C derive from 6 IC sites in 5 animals; the 15 enhancing trials derive from 6 IC sites in 5 animals. **Panel E:** Derivation of effect categories by comparing long-lasting shock-evoked effects on CM and CAP. Each point represents post-shock ΔCAP and ΔCM magnitudes for a different trial. Two trials evoking *MOC[slow]* effects via OCB stimulation are included for comparison (stars). All other trials are for IC electrode sites. Effect categories are grouped by dotted circles and are labeled. The *CAP[-]CM[+]* category includes 5 trials from each of two electrode sites (numbered "1" and "2"). See text for further details.

Stimulation with the time-function paradigm at several IC sites produced long-lasting changes in CAP that were not accompanied by changes in CM (e.g., Figs. 7A,B). As seen with the CAP-level paradigm, those long-lasting CAP changes were suppressive for some IC sites and enhancing for others. Furthermore, in contrast to the *MOC[fast]* during-shocks effects in Figure 6, the long-lasting ΔCAP for trials in Figures 7A and B was similar in magnitude for low-level and high-level tone pips at 13 kHz, reminiscent of the level-independent, long-lasting effects in Figure 4B and 4C. We assume these long-lasting CAP effects captured by the time-function paradigm (e.g., Figs. 7A and 7B) represent the same phenomena as the *CAP[+]* and *CAP[-]* effects captured by the level function paradigm (e.g., Figs. 4B/E and C/F). The case is clearest for the long-lasting suppression data in Figure 4C and 7B: both were produced by stimulation at the same IC site in the same animal.

Quantifying and classifying long-lasting effects based on ΔCAP and ΔCM

The large number of measurements performed by the second paradigm enabled more sophisticated classification of the effects of IC stimulation. Classification based upon the sign of changes in CAP and CM proved to be sufficient to establish distinct categories that were then made more robust by additional response metrics available (e.g., CAP laterality, DPOAE magnitude, and RW noise magnitude). The robustness of these distinct classes allows results of different pathway lesions, in different animals, to be interpreted together for a single effect class.

In order to classify all IC sites at which they were evoked, post-shock CAP data from the contralateral ear at 3kHz was fit by a single exponential (solid line in Figs. 7A and 7B). The resulting magnitudes and time-constants from trials with sufficient data to

allow a reasonable fit (n=119) are shown in Figures 7C and 7D. The sign of the ΔCAP was never different between the two ears: quantitative differences in the laterality of the effects are considered in the next section. Note that a single IC site might, in some cases, generate several repeated trials. When multiple trials were conducted at a single site, the sign of ΔCAP was always the same across trials.

Trials with post-shock CAP enhancement or suppression greater than 0.25 dB (Fig. 7C) were considered to be significant and were investigated further; this included 15 trials with post-shock enhancement and 21 with post-shock suppression. The offset time-constants for enhancing versus suppressing trials were non-overlapping (Fig. 7D): those for long-lasting CAP enhancement ranged from 3 - 20 min; whereas those for long-lasting CAP suppression ranged from 0.5 – 2.5 min.

Long-lasting effects of IC stimulation were further sub-divided based on the behavior of the CM: in Figure 7E, the magnitude and sign of the post-shock ΔCAP, evoked with 13 kHz pips 50 dB re threshold, are plotted versus magnitude and sign of ΔCM.

All trials with long-lasting CAP enhancement had $|\Delta CM| < 0.2$ dB, and are considered as one category: *CAP[+]CM[0]* . These 15 trials were from 6 IC sites (Fig. 8A, unfilled triangles) from 5 guinea pigs. One ΔCAP-vs.-time function from each of these 6 sites is illustrated in Fig. 8B: in the post-shock period, the behavior of the ΔCAP is quite reproducible across sites. In contrast, during the shock epoch, the behavior of the ΔCAP is heterogeneous: two of the sites produced fast-onset CAP suppression that decayed dramatically as the shock trains continued. Alternatively, at other sites, there was minimal or no rapid change in CAP upon shock onset, but rather a slow, steady rise in CAP amplitude progresses throughout the shock epoch.

Figure 8

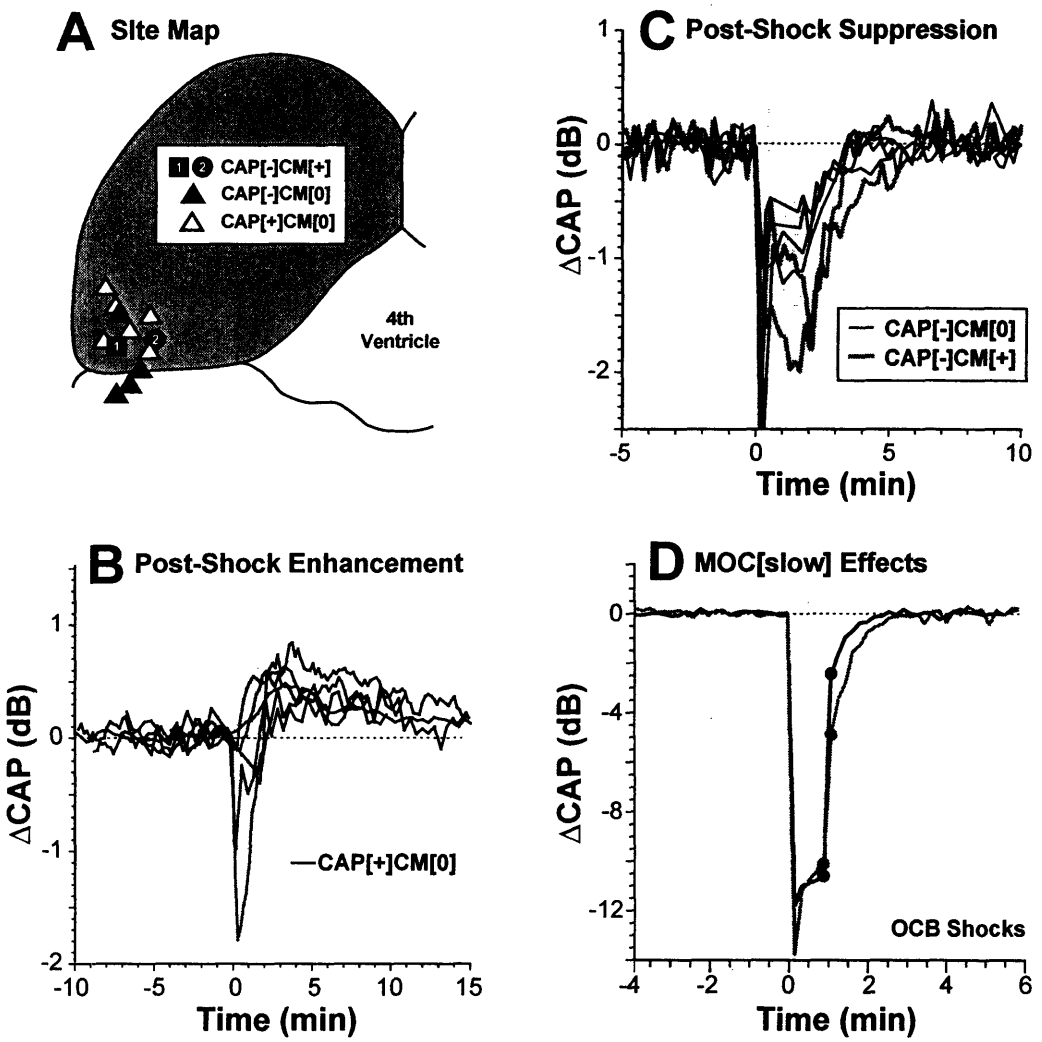


Figure 8: Topographic organization **(Panel A)** of the 12 IC electrode sites producing long-lasting cochlear effects (as categorized in Figure 7E), and superposition of ΔCAP-vs-time functions for one trial from each site, for IC-evoked CAP enhancement (CAP[+]CM[-]; **Panel B)** or suppression (CAP[-]CM[0] and CAP[-]CM[+]; **Panel C**). Data from two cases of MOC[slow] effects elicited with OCB electrodes are included for comparison **(Panel D).** To aid in differentiating MOC[fast] from MOC[slow] effects, filled circles indicate the ΔCAP for the last trial during the shock epoch and the first trial after the shock epoch for each trial in Panel D For all panels, ΔCAP is derived as described in Figure 6, and only data from 3-kHz tone pips are displayed. The orientation of the IC schematic is described in the caption for Figure 5G.

As Figure 7E illustrates, some trials with long-lasting CAP suppression also showed minimal change in post-shock CM (less than 0.2 dB). However, in other cases, there were large CM enhancements. Significant CM suppression was never seen.

Trials with $|\Delta CM| < 0.2$ dB (Fig. 7E) were grouped into a second category: *CAP[-]CM[0]*. The 11 trials in this group were from 4 IC sites (Fig. 8A, filled triangles) from 3 guinea pigs. Example ΔCAP-vs.-time functions from each of these 4 sites are shown as black lines in Figure 8C. For these trials, the post-shock behavior of the ΔCAP is quite homogenous. The ΔCAP during the shock epoch is also similar across sites, but quite complex in nature. Immediately upon shock onset, there is a rapid and strong CAP suppression, which then decays dramatically between the first and second points (as typical for IC stimulation, e.g., Fig. 6B), after which there can be a slower rise in suppression continuing to grow until shock offset. This behavior may arise from the interaction between two different suppressive processes, with different time constants and different origins. This issue will be re-visited in last section of the Results, where the effects of selective interruption of the MOC system will be presented.

The remaining ten IC trials (Fig. 7E) exhibited long-lasting CAP suppression coupled with significant CM enhancement: referred to by the shorthand *CAP[-]CM[+]*. These trials derive from two sites in two animals. Their ΔCAP-vs.-time functions (gray lines in Fig. 8C) also show complex behavior during the shock epoch, with an initial rapidly rising and rapidly decaying suppression, followed by a second wave of suppression with a slower onset time constant.

For comparison, two 4thV stimulation trials, from two animals, representing 4thV-evoked *MOC[slow]* effects (which also have CAP suppression and CM enhancement) are also included in Figure 7E. This *MOC[slow]* effect is qualitatively similar after the

shocks to the IC-evoked *CAP[-]CM[+]* effect; however, during the shocks, the effect magnitude is four times larger than after the shock; in other words, upon termination of the shocks, ΔCAP suppression abruptly falls to 1/4 its during-shocks magnitude (pairs of filled symbols in Fig. 8D).

Table 1 summarizes the results of this section for IC stimulation (columns A-C) and 4thV stimulation (column F), illustrating the sign of CAP and CM (rows 5 and 7), as well as the offset time-course (row 9). In the next section, additional cochlear responses, shown as the headings of Table 1 rows 8 – 11, reinforce the robustness of the classes of effects defined on the basis of changes in CM and CAP.

Clustering of Effect Categories Along Additional Response Dimensions

Additional measurement dimensions presented in this section reinforce the categories established on the basis of magnitude and sign of the long-lasting changes in CAP and CM. These additional dimensions include: 1) the level and frequency dependence of ΔCAP; 2) the relative size of ipsilateral versus contralateral ΔCAP; 3) effects on other OHC-based response (ΔDPOAEs versus ΔCM); and 4) effects on other neurally based response (ΔRW noise versus ΔCAP). Along each of these additional dimensions, elements within each group tend to cluster together and, conversely, elements of different groups tend to be non-overlapping. Furthermore, the behavior of groups with selective neural changes (i.e., *CAP[+]CM[0]* and *CAP[-]CM[0]*) tends to be unlike that seen when evoking MOC "fast" or "slow" effects; whereas the *CAP[-]CM[+]* group tends to be similar to the 4thV-evoked *MOC[slow]* effects.

1) Level and frequency dependence of ΔCAP

The level and frequency dependence of the ΔCAP from all IC stimulation sites are summarized in Figure 9, with IC sites showing long-lasting CAP suppression (Panel A) plotted separately from long-lasting enhancement (Panel B).

The level dependence is quantified by computing, for each trial, the ratio of the ΔCAP measured with the low-level versus the high-level tone pips at 13 kHz. Recall from analyses of CAP-level functions (Fig. 4D), that for *MOC[fast]* effects this level ratio should be >> 1: i.e., more suppression at low sound levels than at high sound levels. Two trials in Figure 9A (stars) were evoked by 4thV stimulation, and thus represent *MOC[slow]* effects, which show the same large level dependence as *MOC[fast]* effects. The IC-evoked trials from both *CAP[-]CM[+]* sites show a large level dependence, and, in this way, behave like MOC effects evoked by 4thV stimulation. In contrast, both groups with selective neural effects (i.e., *CAP[+]CM[0]* and *CAP[-]CM[0]*) show relatively little level dependence. In this way, they act differently from MOC effects, and similar to the long-lasting suppressive and enhancing effects illustrated in Figure 4C and 4B. Level dependence is summarized in row 11 of Table 1 as the ratio of ΔCAP measured at 50 dB versus 15 dB re CAP threshold. CAP effects described as "level independent" or "constant %" had a ratio of 0.5 to 2.5 versus 4 to 14 for those with "much greater suppression at low sound levels".

The frequency dependence of the ΔCAPs is quantified by computing the ratio of ΔCAPs for the moderate-level 3-kHz tone pip and the high-level 13-kHz tone pip. In general, trials from the *CAP[+]CM[0]* group show somewhat greater effects at 3 kHz than at 13 kHz (ratios > 1); whereas the *CAP[-]CM[0]* group tends to show comparable ΔCAPs at the two test frequencies (ratios ~1). With respect to the *CAP[-]CM[+]* group,

the frequency dependence differs markedly for the two sites: the large low-frequency bias at site 2 makes these results different from all others evoked in the ventrolateral corner of the IC.

Figure 9

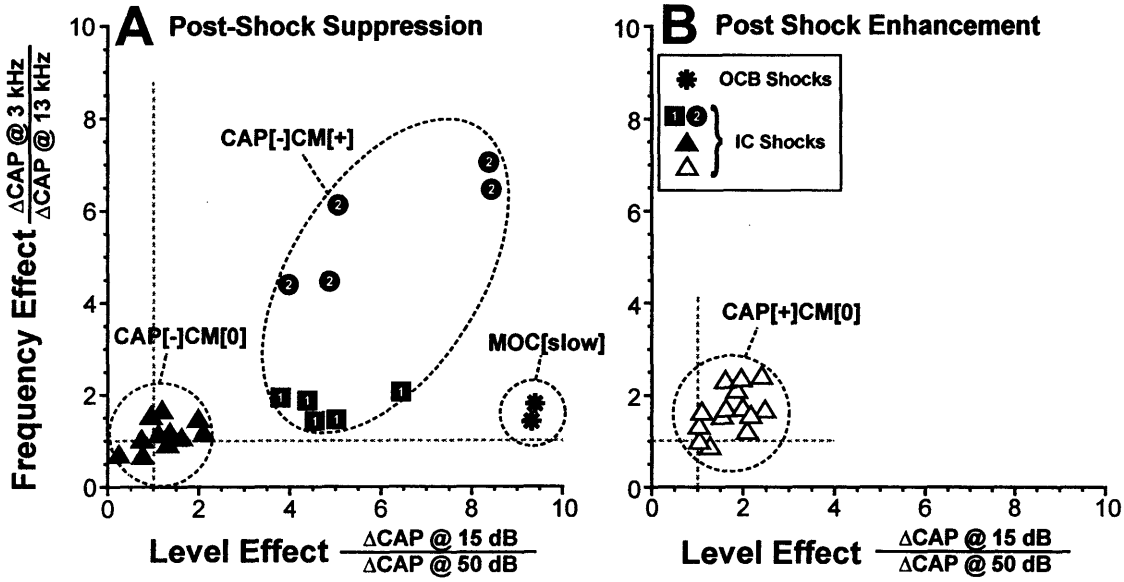


Figure 9: Level and frequency dependence of the long-lasting, IC-evoked changes in CAP amplitude: CAP suppression in **Panel A;** CAP enhancement in **Panel B.** Effect categories and other conventions for data display are defined in Figure 7E. Symbol key in Panel B also applies to Panel A. The CAP level effect is defined as the ratio of the ΔCAP measured for 13 kHz pips at 15 dB re CAP threshold to that measured at 50 dB re threshold. The CAP frequency effect is defined as the ratio of the ΔCAP measured for 3 kHz pips at 35 dB re threshold to that measured from 13 kHz pips at 50 dB re threshold. ΔCAP is defined by exponential curve fitting as described in Figure 7.

2) Contralateral versus Ipsilateral ΔCAP

As summarized in Figure 10, trials with IC-evoked long-lasting and selective changes in CAP amplitude, i.e., the *CAP[+]CM[0]* (Panel B) and *CAP[-]CM[0]* (Panel D) groups, showed little difference between the two ears in the magnitude of the ΔCAP. Indeed, data from *CAP[-]CM[0]* sites lie very close to the 45° line indicating equilateral effects. Although the *CAP[+]CM[0]* sites show a slight contralateral bias, the contralateral ΔCAPs are larger than ipsilateral by only a factor of ~1.2. In contrast, both *CAP[-]CM[+]* sites show a large bias in magnitude of the long-lasting ΔCAP towards the contralateral ear. In this respect, the *CAP[-]CM[+]* effects (Panel D) are similar to the IC-evoked (fast) effects seen during the shock epochs for almost all IC sites (Figs. 10A and C). The strong contralateral bias for the (fast) during-shocks CAP suppression at all IC sites is consistent with effects mediated by an ipsilateral IC-to-MOC projection (Thompson and Thompson 1993) followed by a 2:1 contralateral bias in the MOC to cochlear projections (Robertson 1985).

Another similarity between *MOC[fast]* effects and the fast-onset CAP suppression seen during IC shocks (Figs. 10A and 10C) is that, for both phenomena, ΔCAP is highly level dependent: in Figure 10A or 10C, the ΔCAP for low-level tone pips at 13kHz (open circles) is always greater than ΔCAP for high-level tone pips at 13kHz (filled circles). This stands in contrast to the lack of level dependence for the (slow) post-shocks effects seen in both *CAP[-]CM[0]* and *CAP[+]CM[0]* groups, as described above (Fig. 9), and as also seen in the data in Figure 10B and D. Row 10 of Table 1 summarizes CAP laterality as the ratio of the contralateral / ipsilateral magnitude seen for each effect class.

Figure 10

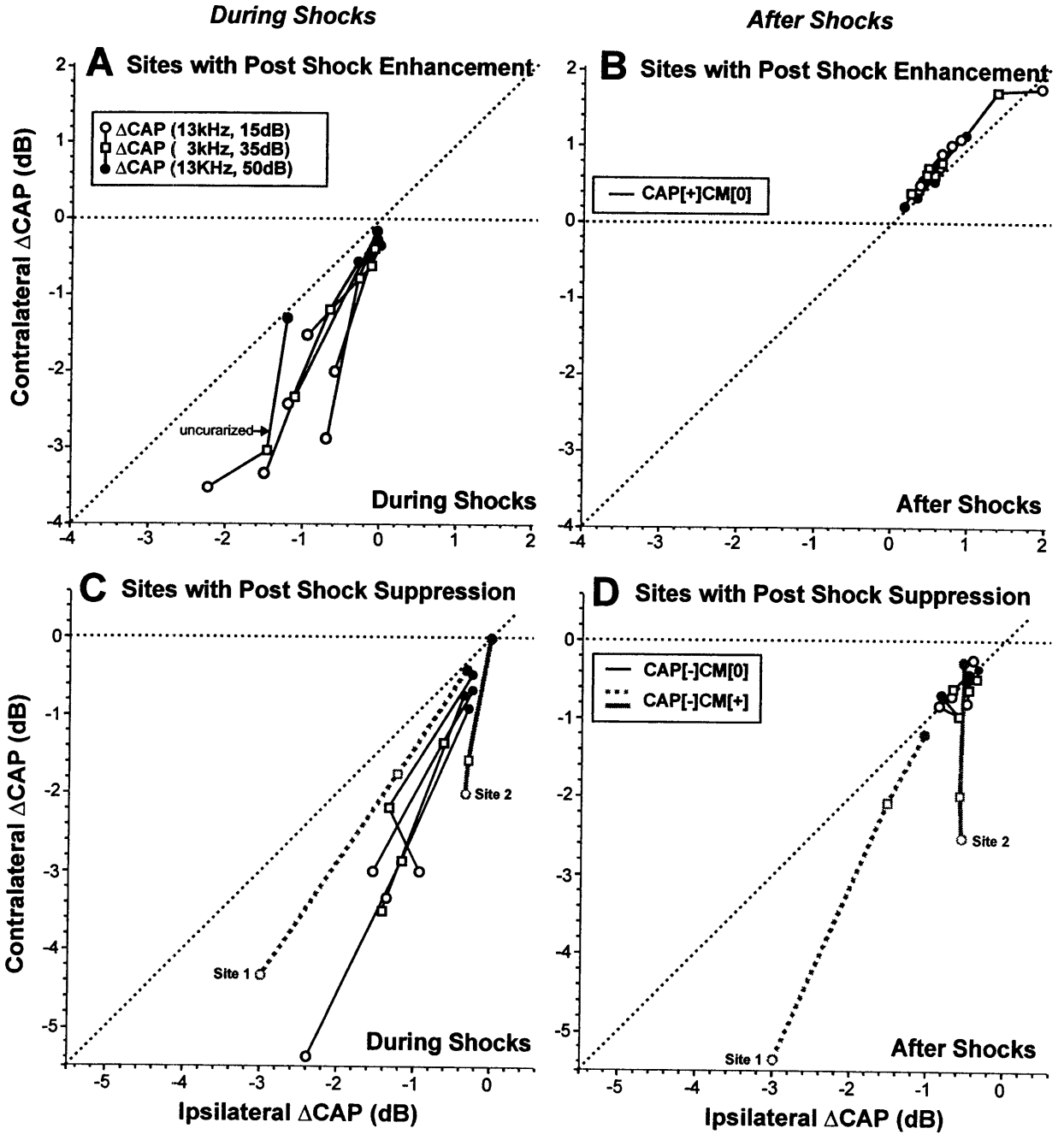

During Shocks
After Shocks
A Sites with Post Shock Enhancement
ΔCAP (13kHz, 15dB)
ΔCAP (3kHz, 35dB)
ΔCAP (13KHz, 50dB)
uncurarized
During Shocks
B Sites with Post Shock Enhancement
CAP[+]CM[0]
After Shocks
C Sites with Post Shock Suppression
Site 2
Site 1
During Shocks
D Sites with Post Shock Suppression
CAP[-]CM[0]
CAP[-]CM[+]
Site 2
Site 1
After Shocks
Contralateral ΔCAP (dB)
Ipsilateral ΔCAP (dB)

Figure 10: Comparison of the laterality of IC-evoked effects on CAP, during (**Panels A and C**) vs. after (**Panels B and D**) the shock epochs. Data include one trial from each IC site evoking long-lasting suppression (**Panels C and D**) or enhancement (**Panels A and B**) of the CAP, as described in Figure 7C. Trials are categorized as shown in Figure 7E, based on sign and magnitude of post-shock ΔCAP and ΔCM. One trial from each site is plotted in the row appropriate to the sign of the long-lasting CAP change. Data from the three tone-pip frequency/level combinations in a single trial are connected by a line. The magnitude of the post-shock ΔCAP is derived as described in Figure 7. The magnitude of each during-shock ΔCAP is defined as the ratio, in dB, of the first CAP amplitude measured after shock onset to that of the mean pre-shock CAP to the same frequency/level pips.

3) Pre-synaptic OHC-based measures

ΔDPOAEs versus ΔCM. Analysis of the magnitude of long-lasting changes in the DPOAE, in concert with the ΔCM data, provide additional insight into long-lasting shock-evoked effects at the level of OHCs. Figure 11 summarizes the relationships between the ΔCM and ΔDPOAE (magnitudes and signs) for the same set of IC stimulation sites summarized in Figures 7, 8, 9 and 10. Consistent with a post-synaptic effect, the *CAP[-]CM[0]* and *CAP[+]CM[0]* groups exhibited negligible post-shock changes in DPOAE magnitude: all data points are clustered around zero values for both ΔCM and ΔDPOAE (Figs. 11A and 11B). In contrast, the *CAP[-]CM[+]* group exhibited non-zero post-shock ΔDPOAEs. As seen for ΔCAP frequency dependence (Fig. 9A), the effects at the two sites in this group were significantly different. Site 1 showed long-lasting DPOAE suppression ~4 times greater than the CM enhancement (Fig. 11A), In contrast, site 2 showed enhancement of both DPOAE and CM of similar magnitude. Once again, the behavior at site 1 was more similar to *MOC[slow]* effects evoked at the 4thV, which also showed long-lasting DPOAE suppression of significantly greater magnitude than the CM enhancement (~5-6 times). Rows 7 and 8 of Table 1 summarize the sign of changes in CM and DPOAE for each effect class.

Figure 11

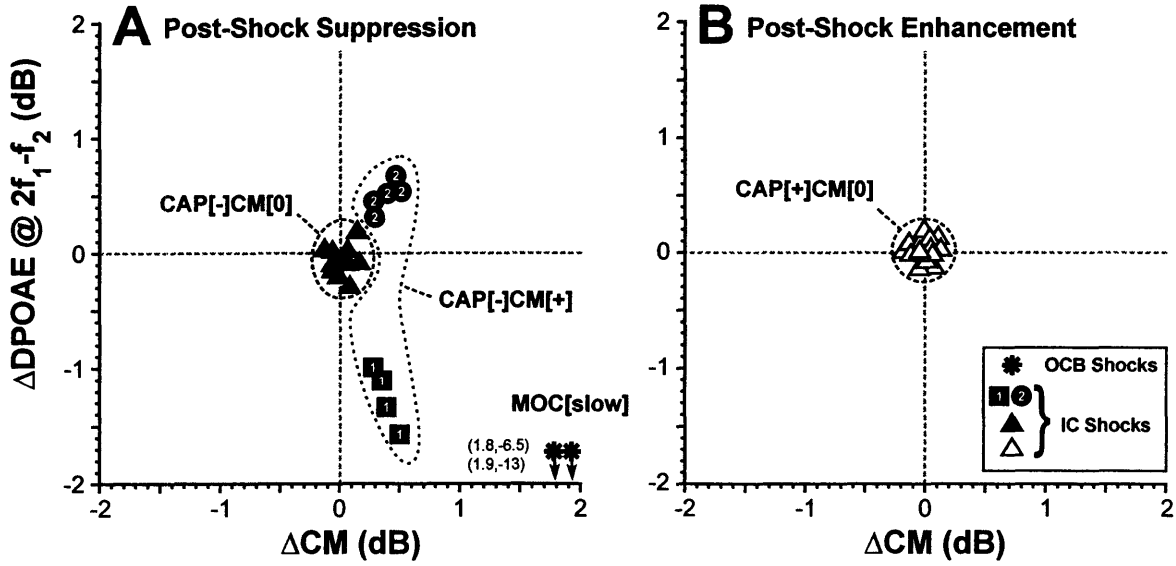


Figure 11: Relation between long-lasting changes in CM vs. DPOAE evoked by IC stimulation for all trials with long-lasting CAP suppression **(Panel A)** or CAP enhancement **(Panel B)**. Conventions for data display are as described in Figure 7E. The values for the OCB-evoked MOC[slow] effects are off scale: true coordinates are indicated in the parentheses. Values for ΔDPOAE and ΔCM were derived by exponential curve fitting as described for the ΔCAP in Figure 7. For DPOAE measurements, f2 was at 13 kHz, and CM was click evoked (See Methods).

4) Post-synaptic neurally based measures

ΔRW Noise versus ΔCAP. For long-lasting effects modulating CAP but not CM (i.e., *CAP[+]CM[0]* and *CAP[-]CM[0]*), RW noise was affected to a similar degree as the CAP (Figure 12, Panels E and F). For effects suppressing CAP and enhancing CM, i.e., CAP[-]CM[+], RW noise was suppressed by a small fraction of CAP suppression (Figure 12, Panels G and H). Figure 13 more completely shows the relationships of ΔRW noise versus ΔCAP for the long-lasting effect classes.

As illustrated in the top row of Figure 12, the spectrum of the RW noise, which was always recorded in the absence of exogenous sound, exhibits a large peak at 800-1000 Hz. This peak corresponds to the spectral peak of the electrical contribution of single auditory nerve spikes to the RW potential, as determined by cross-correlation techniques (Kiang et al. 1976), and is eliminated by selective elimination of auditory nerve fibers with kainic acid (Dolan et al. 1990). It is also eliminated with tetrodotoxin, but not by ablating the cochlear nucleus, providing further evidence that the 800-1000 Hz peak of the RW noise arises from auditory afferent firing (McMahon and Patuzzi 2002). For each effect class, IC stimulation modulated this neural component of the RW noise (see the top row of Fig. 12; RW noise spectra before (open symbols) and ~30 seconds after the shock epoch (filled symbols)). To estimate the shock-induced changes in spontaneous rates in the auditory nerve as a function of time, the average spectral energy in the 800-1000 Hz band was extracted from the RW noise spectra obtained at 13 second intervals (Fig. 3), and the change in amplitude (dB re average pre-shock values) is plotted as a function of time, where it can be compared to the ΔCAPs for the same trials (Fig. 12, Panels E-H).The trends shown in Figure 12 are illustrated more completely by the scatterplots in Figure 13, where the magnitude of ΔRW noise and ΔCAP are compared for all IC sites. Effects categorized as *CAP[-]CM[+]* exhibit RW noise suppression that is 1/5 to 1/20 the ΔCAP suppression in the same trials. Recall that for this group, the multiple trials

Figure 12

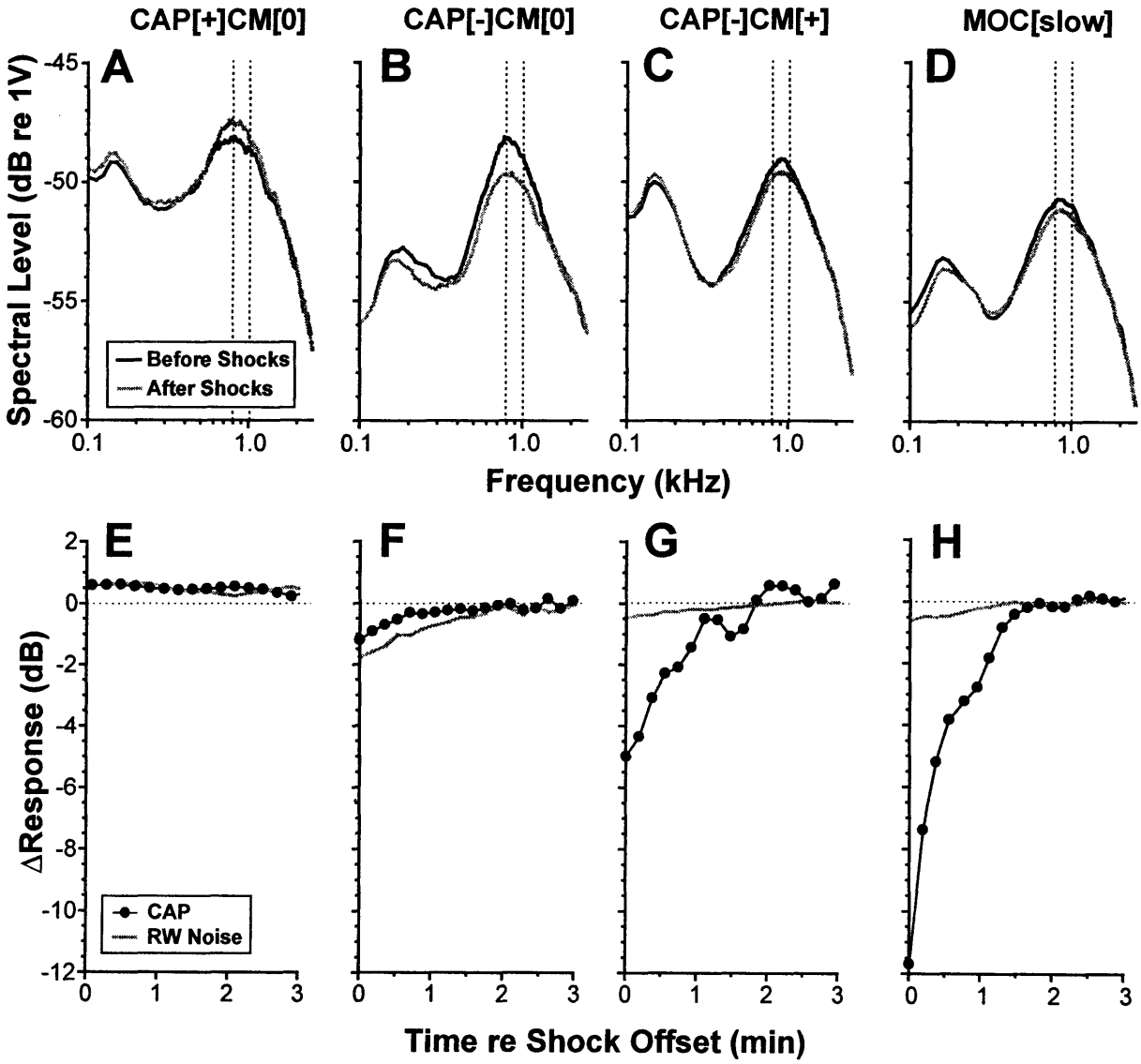


Figure 12: Long-lasting changes in RW noise spectrum evoked by IC stimulation can include suppression or enhancement of the 900 Hz peak, which represents ensemble spontaneous discharge in the auditory nerve. **Panels A-D:** RW noise spectra before vs. after the shock epoch for a representative trial from each of the IC effect categories, compared with one trial with OCB stimulation evoking MOC[slow] effects. In each panel, one pre-shock spectrum is compared to the average of two spectra obtained immediately after the end of the shock epoch. Symbol key in Panel A applies also to Panels B - D. Dashed vertical lines illustrate the frequency band over which spectral values are averaged to extract a single-valued metric of ensemble auditory nerve activity (as used in panels E-H. **Panels E-H:** compare post-shock changes in the 800-1000 Hz component of RW noise as a function of time to those seen for the CAP in the same trial shown in the panel above. In each panel, the gray lines represent the single-valued metric of RW noise described above, converted into a ΔResponse in dB as described in Figure 6; the filled circles represent ΔCAP (in dB) for 13-kHz tone pips at 15 dB re threshold.

shown represent data from only two sites: within each site, the ratio of ΔCAP to ΔRW noise remains roughly constant across the multiple trials. For the CAP[-]CM[0] group, the ratio of ΔRW noise to ΔCAP was never smaller than 0.8, and could be as large as 4. For the CAP[+]CM[0] group, ΔRW noise was typically similar to ΔCAP, however, there were a few sites that did not exhibit large changes in ΔRW noise. The relative change in RW noise for each effect class is denoted by the size of the arrow in row 6 of Table 1.

Figure 13

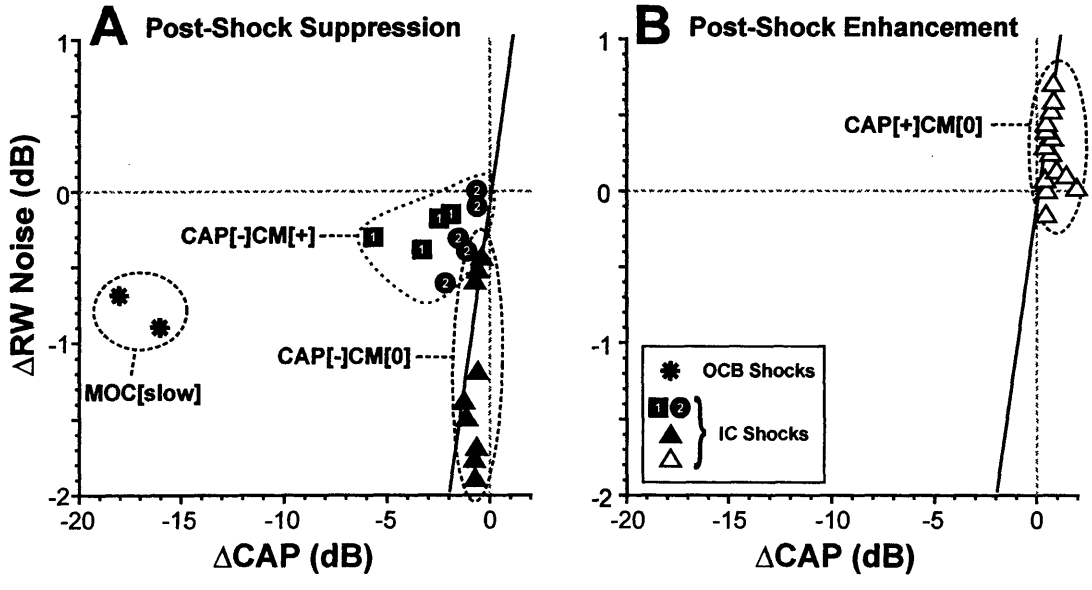


Figure 13: Relation between long-lasting changes in RW noise and CAP evoked by IC stimulation for all trials with long-lasting CAP suppression **(Panel A)** or CAP enhancement **(Panel B)**. Conventions for data display are as described in Figure 7E. Two trials from the CAP[-]CM[0] group were removed because the animal had a spontaneous otoacoustic emission. Values for the ΔCAP were based on data obtained for 13 kHz tone pips at 15 dB re threshold. Values for ΔRW noise were derived by taking the average spectral values between 800 and 100 Hz (See Figure 12) for spectra obtained immediately after shock offset and converting to dB as described in Figure 6. The solid line in each panel indicates ΔRW = ΔCAP. One site 1 CAP[-]CM[+]' trial was excluded due to artifact in the RW noise.

Repeatability of effects with multiple trials at the same site

All categories of long-lasting effects were reproducible when multiple trials were carried out at a single site (Fig. 14). Furthermore, the effects elicited by repeated trials at one site were qualitatively similar. The repeatability of these effects allowed comparisons before and after lesions were placed to interrupt OC or autonomic fiber pathways.

For the examples illustrated in Figure 14, the inter-trial period varied from 5 to 15 minutes. Thus, for the *CAP[+]CM[0]* effects, which showed offset time constants as long as 20 min (Fig. 7D), the CAP enhancement was sometimes cumulative, i.e., the effects of each shock epoch built on the enhancement of the previous one. In the example shown in Figure 14A, the inter-trial period was 10 minutes: note, in this case, the parallel and comparable enhancement of CAP amplitude and RW noise contrasting with the lack of shock-evoked effect on the DPOAE.

The offset time constants for both CAP suppressive categories were shorter (Fig. 7D) than the minimum interval between trials (5 minutes). Thus, it is unknown if they are cumulative; however, the data in panels B, C and D show that they are also repeatable. Note, for both of the *CAP[-]CM[+]* sites (Panels C and D), there is particularly complex behavior of the DPOAE measure: a during-shock suppression followed by a post-shock enhancement for site 1 and a during-shock enhancement and a post-shock suppression for site 2. Both of these complex behaviors appear repeatedly across the multiple trials at the same site.

Figure 14

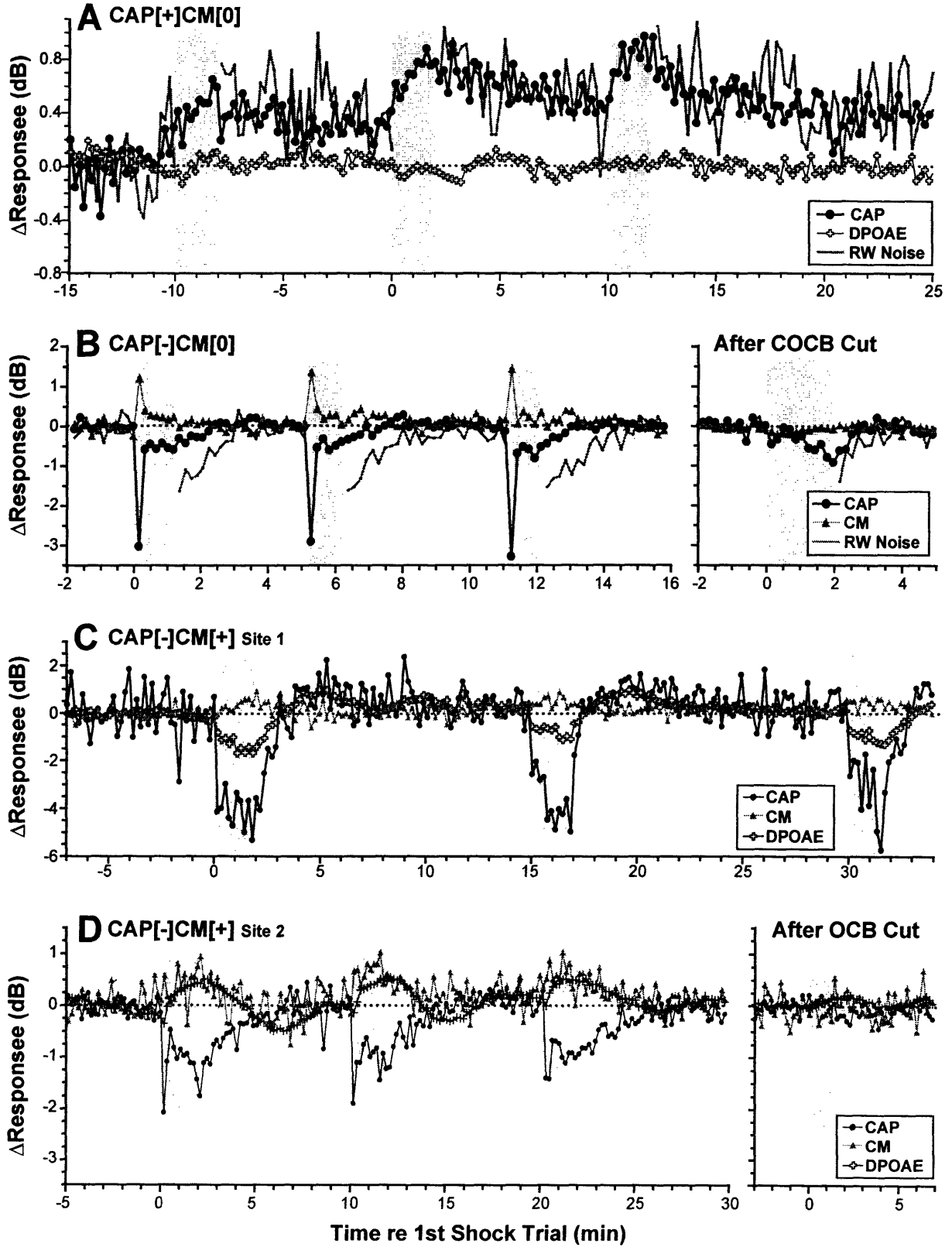

A CAP[+]CM[0]
ΔResponsee (dB)
CAP
DPOAE
RW Noise
B CAP[-]CM[0]
After COCB Cut
CAP
CM
RW Noise
C CAP[-]CM[+] Site 1
CAP
CM
DPOAE
D CAP[-]CM[+] Site 2
After OCB Cut
CAP
CM
DPOAE
Time re 1st Shock Trial (min)

Figure 14: Repeatability of long-lasting IC-evoked effects on cochlear response is illustrated for at least one site from each of the effect categories (**Panels A, B, C and D**). For each site, the timing of the repeated 2-minute shock epochs is shown by the gray shading. For each site, the contralateral ΔCAP is shown along with simultaneously measured ΔCM, ΔDPOAE or ΔRW noise (See symbol key at the right of each row of panels). The tone-pip level and frequency used for CAP measures was 13 kHz at 50 dB, 3 kHz at 35 dB, 13 kHz at 15 dB and 3 kHz at 35 dB for panels A, B, C, and D, respectively. ΔCAP, ΔCM and ΔDPOAE data are calculated as in Figure 6, and the ΔRWnoise is calculated as in Figure 12. For two of the sites (Panel B and D), a final shock epoch is illustrated at the right, obtained after lesioning the crossed OCB at the midline (Panel B) or the entire OCB at the sulcus limitans (Panel D). Values of ΔRWnoise are not obtained during the shock epoch, because the gaps in the shock train are not long enough (Figure 3). See text for further details.

Effect of Sectioning Autonomic or Olivocochlear Pathways

The effects on cochlear responses elicited by IC shocks could be mediated by a number of pathways including: 1) systemic effects on cardiovascular or respiratory system, 2) the autonomic neural projections to the ear, 3) the middle ear muscle pathways and/or 4) the olivocochlear projections to the ear, either the MOC or the LOC system. In the sections that follow, evidence is considered for and against mediation of the IC-evoked cochlear effects by each these each possible routes.

Cardiovascular or respiratory effects of IC shocks

Animals were paralyzed and artificially ventilated in almost all experiments, thus changes in respiration rate would not be elicited by the electrical stimulation. Heart rate was continuously monitored throughout all experiments with the time-function paradigm. IC shocks at times affected heart rate, sometimes increasing, sometimes decreasing it. The change in heart rate, however, was not correlated with the change in cochlear responses (Fig. 15C): some of the largest IC-evoked changes in heart rate were seen with small ΔCAPs; and some of the largest ΔCAPs were seen without significant change in heart rate. The 190 trials represented in Figure 15C are not coded by effect category; however, no correlation was seen within any of the three effect groups defined in Figure 7.

Removing autonomic projections to the cochlea

The superior cervical ganglion (SCG) is the major source of autonomic innervation to the inner ear, and the projections are exclusively ipsilateral (Densert and Flock 1974; Spoendlin 1981; Brown 1987; Hozawa, Kimura et al. 1989). To rule out a role for this

Figure 15

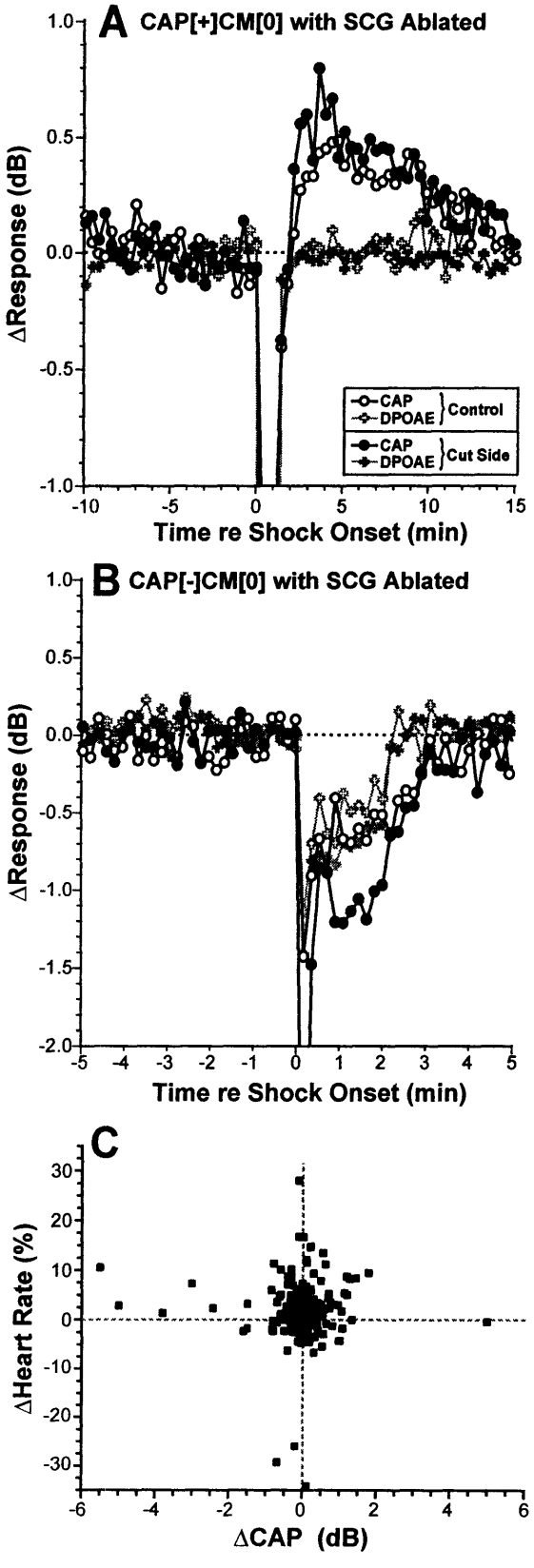


Figure 15: The presence of long-lasting IC-evoked cochlear effects after unilateral ablation of the superior cervical ganglion **(Panels A and B)**, and the lack of correlation between ΔCAP and changes in heart rate **(Panel C)**, suggest that autonomic system is not directly involved in the generation of these effects. **Panel A and B:** CAP shown is for 3-kHz tone pips at 35 dB re threshold. DPOAEs were evoked with f2=13 kHz, at primary levels 20 dB above threshold. **Panel C:** Data are shown for each of the 190 shock trials at IC electrode sites. CAP values are derived from the 13 kHz tone pips at 15 dB above threshold. Changes in CAP and heart rate are computed by averaging values in a 2-minute window immediately before vs. a 2-minute window immediately after the shock epoch. ΔCAP is expressed in dB as described by the general equation in the caption to Figure 4.

feedback system in generating the observed effects of IC stimulation, the SCG was removed unilaterally in three guinea pigs before exposing the ipsilateral IC and placing the stimulating electrodes. All of these experiments used the time-function paradigm. In two cases, IC stimulation sites were identified at which long-lasting CAP effects could be repeatedly evoked. In both cases, these robust repeatable effects were observed bilaterally. In one case (Fig. 15A), effects were of the *CAP[+]CM[0]* group; in the other case, *CAP[-]CM[0]* (Fig. 15B). In both cases, the effects were of similar magnitude and time course bilaterally, consistent with laterality previously established for these phenomena in animals with SCG intact bilaterally (Fig. 9). *CAP[-]CM[+]* effects were not found in any of the 3 animals in which the SCG was ablation. The SCG lesion results are summarized in row 12 of Table 1.

Identifying contributions of middle ear muscles

Middle ear muscles are activated by shocks to the IC (Mulders and Robertson 2000), and are likely to also be activated by shocks near the LSO, since stapedius and tensor tympani motoneurons are located in and near the superior olivary complex (Joseph et al. 1985). To minimize the possible contribution of the middle ear muscles to any effects elicited by electrical stimulation of the IC, most of the animals were paralyzed and artificially ventilated (See Methods). In addition, an independent metric of middle ear contraction was available for all experiments done with the time-function paradigm. Every 13 seconds, in that paradigm, a measure of the ear canal sound pressure waveform was digitized and DPOAE components were extracted, as well as the sound pressures of the primary tones. When animals were not curarized, the IC shocks often evoked large changes in both DPOAEs and primary tones extracted from the ear-canal sound pressure in response to the primaries, as is expected from the impedance change seen in response

to stiffening the ossicular chain (Pang and Peake 1986). After curarization, the changes in primary levels were always insignificant (less than 0.1 dB) during and after the shock epochs.

Cutting the crossed versus the entire OCB

The projections from MOC and LOC systems to ipsilateral and contralateral cochleas have been well described in the guinea pig (Robertson 1985). Since roughly 70% of the MOC-to-cochlea projections are contralateral, whereas 99% of the LOC projections are ipsilateral, cuts to the crossing fibers of the OCB at the midline will interrupt 70% of the MOC innervation to both ears while sparing almost all the LOC projections (Fig. 17). Cuts placed more laterally on the floor of the 4thV (near the sulcus limitans), can interrupt the entire OCB to one ear only (Kujawa and Liberman 1997). Verification of crossed OCB lesion by midline incisions was accomplished, in three out of three cases examined, by inspection of aceylcholinesterase-stained brainstem sections (e.g., Fig. 16A). In such material, the cholinergic OCB is clearly visible and the cut ends at the incision site can be unambiguously noted. Verification of the lateral lesion, designed to cut the entire OCB, was successful in 1/1 cases processed histologically.

Both midline and lateral OCB incisions were carried out, with IC-evoked effects on cochlear responses measured before and after such manipulations. Given the unpredictability of finding any particular effect type when placing the IC electrodes, it was not possible to study all permutations of lesion locus superimposed on all permutations of effect category. Nonetheless, the ensemble OC-lesion data, summarized in Figures 14 and 16, strongly suggest that the fast CAP suppression seen in both CAP-level and time-function paradigms is mediated by the MOC system, whereas the long-

Figure 16

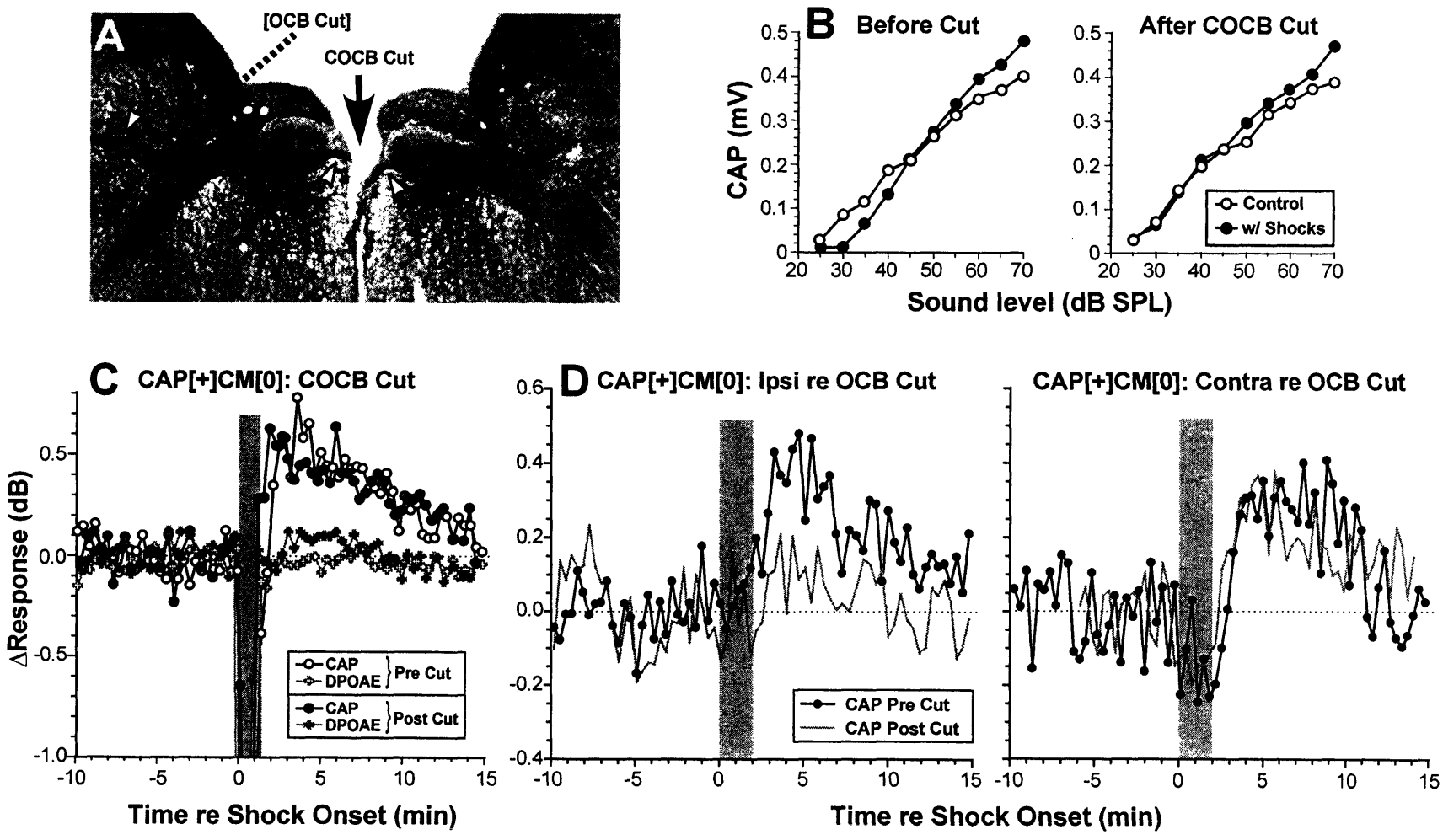

A
[OCB Cut]
COCB Cut
B Before Cut
After COCB Cut
CAP (mV)
Control
w/ Shocks
Sound level (dB SPL)
C CAP[+]CM[0]: COCB Cut
ΔResponse (dB)
CAP
DPOAE
Pre Cut
Post Cut
Time re Shock Onset (min)
D CAP[+]CM[0]: Ipsi re OCB Cut
CAP Pre Cut
CAP Post Cut
CAP[+]CM[0]: Contra re OCB Cut
Time re Shock Onset (min)

Figure 16: Effects of two types of OC lesions on long-lasting IC-evoked effects are consistent with an LOC role. **Panel A**: Photomicrograph of a transverse section through the brainstem of a guinea pig, stained to reveal cholinergic fibers of the OCB (open-headed arrows). At the midline, the bundle has been cut in this case (filled arrow), verifying the completeness of the transection. The dashed line superimposed on the micrograph illustrates schematically the position and angle of the lateral cut designed to transect the entire OCB to one side. **Panel B:** After a COCB section, low-level suppressive effects of IC stimulation disappear while CAP enhancements at high SPLs remain: compare left and right panels. Tone pips were at 11.3 kHz. The lack of enhancement in below 40 dB SPL may be a result of not sectioning both crossed and uncrossed MOC, and therefore not completely eliminating the low sound level suppressive effects of the MOC. **Panel C**: COCB section does not reduce post-shock CAP enhancement. ΔCAP values shown are for the 3 kHz tone pips at 35 dB re threshold; ΔCAP values for 13 kHz tone pips at 15 and 50 dB SL showed similar effects. ΔDPOAE values are for f_2 = 13 kHz. Because the animal was uncurarized during this particular trial, the during-shocks reduction is DPOAE and CAP was in part due to middle-ear muscle contraction evidence by a correlated reduction in the primaries recorded in the ear canal; after-shocks measurements were unaffected by middle-ear contraction evidence by the fact that DPOAEs and the primary tone measured in the ear canal returned to baseline. **Panel D**: Transection of the entire OCB effectively eliminates the long-lasting shock-evoked CAP enhancements on the side ipsilateral to the cut without affecting the ΔCAP contralaterally. CAP data shown here are for 3 kHz tone pips at 35 dB re threshold.

lasting enhancement or suppression of CAP, in the absence of changes in CM, is mediated by the LOC system.

Consider first the midline OCB incisions, designed to remove 70% of MOC projections without affecting the LOC system. In the case shown in Figure 16B, interruption of the MOC system selectively eliminated the CAP suppression seen at the low sound levels with the CAP-level paradigm, without affecting the CAP enhancement seen at high sound levels. In another case (Fig. 16C), with the time-function paradigm, a midline OCB incision attenuated the fast, during-shocks CAP suppression (not visible due to response scaling) without affecting the magnitude of the long-lasting CAP enhancement in this *CAP[+]CM[0]* trial. A third example, with midline OCB incision and a *CAP[-]CM[0]* trial is shown in Figure 14B: Here, the selective elimination of MOC projections has little effect on the long-lasting suppression of the CAP and RW noise after the end of the shock epoch. However, during the shock epoch, the MOC lesion transforms a complex pattern of CAP suppression consisting of a rapid-onset, rapidly decaying CAP suppression followed by a non-monotonicity and a slow further growth of the suppression into a simpler pattern consisting of only the slowly rising CAP suppression. No midline OCB incisions were made follow stimulation of IC sites producing *CAP[-]CM[+]* effects. The results of midline OCB incision experiments are summarized in row 13 of Table 1.

Consider last, the lateral OCB incisions designed to interrupt both LOC and MOC projections to one ear. In one animal, a lateral OCB incision essentially eliminated the IC-evoked long-lasting CAP enhancement in a site eliciting *CAP[+]CM[0]* effects, without substantially affecting the ΔCAP measured contralateral to the incision (Fig. 16). In another animal (Fig. 14D), a lateral OCB incision eliminated the long-lasting CAP

suppression and CM enhancement from IC stimulation evoking *CAP[-]CM[+]* effects. *CAP[-]CM[0]* effects were not evaluated with complete OC lesions. Lateral OCB lesions unilaterally eliminated MOC[fast] effects evoked with both IC and 4thV shocks (data not shown). The lateral OCB incision results are illustrated in row 14 of Table 1 along with previously reported results (column F).

The ensemble OC-lesion data, summarized in Figures 14 and 16, strongly suggest that the fast CAP suppression seen in both CAP-level and time-function paradigms is mediated by the MOC system, whereas the long-lasting enhancement or suppression of CAP, in the absence of changes in CM, is mediated by the LOC system. Row 15 of Table 1 illustrates this conclusion.

DISCUSSION

Summary of Results

This research sought to activate the LOC system indirectly via a higher-level auditory center, the inferior colliculus, in anesthetized guinea pigs, while bilaterally measuring cochlear potentials. The results demonstrated that stimulation at some IC sites produced changes in cochlear responses fundamentally different from those seen when stimulating OC the bundle at the 4thV. These changes fell into two novel effect classes: the first enhanced neural potentials; the second suppressed neural potentials. As Columns A and B of Table 1 summarize, these two classes of effects were marked by long-lasting "constant %" changes in CAP, accompanied by similar magnitude changes in RW noise, but no change in CM or DPOAEs. These two classes of effects, evoked with IC stimulation, were unchanged by SCG ablation or by sectioning the crossed OC bundle (70% MOC, 1% LOC). Unilaterally sectioning the OC bundle in one experiment unilaterally eliminated the neural effects. This ensemble of cochlear responses and pathway lesions results is consistent with activation of two distinct subdivisions of the LOC system, and is inconsistent with other known efferent pathways to cochlea.

LSO stimulation reproduced the effects seen with IC simulation. Two classes of long-lasting "constant %" changes in CAP were observed in the ipsilateral cochlea (Table 1, columns D and E), consistent with the 99% ipsilateral LOC projection from the LSO (Robertson 1985).

The remaining IC sites produced either "slow" or "fast" CM enhancement accompanied by correlated DPOAE modulation and CAP suppression that was largest at

low sound levels (Table 1 columns C and G). It is well established that the MOC system produces effects with these characteristics (Sridhar et al. 1995; Guinan 1996)

IC-evoked activation of the MOC system

MOC[fast] effects

Previous studies have shown that electrical stimulation of the IC can elicit cochlear effects similar to those evoked by shocking the 4thV. For example, Rajan has shown that IC shocks can protect the inner ear from acoustic injury in similar ways to that seen with 4thV stimulation (Rajan 1990). More recently, Robertson and colleagues have shown that IC stimulation can elicit CAP suppression in both ipsilateral and contralateral cochleas (Mulders and Robertson 2000). The cochlear effects in the latter study were qualitatively similar to those we categorized as *MOC[fast]* (Column G Table 1): i.e., both show fast CAP suppression that is 1) consistent with an offset time constant of ~100 ms; 2) larger at low SPLs than high SPLs; 3) larger in the contralateral than the ipsilateral ear; and 4) associated with CM enhancement and DPOAE suppression. In all respects, these phenomena are similar to "classic" MOC suppressive effects elicited when stimulating the 4thV (Column H in Table 1).

Further evidence was provided via lesions to the MOC pathway. As schematized in Figure 17A, midline OCB lesions should interrupt only MOC projections while sparing the LOC projections. Correspondingly, cutting the OCB at the midline (Figs. 14B and 16B) selectively eliminated the fast, during-shocks CAP suppression, without eliminating either the slower, long-lasting enhancements (Fig. 16B) or suppression (Fig. 14B), which were also evoked by the IC shocks.

Figure 17

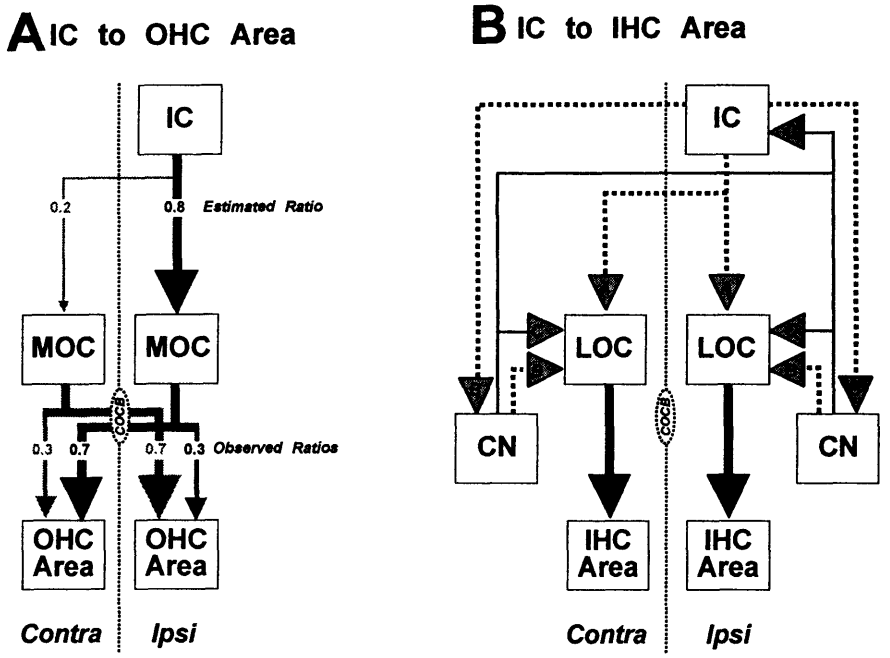


Figure 17: Schematics illustrating the known (solid lines) and putative (dashed lines) neural circuitry underlying the IC-evoked effects on cochlear function. **Panel A:** IC evoked effects in OHC area. Observed ratios cited for MOC to OHC projections are from published studies (Robertson et al. 1987). Estimated ratios cited for IC to MOC projections are derived from laterality of MOC[fast] effects seen after sectioning the COCB. See text for further details. **Panel B:** IC-evoked effects in IHC area. The lettered arrowheads are described in the text.

Data on descending projections from the IC, especially with respect to laterality, are consistent with the classification of these IC-evoked, during-shocks effects as MOC in origin. As schematized in Figure 17A, the IC-to-MOC projections in guinea pig are predominately ipsilateral, although precise ratios are hard to derive from the existing data (Thompson and Thompson 1993). The projections from MOC regions to outer hair cells, in turn, are known to be roughly 70% contra: 30% ipsi in guinea pig (Robertson 1985). Given the bilateral symmetry of MOC projections, such a wiring diagram would predict that 1) stimulation of the IC ipsilaterally should lead to larger effects in the contralateral ear (as was observed - Figs. 5A, 10A, and 10C), and 2) after midline section of the COCB, contralateral effects should be reduced as a function of the ratio of ipsi:contra IC-to-MOC projections. For example, if the descending IC projections were exclusively ipsilateral, the midline cut should remove all contralateral effects. Our observation that COCB section reduced the IC-evoked rightward shift in the contralateral CAP to 10-20% of its precut dB shift (data not shown, n=3 histologically verified animals) is consistent with roughly a 80:20 ratio for ipsi versus contra descending IC projections. Such a value is not inconsistent with existing anatomical data (Thompson and Thompson 1993). Such a small proportion of contralateral IC-to-MOC projections would not greatly modify the expected ratio of contra : ipsi cochlear effects from IC shocks (70:30 in the absence of any contralateral IC-MOC projections versus 62: 38 with a small (20%) contralateral descending IC projection).

The laterality of *MOC[fast]* effects evoked by IC stimulation is complicated by the fact that the ipsi versus contra cochlear projections of the MOC system have different distributions along the cochlear spiral. The ipsilateral projection is skewed towards the apex (low-frequency) of the cochlea when compared to the contralateral projection, in cat (Guinan et al. 1984). Using the density of MOC terminals for each cochlear

frequency given by Guinan and Warr, and assuming that IC stimulation evenly activated the MOC system in an 80 : 20 (ipsi : contra) ratio, we can predict the laterality of effects as a function of frequency. The ratio of ipsi:contra cochlear effects of IC stimulation should approach equality as the acoustic stimulus excites increasingly apical cochlear locations and approach 1:3 (ipsi:contra) for stimuli that evoke basal cochlear locations. Such a trend was indeed observed (Fig. 5A).

A final layer of complexity that must be considered is the possibility that the descending IC-to-MOC projections mirror the tonotopic organization of the ascending projections to the IC (Ota and Dolan 1999). In one experiment, we compared the magnitude of *MOC[fast]* effects for IC electrode sites in the ventral, middle and dorsal regions of the IC (Fig. 5B) and saw that the cochlear region maximally affected shifted to lower frequencies as the electrode site moved more dorsally. A similar tonotopic organization has been described for the sound-evoked responses of IC principal neurons (Merzenich and Reid 1974). Although more detailed experimentation is required to systematically characterize this parallel tonotopicity, the existing evidence overwhelmingly supports the notion that the *MOC[fast]* effects in the present study represent the activation of MOC neurons by a descending IC to MOC projection.

MOC[slow] effects

If IC stimulation can activate the MOC system, then IC shocks should also be able to elicit the "slow" effects of MOC activation demonstrated in experiments with 4thV stimulation via electrodes at the floor of the 4thV (Sridhar et al. 1995). These slow effects comprise a long-lasting CAP suppression mirrored by a CM enhancement; effects on DPOAEs or RW noise have not been previously documented. Both fast and slow

effects of the MOC system are blocked with equal effectiveness by pharmacologic antagonists of the α9 cholinergic receptor on OHCs (Sridhar et al. 1995). Sridhar et. al. hypothesized that slow effects arise from a wave of calcium-induced calcium release occurring secondarily to the Ca influx induced by ligand binding to the α9 receptor (Sridhar et al. 1997).

MOC[slow] effects were documented in the present study with electrodes on the 4thV. The CAP suppression is mirrored by a comparable suppression of the DPOAE and a small suppression of the RW noise (See Table 1). Both results are consistent with expectations. The DPOAE should be suppressed given that the MOC effects on OHCs result in a reduction of cochlear mechanical amplification (Murugasu and Russell 1996). Similarly, a small reduction of RW noise is not unexpected given that MOC effects on the cochlea include a small decrease in spontaneous discharge rates in auditory nerve fibers (Guinan and Gifford 1988b), thought to be mediated by the decrease in EP associated with the increased conductance through the OHCs (Guinan and Stankovic 1995).

As indicated in Table 1 (Column C), we hypothesize that the *CAP[-]CM[+]* category elicited via IC shocks represent *MOC[slow]* effects. The fact that this effect disappeared after complete OC section (Fig. 14D) is consistent with an olivocochlear origin. The similarity of effects to those seen with 4thV stimulation (compare columns C and F of Table 1) constitutes the evidence that this category represents *MOC[slow]* effects. Clearly, the sign and magnitude of the ΔCAP, ΔCM and ΔRW noise are comparable. The fact that the DPOAE was suppressed at site 1 and enhanced at site 2 for this effect category (Fig. 11A) is not inconsistent with MOC mediation, since other studies have shown that shock- and sound-evoked MOC activation can sometimes enhance or

suppress DPOAE magnitudes (Siegel and Kim 1982; Liberman et al. 1996). Similarly, the differences in frequency and offset time-constant are not inconsistent with MOC mediation given that IC to MOC projections appear to be tonotopically organized and the mediating neural circuits could lengthen the duration of the effect. Further comparison from the summary Table 1 shows that IC-evoked *CAP[-]CM[+]* effects and 4thV-evoked *MOC[slow]* effects are also similar in that their ΔCAPs show a strong level dependence, with effects greater at low stimulus SPLs, as expected for an MOC effect that decreases gain of the cochlear amplifier. Although this effect category was not often evoked, the simplest interpretation is that it represents activation of *MOC[slow]* effects via IC-to-MOC projections.

IC- or LSO-evoked activation of the LOC system

Distinguishing LOC from MOC effects

Electrode sites in the IC and the LSO produced long-lasting suppression or enhancement of the CAP (Columns A,B, D, and E in Table 1; Figs. 7A and 7B). Using the CAP-level function paradigm, we showed that these effects differed from MOC effects (whether "fast" or "slow"). First, the ΔCAP was level-independent, corresponding to a constant % change regardless of tone pip SPL (Fig. 4). Second, the ΔCAP was unaffected by COCB section (Figs. 14B, 14B), which should eliminate 70% of the MOC innervation without significantly affecting the LOC innervation of the ear (Fig. 17). When stimulating in the LSO (Table 1: Columns D and E), such effects were only seen in the ipsilateral ear consistent with LOC projections and inconsistent with the MOC projections (Fig. 17B). When stimulating in the IC (Table 1: Columns A and B),

such effects were evocable only with electrodes in the ventrolateral corner of the nucleus (Fig. 4G), consistent with the idea that different regions of IC have different descending projections (See below).

The full constellation of cochlear effects associated with this long-lasting suppression or enhancement of the CAP was consistent with a site of action corresponding to the major LOC peripheral targets on IHCs and the type-I afferent fibers which contact them (Liberman 1980; Liberman et al. 1990). The neurally-based ΔCAP was mirrored by ΔRW noise of similar sign and time course (suggesting long-lasting modulation of spontaneous discharge in the auditory nerve or alterations in the electrical properties of the auditory nerve terminals (McMahon and Patuzzi 2002)), whereas both OHC-based metrics, ΔCM and ΔDPOAE, were unaffected by the shocks. Furthermore, the level-independent nature of the ΔCAP is similar to effects reported with cochlear perfusion of putative LOC neurotransmitters.

A final argument against MOC involvement can be made for the CAP enhancing group. MOC-mediated enhancement of the CAP response to transient stimuli, such as tone pips, is only seen in the presence of continuous background noise ((Kawase and Liberman 1993); See below). Background noise was not present in this study, and, furthermore, MOC activation via OCB stimulation failed to produce CAP enhancement in the same animals that showed IC-evoked CAP enhancement effects.

Distinguishing OC effects from other feedback pathways

Having reviewed the evidence for LOC mediation and against MOC mediation of long-lasting modulation of cochlear neural responses, consider the arguments for OC mediation versus other feedback pathways to the ear.

A role of the middle-ear muscles is effectively ruled out by the demonstration that the curarization procedures eliminate the shock-evoked changes in middle-ear impedance, but does not eliminate the putative LOC effect. Unchanging middle-ear impedance was confirmed throughout the experiment by measuring the sound pressure in the ear canal produced by the primary tones used to evoke DPOAEs. Similar measures of muscle-induced impedance changes constitute the basis for clinical devices used to measure the middle-ear muscle reflex (Bel and Causse 1976); thus the test applied here is a well-studied and robust one.

A possible role for the autonomic system in the observed effects needs to be carefully considered. The autonomic system primarily innervates cochlear blood vessels (see below). However, a component of the superior cervical ganglion (SCG) forms a plexus of "vessel-independent" terminations among the peripheral axons of ipsilateral auditory nerve fibers (Densert and Flock 1974; Spoendlin 1981). These autonomic fibers appear to have an enhancing effect on ipsilateral neural potentials: unilateral stimulation and chronic ablation of the SCG can respectively enhance (Pickles 1979), and cause "constant %" reductions in (Hultcrantz et al. 1982) ipsilateral cochlear CAP. To rule out a role of the vessel-independent sympathetic fibers arising from the SCG, we showed that both *CAP[+]CM[0]* and *CAP[-]CM[0]* effects were still demonstrable bilaterally after unilateral removal of the SCG (Figs. 15A and 15B).

The autonomic innervation of the cochlea's vasculature derives from three sources. First, superior cervical ganglion fibers are intimately associated with ipsilateral blood vessels in the modiolus (Spoendlin and Lichtensteiger 1967). Second, stellate ganglion fibers are bilaterally associated with the labyrinthine and cochlear artery (Spoendlin 1981). Third, the trigeminal ganglion system appears to give rise to ipsilateral vessel-associated fibers in the modiolus, the interscalar septae and the stria vascularis (Vass et al. 1998). Cochlear effects of electrical stimulation of the trigeminal ganglion have not been studied; however, given that the fibers are associated with blood vessels, including those in the stria vascularis (Vass et al. 1998), trigeminal ganglion is likely to affect blood flow, and in turn, the EP. Stimulation of the SCG and the stellate ganglion have been shown to reduce cochlear blood flow (Laurikainen et al. 1993; Ren et al. 1993).

Several pieces of evidence suggest that a change cochlear blood flow is unlikely to mediate *CAP[+]CM[0]* or *CAP[-]CM[0]* effects. First, cochlear vascular changes, mediated by either type of vessel-associated autonomic fiber system, would likely affect EP, and would be expected to change OHC-based metrics of cochlear function, not only neural based metrics. CM and DPOAE have been shown to be vulnerable to changes in EP (Mills et al. 1993; Tsukasaki et al. 2000)). Betahistine hydrochloride, which improves cochlear blood flow by dilating feeding vessels, increased both CAP and CM (Laurikainen et al. 1997), and DPOAEs have been shown to be sensitive to reductions in cochlear blood flow (Mom et al. 1999). Secondly, several attempts to stimulate the SCG have failed to change CAP (Baust et al. 1964; Lee and Moller 1985; Wada et al. 1995), whereas there appears to be no report where SCG stimulation failed to affect cochlear blood flow. It follows that changes in neural potentials only occur after large changes in cochlear blood flow, consistent with evidence that cochlear blood flow is highly autoregulated (Brown and Nuttall 1994; Wangemann 2002). Consistent with evidence

of autoregulation, IC shocks at times produced changes in heart rate, as large as + / - 30%, with no effect on CAP (Figure 15C). Third, CAP[+]CM[0] effects were seen with no change in heart rate (Figure 15C); no correlation between heart rate and CAP effects was observed across our database of effects. Lastly, an OC role in mediating the long-lasting selective modulation of cochlear neural potentials is most convincingly demonstrated by the fact that the effects disappear unilaterally after unilateral transection of the OCB in the brainstem (Fig. 16D).

Taken together, this evidence strongly supports the notion that we have succeeded in activating the LOC system via shocks delivered in either the IC or the LSO. Although previous studies have evaluated cochlear effects of shocking the IC (Mulders and Robertson 2000) or in the vicinity of the LSO (Gifford and Guinan 1987), none has suggested that the LOC system was activated. Note that previous investigations in the LSO or IC were not designed to evaluate small, or long-lasting changes in cochlear response. Previous IC studies may not have stimulated the ventrolateral corner, where the putative LOC effects were evoked in the present study. Although (Mulders and Robertson 2000) note in passing that IC stimulation sometimes enhanced the CAP evoked by 16 kHz tone pips, they suggested the effect was the MOC-mediated anti-masking phenomenon previously reported for OCB stimulation (Nieder and Nieder 1970).

Several themes emerge from this set of results. First is that IC stimulation can activate the LOC system. Second is that the LOC system can both enhance and suppress peripheral neural activity. Third, is that changes produced in the CAP have a similar dB magnitude for stimuli across a wide range of sound levels. These themes are explored further in the next section and strengthened by cross-study comparison.

IC connections to the LOC system?

The current study provides evidence for a direct or indirect IC-to-LOC pathway, yet no definitive anatomic demonstration exists either for or against an IC-to-LOC projection. Double labeling of OC and IC neurons illustrated explicit IC projections to the MOC system (Thompson and Thompson 1993), but failed to demonstrate IC synapses on LOC somata, possibly because LOC neurons primarily receive inputs on their dendrites (Lu et al. 1987). Furthermore, the observation of sparse IC projection into LSO region may in part reflect limited focal anterograde tracer injections that excluded much of the external nucleus, which is the most densely labeled IC region following injections of retrograde tracers into the superior olivary complex (Faye-Lund 1986). Consistent with the possibility of a direct IC-to-LOC pathway, one study in cat found significant degeneration in the LSO region bilaterally following IC ablation (VanNoort 1969), however, it is unknown whether these fibers terminate on LOC dendrites.

As schematized in Figure 17B, a number of routes could mediate indirect IC activation of the LOC neurons. In addition to a direct descending projection from IC to LOC ("A" arrows in Fig. 17B), there might be a more indirect route, e.g., descending IC to cochlear nucleus projection followed by an ascending cochlear nucleus to LOC projection ("B" arrows in Fig. 17B). Numerous other nuclei in the auditory brainstem could be involved. It is notable, in this regard, that, even with respect to the IC-to-MOC projections, the number of MOC neurons with identified IC terminals was so small in the tracer study (n=15 out of hundreds of sections examined; Thompson and Thompson 1993), that the authors suggested there might be additional projections via intervening

nuclei (e.g., lateral lemniscus), given the apparent ease with which MOC-like effects can be elicited via stimulation in central nucleus of the IC.

Lastly, it is possible that our electrodes in the ventrolateral IC, positioned where the nucleus meets the fiber tract of the lateral lemniscus, are actually antidromically activating an ascending projection to the IC that also sends a branch to the LOC cells in the LSO ("C" arrows in Fig. 17B). Present results can shed little light on the pathways involved, except that the near equality of ipsilateral and contralateral effects (See Table 1) require bilateral projections somewhere in the pathway. As discussed below, the heterogeneity of putative LOC effects suggests that different subgroups may exist; thus the neural circuits feeding them may also differ.

Putative subsystems within the LOC pathway

Current observations that the LOC system, activated indirectly with IC stimulation, can produce dual and complementary effects in the periphery (i.e., long-lasting suppression or enhancement of neural responses) fits well with results of neurochemical, pharmacological, and neuroanatomical studies. The LOC system is heterogeneous with respect to neurotransmitters, with evidence (reviewed by (Eybalin 1993)) that LOC neurons may contain acetylcholine, GABA, catecholamines (e.g., dopamine), as well as peptides (e.g., enkephalins, CGRP and urocortin). The application of these putative transmitters to hair cell systems can have excitatory (e.g., acetylcholine (Felix and Ehrenberger 1992), dynorphins (Sahley and Nodar 1994)), and inhibitory (e.g., GABA (Arnold et al. 1998), enkephalin (Burki et al. 1993), and dopamine (d'Aldin et al. 1995)), or mixed (e.g., CGRP (Bailey and Sewell 2000)) effects on neural responses.

The idea of a dual LOC peripheral action also fits with anatomical evidence of at least two classes of neurochemically distinct LOC neurons, e.g., cholinergic versus GABAergic in rat (Vetter et al. 1991), and two morphologically distinct LOC neurons in the periphery (unidirectional versus bidirectional with respect to cochlear projection patterns (Brown 1987)), and in the brainstem (intrinsic versus shell with respect to soma location in the brainstem (Vetter and Mugnaini 1992)). Intrinsic LOC neurons appear to give rise to peripheral projections "unidirectional" IHC efferents, while LOC shell neurons give rise "bidirectional" peripheral projecions (Warr et al. 1997).

Given that both suppression and enhancement of CAP appear to result from activation of the LOC system, and given that different polarities of modulation were reproducibly elicited at different electrode sites within the IC or LSO, the LOC system appears unlikely to be comprised of a homogeneous population of neurons with identical transmitter content and uniform peripheral targets.

One simple possibility is that two subgroups of LOC neurons have different neurotransmitter content (e.g., GABAergic versus cholinergic, Vetter et al. 1991) and different driving circuitry, but similar peripheral targets. Recent immunohistochemical evidence favoring the notion that individual LOC fibers colocalize all neurotransmitter candidates (Safieddine et al. 1997; Maison et al. 2003a) suggests an alternate scenario.

Another possibility is that two putative subgroups have similar neurotransmitter content, but differ in driving circuitry and peripheral targets, e.g., one synapses with auditory nerve fibers and another with the inner hair cell (Liberman et al. 1990). The peripheral terminals of the LOC make numerous axo-dendritic synapses on cochlear afferents in the IHC area. Additionally there is also a small but consistent contingent of synaptic connections with the IHC itself, and numerous terminals in intimate contact

with IHCs without clear synaptic specializations (Liberman et al. 1990). Thus, both pre- and post-synaptic effects of the LOC system in the inner hair cell area must be considered in parsing out the complex effects reported here.

A further level of complexity is introduced by the fact that cochlear afferents are not a homogeneous population. Rather, they are subdivided into 2 or 3 functional subgroups, differing systematically in spontaneous discharge rate (SR), threshold sensitivity, position of synaptic contact around the IHC circumference, as well as nature and complexity of their central projections to the cochlear nuclei (Liberman 1982; Liberman 1991). Consistent with possible SR-based differential targeting, the existing anatomical data show clearly that the Low-SR fiber group is much more richly innervated by the LOC system (Liberman et al. 1990).

Inferences about LOC effects from previous studies

Many manipulations related to the LOC system, including those in the current experiments, modulate cochlear neural responses such that amplitudes are decreased/increased by a constant % over a wide range of input sound-pressure levels, including in the absence of exogenous acoustic stimulation, i.e., spontaneous potentials. These manipulations include 1) perfusion of putative LOC transmitters (or their agonists) through the cochlear scalae (d'Aldin et al. 1995), 2) genetic elimination of putative LOC transmitters or their receptors in knockout mice (Maison et al. 2003b), and 3) lesions to LOC projections to the cochlea (LePrell et al. 2003). Consideration of the present results in the context of this pre-existing work suggests which transmitter/receptor interactions underlie the excitatory and inhibitory effects of LOC activation described here.

Candidate pathways for both the excitatory and inhibitory effects are suggested by previous work on exogenously applied transmitter and/or receptor agonists. For example, in the mammalian cochlea, inhibitory effects on either spontaneous spike rates, or glutamate-evoked spike rates in the IHC area, have been reported after cochlear perfusion of GABA (Arnold et al. 1998), dopamine (Burki et al. 1993; Ruel et al. 2001), or enkephalin (Burki et al. 1993), an endogenous opioid that interacts with the mu-opioid receptors. With respect to dopaminergic effects, cochlear perfusion of pirebidil (an agonist of the D1 and D2 receptor classes) also suppressed CAPs (d'Aldin et al. 1995). When the pirebidil data are replotted as dB shifts (Fig. 18A), the ΔCAPs are level-independent, as was characteristic of the putative LOC suppressive effects in the present study (e.g., Fig. 4). Thus the dopaminergic pathway is a candidate for the *CAP[-]CM[0]* effects reported here.

Excitatory effects on either CAP amplitude or IHC subsynaptic spiking activity can be elicited by application of either pentazocine (a kappa-opioid receptor agonist; (Sahley and Nodar 1994)) or acetylcholine (Felix and Ehrenberger 1992) respectively. The ΔCAPs elicited by the opioid agonists were larger at low SPLs than at high SPLs, as seen in the replotted data in Figure 18A. In this respect, they differ from the putative excitatory effects of LOC activation reported here.

CGRP is a peptidergic transmitter that co-exists in the LOC system with acetylcholine (Vetter et al. 1991). Effects of CGRP perfusion have not been reported in the mammalian cochlea; however, in the Xenopus lateral line, CGRP application increases spontaneous activity in afferent fibers (Bailey and Sewell 2000). In the mammalian cochlea, the effects of CGRP deletion have been studied in a transgenic mouse line (Maison et al. 2003b). In the homozygous CGRP-null mice, there was a

Figure 18

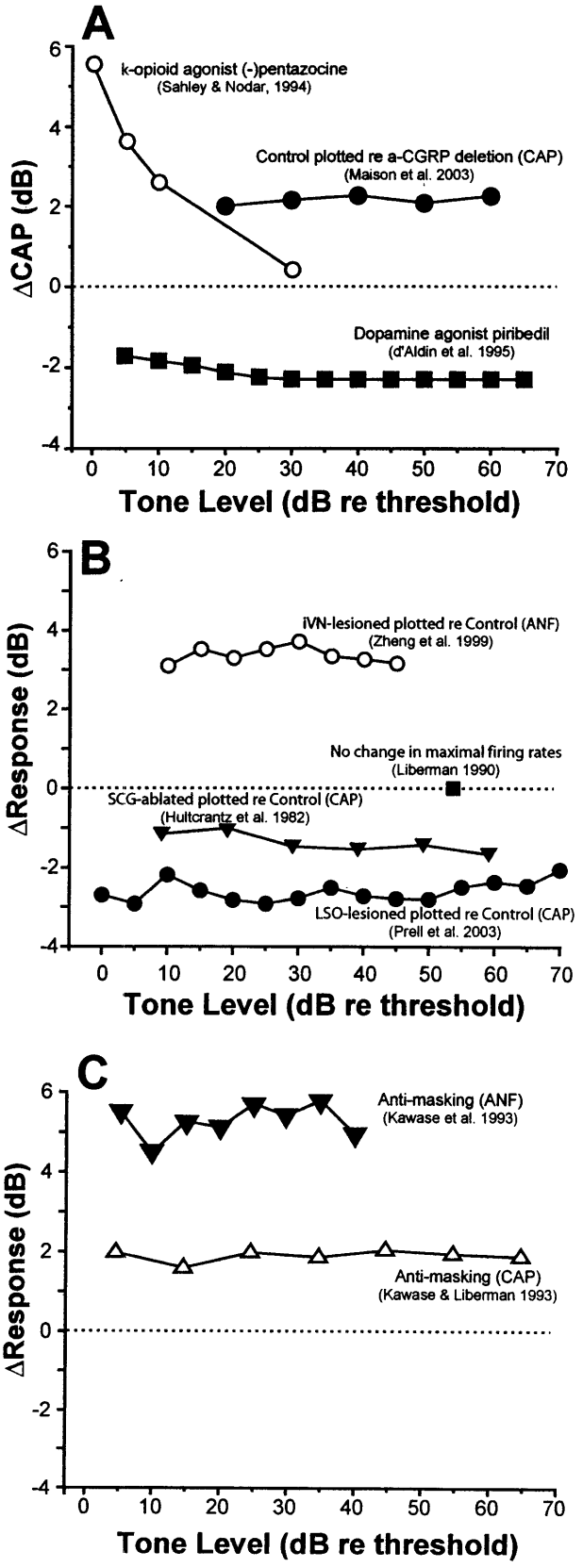


Figure 18: A number of manipulations in the auditory periphery result in level-independent, constant-precentage shifts in neural responses. **Panel A:** Changes in CAP or ABR amplitudes, plotted in dB, seen in other studies following intracochlear perfusion of, or genetic deletion of, putative LOC neurotransmitters or their agonists. Perfusion of the opioid-agonist pentazocine enhanced CAP (Sahley and Nodar 1994), whereas the dopamine-agonist pirebidil suppressed CAP (d'Aldin et al. 1995). Deletion of aCGRP from the cochlea decreased CAP (Maison et al. 2003a), therefore the effects of CGRP are shown as enhancements. **Panel B:** Changes in CAP amplitudes or ANF firing rates, plotted in dB, following lesions to the OC or autonomic pathway. Lesionsing the LSO with Melittin (Prell et al. 2003) and ablating the SCG (Hultcrantz et al 1982) decreased CAP in guinea pigs, whereas sectioning the inferior vestibular nerve (Zheng et al. 1999) enhanced ANF firing in chinchillas, and sectioning the OCB with an incision along the sulcus limitans (Liberman 1990) had no effect on CAP measured at suprathresholds in cats. **Panel C:** Changes in CAP amplitude, or driven rate in auditory nerve fibers, plotted as dB shifts seen in other studies following activation of the MOC system by addition of contralateral noise (Kawase et al. 1993; Kawase and Liberman 1993).

significant decrease in the amplitude of neural potentials auditory brainstem responses, in the absence of changes in the amplitude of the DPOAEs, consistent with selective effects on the IHC or cochlear afferent fibers. Replotting the ΔABRs, i.e., the difference in amplitude between wildtype and CGRP null littermates, as percentage change in neural amplitudes reveals a level-independent effect. The CAP decrease in the knockout ears suggests an excitatory role for CGRP in the mammalian cochlea. The level-independence of the modulation is characteristic of the putative LOC-mediated excitation reported here. Thus, activation of the CGRPergic pathway is a reasonable candidate for the *CAP[+]CM[0]* effects reported here.

If there are complementary excitatory and inhibitory LOC subsystems operating in the normal ear, the effects of de-efferentation might be complex and variable. Studies lesioning the LOC system observed constant % decreases (LePrell et al. 2003), constant % increases (Zheng et al. 1999), and no change (Liberman 1990) in sound driven neural potentials. Figure 18B summarizes these lesion studies. LePrell et al. provide the only selective LOC lesions, and deduce that the LOC system can have tonic enhancing effects in guinea pig (LePrell et al. 2003). Zheng el al.'s constant % enhancement suggests a tonic suppressive LOC system in chinchilla (Zheng et al. 1999). This result is inconsistent with disruption of SCG projections that anastomose to the vestibular nerve; chronic disruption of SCG projections *reduces* cochlear potentials (Hultcrantz et al. 1982). It is also inconsistent with MOC system given that 1) removal of anti-masking would result in decreased, not increased firing rates, 2) the magnitude of the changes in afferent firing rates measured at high sound levels were greater than those seen with stimulation of entire OC bundle (7% on average; Guinan and Gifford 1988), and 3) sectioning the crossed OC fibers had no effect on cochlear potentials (current experiments, data not shown, n=9). The lack of change in driven rates in the

anesthetized cat (Liberman 1990) suggests that in some species, suppressing and enhancing LOC effects may balance, such that removing both has no significant effect.

All studies examining spontaneous rates following OCB lesions report a decrease in spontaneous discharge rates and hypothesize that these long-term changes are due to loss of LOC innervation (Liberman 1990; Zheng et al. 1999). In the normal ear with no exogenous acoustic stimulation, excitatory LOC influences may outweigh inhibitory ones, such that loss of both subsystems results in a net decrease in spontaneous neural activity.

Implication for mechanisms of action and for LOC function(s)

The magnitudes of the observed effects on CAP were small, on the order of 10%; however, the effects of the LOC on individual fibers may be significantly larger. First, the LOC efferents are known to disproportionately synapse on the low spontaneous rate afferent fibers on the modiolar side of the IHC (Liberman et al. 1990). Thus, there could be large effects on this subpopulation of afferents without large changes in the CAP, which is dominated by the more numerous high-spontaneous rate fibers. Secondly, IC stimulation is likely to only partially activate the LOC system, in the same way that IC stimulation appears to result in limited activation of the MOC system (Rajan 1990; Mulders and Robertson 2000). Thirdly, the anesthesia regimen may suppress the activity of the LOC system, and reduce its peripheral effects relative to those in an awake animal. Thus, the potential effects of the LOC system in individual fibers in awake animals is likely to be much larger than the observed changes in CAP when stimulating the IC.

Characterization of putative LOC effects as involving constant-percentage changes in neural firing rates has heuristic value in providing an important criterion by which to differentiate MOC from LOC effects. This characterization is also valuable for what it implies about the mechanisms underlying the effect, as well as a possible functional role for the LOC system.

Manipulations that alter the degree of adaptation at the IHC / afferent synapse can produce constant-percentage changes in auditory-nerve discharge. Adaptation in the auditory nerve arises in synaptic transmission between inner hair cell and cochlear afferent, involving a shift in the equilibrium between the rate of synaptic-vesicle release and the rate of vesicle resynthesis/re-mobilization into the readily releasable pool (Smith et al. 1983; Moser and Beutner 2000). In response to a prolonged tone, sound-evoked discharge in single fibers decreases exponentially; and, as sound pressure increases, the onset and steady-state rates increase in constant ratio (Smith 1979). In a simultaneous masking paradigm, neural discharge to a brief probe tone is decreased by addition of a simultaneous masker, because it increases the degree of adaptation (vesicle depletion) at the synapse. Anti-masking can occur by reducing the ongoing response to the masker via activation of the MOC system. Though this anti-masking mechanism works via the OHC-based cochlear amplifier, it translates into a reduction in adaptation level at the IHC / afferent synapse, which is reflected by a constant-percentage increase in CAP amplitude as well as in driven rates in auditory nerve fibers (Fig. 18C).

Hypothetically, any process within the IHC that increases the number of vesicles in the readily releasable pool should mimic a change in adaptation level and produce a level-independent, constant-percentage change in neural response. Although these arguments suggest that IHC-based effects can mimic the putative LOC effects evoked in

the present study, changes in the behavior of post-synaptic receptors on the afferents could also produce the level-independent effects seen.

With respect to possible LOC function, present results suggest that LOC feedback provides a means to modulate neural discharge rates in a way that could compensate for alterations in adaptation level in the auditory nerve. Such a capability is intriguing in light of the fundamental role of the LSO (the nucleus of origin of the LOC system) in computing the location of sounds in space from intensity differences at the two ears that, in turn, are coded as interaural differences in response rates in the ipsilateral versus contralateral auditory nerves. It is possible that an LOC-based feedback system, with the power to raise and lower afferent response rates from either ear in level-independent fashion, is required to maintain the appropriate balance in neural outflow from the two ears, and thus to maintain the accuracy of the binaural computations performed by the LSO (Guinan 1996). Such binaural accuracy must be maintained in the face of sub-optimal listening environments, including, for example, the presence of asymmetrical masking noises, which could cause asymmetric changes in adaptation levels between the two ears. Given the long time-constants of the putative LOC effects revealed in the present study, it is also interesting to speculate that LOC feedback could be useful in maintaining the accuracy of binaural intensity comparisons in the face of slowly shifting cochlear thresholds, as might arise during the onset and recovery from a unilateral middle-ear infection. The psychophysical performance of animals with chronic cochlear de-efferentation should be revisited with tests assaying the sound localization abilities in suboptimal listening environments.

Lastly, the LOC system appears to exert the largest reduction in cochlear responses evoked with very high SPL acoustic stimuli, positioning it to reduce the damage to

overstimulated afferent fibers. Indeed, dopamine agonist piribedil, which produced constant % suppression (Fig. 18A), reduced the damage to radial dendrites caused by noise exposure. Furthermore, unilateral OCB incision unilaterally increased hearing loss during noise exposure (Kujawa and Liberman 1997; Attanasio et al. 1999), whereas sectioning 70% of the MOC system did not increase hearing loss. Evaluation of hearing loss following unilateral LSO lesions would further establish LOC system's protective role.

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