A Novel Effect of Cochlear Efferents: In Vivo Response Enhancement Does Not Require α9 Cholinergic Receptors

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Maison SF, Vetter DE, Liberman MC. A novel effect of cochlear efferents: in vivo response enhancement does not require α9 cholinergic receptors. J Neurophysiol 97: 3269–3278, 2007. First published March 7, 2007; doi:10.1152/jn.00067.2007. Outer hair cells in the mammalian cochlea receive a cholinergic efferent innervation that constitutes the effector arm of a sound-evoked negative feedback loop. The well-studied suppressive effects of acetylcholine (ACh) release from efferent terminals are mediated by α9/α10 ACh receptors and are potently blocked by strychnine. Here, we report a novel, efferent-mediated enhancement of cochlear sound-evoked neural responses and otoacoustic emissions in mice. In controls, a slow enhancement of response amplitude to supranormal levels appears after recovery from the classic suppressive effects seen during a 70-s epoch of efferent shocks. The magnitude of post-shock enhancement can be as great as 10 dB and tends to be greater for high-frequency acoustic stimuli. Systemic strychnine at 10 mg/kg eliminates efferent-induced suppression, revealing a purely enhancing effect of efferent release from efferent terminals are mediated by α9/α10 ACh receptor complexes.

Previous in vivo studies hinted at the existence of MOC-evoked response enhancements: after long (60-s) epochs of MOC stimulation, cochlear responses recovering from slow-effect suppression sometimes “overshoot” the pre-shock baseline (see Sridhar et al. 1995; Fig. 6) to supranormal amplitudes and require >100 s to return to baseline levels. Similarly, in a study of MOC effects on basilar membrane vibrations, in addition to fast and slow suppressive effects evoked by MOC shock trains, the authors noted that a rebound period of hypersensitivity was often observed after recovery from slow inhibition (see Cooper and Guinan 2003; Fig. 3C). In the absence of evidence to the contrary, this overshoot/rebound was assumed to be part of the recovery process of slow effects rather than a separately evocable component of efferent effects on the inner ear.

Here, we show that this MOC-evoked slow enhancement is a robust and repeatable phenomenon in the mouse and present evidence that it represents a fundamentally different phenomenon from the fast and slow suppressive effects of ACh previously described. We show systemic strychnine can completely eliminate MOC-evoked suppression without any diminution of slow enhancement. We also reanalyzed MOC-evoked effects in mice with targeted deletion of the α9 nicotinic ACh receptor (Vetter et al. 1999) and show a similar phenotype: i.e., loss of suppression with a robust slow enhancement remaining in the most sensitive animals. We consider the possibility that this novel phenomenon reflects noncholinergic effects of the MOC system and/or the involvement of peripheral targets other than OHCs.

METHO

Experimental procedures

CBA/CaJ and C57Bl/6 mice were obtained from Jackson Laboratories. Mutant lines were supplied from a variety of different labora-

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Distortion product otoacoustic emission and compound action potentials response

Acoustic stimuli were presented through a custom acoustic assembly consisting of two electrostatic drivers (EC-1, Tucker Davis Technologies) to generate acoustic stimuli and a Knowles miniature microphone (EK3103) to record ear-canal sound pressure. Stimuli were generated and responses were measured with locked digital I-O boards at 4-μs sampling (AO-6052E, National Instruments). For distortion product otoacoustic emissions (DPOAEs), primary tones f1 and f2, with f1/f2 = 1.2 and f2 level 10 dB < f1 level were presented continuously, and the ear-canal sound pressure waveform was amplified (1,000 times) and averaged (8 or 25 consecutive waveform traces), and a spectrum was computed (fast Fourier transform); the process was repeated 2 or 4 times, the resultant spectra averaged, and 2f1-f2 DPOAE amplitude and surrounding noise floor were extracted, a procedure requiring ~1 or 4 s of data acquisition and processing time, respectively. When response averaging was set to 25 traces per spectrum and four spectra per point, the noise floors were from ~5 to ~10 dB SPL, depending on frequency. For compound action potentials (CAPs), 5-ms tone pips at 20/s were presented to the ear canal, and responses from a silver wire on the round window membrane were amplified 10,000 times, referred to a ground in the neck muscles, and fed to the I-O for averaging (16 responses averaged per point).

Medial olivocochlear assay

Animals were anesthetized with urethane (1.20 g/kg, ip). A posterior craniotomy and partial cerebellar aspiration were performed to expose the floor of the IVth ventricle. To stimulate the OC bundle, shocks (monophasic pulses of 150-μs duration presented at 200/s continuously throughout the ~70-s shock epoch in each run of the MOC assay) were applied through fine silver wires (0.4 mm spacing) placed along the midline, spanning the OC decussation. Shock threshold for facial twitches was determined, muscle paralysis was induced with α-α-tubocurarine (1.25 mg/kg ip), and the animal was connected to a respirator through a tracheal cannula. Shock levels were raised to 6 dB above twitch threshold. During the OC suppression assay, f2 level was typically set to produce a DPOAE ~10–15 dB greater than noise floor. To measure OC effects, repeated measures of baseline DPOAE amplitude were first obtained (n = 12), followed by a series of 17 continuous periods in which DPOAE amplitudes were measured with simultaneous shocks to the OC bundle. In some experiments, DPOAE measures and CAP measures were interleaved before during and after the OC shock train.

RESULTS
CAPs versus DPOAEs and the level dependence of MOC effects

Activation of MOC efferents, which project to the cochlea’s outer hair cells (OHCs), can reduce the magnitude of CAPs, the summed activity of cochlear nerve fibers evoked by short tone pips. MOC activation can also reduce DPOAEs (Puria et al. 1996), which arise when two simultaneously presented tones interact with nonlinearities in mechanoelectric transduction and produce distortions in the receptor potential that drive somatic motility in OHCs (Lukashkin et al. 2002). These amplified distortions propagate back through the middle ear, where they produce DPOAEs in the ear canal.

In mice, MOC activation elicits suppression of both CAPs and DPOAEs (Fig. 1, A and B), on both the slow and fast time scales reported in guinea pig (Sridhar et al. 1995). At low sound pressures, cochlear responses are suppressed immediately after onset of MOC activation: the time resolution here is ~6 s/pooint, whereas the onset time constant for this fast effect is ~100 ms (Wiederhold and Kiang 1970). The magnitude of
obtained just before the 4 MOC assays in DPOAE-based metrics (Fig. 1). The 4 primary levels are indicated by labeled arrows: the “notch” in amplitude vs. level function occurred for $f_2 = 65\text{ dB SPL}$.

Response suppression can continue to grow for tens of seconds through the roughly 70- to 80-s shock epoch. This has been called the “slow effect” (Sridhar et al. 1995). After shock offset, there is an abrupt response rebound (cessation of fast effect) that can be followed by a slower return to baseline, requiring tens of seconds (e.g., 60 and 65 dB traces from Fig. 1A): another manifestation of the slow suppressive effects of MOC stimulation. According to previous work in guinea pig, these slow effects also appear largest at lower stimulus levels (Sridhar et al. 1995).

In the mouse, both CAPs and DPOAEs show a prominent “overshoot” during the post-shock recovery (Fig. 1, A and B). This response enhancement peaks 75–150 s after shock cessation and can require hundreds of seconds to return to baseline. Enhancement tends to be larger at lower stimulus levels and is of similar magnitude whether measured through CAPs or DPOAEs (Fig. 1C). In the work that follows, we show only DPOAE data elicited by stimuli at lower sound pressure levels, i.e., with primary tone levels producing a response 10–15 dB above the measurement noise floor (typically corresponding to primary levels of 25–35 dB SPL). At higher stimulus levels (60–70 dB SPL), MOC fast effects on DPOAEs can invert from suppression to enhancement (Siegel and Kim 1982), because of the non-monotonicity, or “notch,” in DPOAE amplitude versus level functions (Fig. 2B) (Lukashkin and Russell 2002). If MOC fast effects are functionally similar to decreasing the stimulus level, during MOC shocks, DPOAE suppression should be seen where the level function slope is positive and enhancement where the slope is negative. This is the pattern observed for the during-shock effects in our MOC assay (Fig. 2). During-shock suppression was seen for all primary levels, except at the notch (65 dB SPL), where the during-shock effect inverted to enhancement. In contrast, the post-shock effect remained an enhancement at all primary levels, suggesting that the two phenomena derive from different mechanisms.

**Frequency and threshold dependence of suppression versus enhancement**

In mammalian ears, fast-onset suppression tends to be largest for frequencies corresponding to the upper basal turn (Guinan and Gifford 1988): in the mouse that translates to the 16- to 22-kHz region (Figs. 3, A–C, and 4). This frequency distribution fits roughly with the density of cholinergic efferent terminals on OHCs, which also peaks in mid-cochlear regions and falls off at more apical and basal cochlear regions (Maison et al. 2003).

Slow-effect suppression is difficult to quantify in the mouse. Indeed, neither the slow, during-shock increase in suppression nor the lingering post-shock suppression (visible in the 60 and 65 dB traces from Fig. 1) is seen in the mean data (Fig. 3, A–C). Rather, mean suppression decays during the shock train, and, on average, the first post-shock data point is above baseline. Slow suppressive effects are visible in individual runs, when the post-shock enhancement is small (Fig. 5C). The prominence of the during-shock decay in suppression and the immediate post-shock jump to large response enhancements (e.g., Fig. 5B) suggest that the enhancement process might be initiated soon after shock-train onset and is growing during the shock epoch. This view is supported by the correlation between during-shock decay of suppression and post-shock enhancement (Fig. 5A) and by additional results presented below.

Enhancement magnitudes are not closely tied to those of fast-onset suppression (Fig. 3, A–C). Indeed, at 8 kHz, the mean data show a subtle but significant enhancement (both during and after the shocks), with virtually no fast suppression (Fig. 3A). Furthermore, fast suppression at 16 kHz (Fig. 3B) has grown as that seen at 8 kHz, whereas enhancement has changed little. In general, slow enhancements tend to increase in magnitude with increasing stimulus frequency (Fig. 4), unlike the fast suppressive effects.

The magnitudes of both fast suppression and slow enhancement show a weak correlation with cochlear sensitivity (Fig. 3, D–F), which seems strongest at the highest stimulus frequency (45.2 kHz). Such a correlation is not unexpected for fast suppression, given that 1) this suppression arises when ACh release turns down the contribution of the OHC-based cochlear amplifier to cochlear sensitivity and 2) that most elevations of baseline cochlear thresholds, whether chronic or acute, also arise from loss of OHC “gain” (Patuzzi et al. 1989). However, we assume that much of the variation in suppression strength arises from differences in the effectiveness of the electrical stimulation in activating all MOC fibers.
Effects of strychnine, LOC destruction, or deletion of cholinergic or GABAergic receptors

Strychnine is among the most potent blockers of MOC fast and slow suppression in vivo (Sridhar et al. 1995) and of ACh-induced currents in hair cells (Fuchs and Murrow 1992; Housley and Ashmore 1991) and oocytes transfected to express α9/α10 receptor complexes in vitro (Elgoyhen et al. 2001; Weisstaub et al. 2002). Because it crosses the blood–brain barrier, it also has the advantage of systemic administration.

Here, we show that strychnine completely blocks MOC-evoked suppression without changing post-shock enhancement (Fig. 6A). Within 5 min after strychnine injection, fast suppression is attenuated and, by 20 min, seems to have been abolished; however, the magnitude and time-course of the enhancement seem unchanged. At higher concentrations, the slow enhancement is also blocked by strychnine (Fig. 6B); however, there is a 20-fold difference in the EC50 for strychnine block of suppression (0.75 mg/kg) versus enhancement (15 mg/kg).

As a further test of the independence of fast suppression and slow enhancement, we re-evaluated MOC-evoked effects in a mouse with targeted deletion of the α9 cholinergic receptor, previously shown to lack MOC-mediated suppression (Vetter et al. 1999). Recognizing that large enhancements are not always seen in control ears and that they tend to be largest in ears with good threshold sensitivity (Fig. 3, E and F), we studied a number of α9-null ears. We found a robust MOC-mediated enhancement in those ears with the best sensitivity (Fig. 6C): its magnitude and time-course were remarkably similar to that seen in control ears after strychnine treatment (Fig. 6A). Similar results, i.e., a slow enhancement of cochlear responses with no sign of fast or slow suppression, were seen in mice with targeted deletion of the α10 nicotinic ACh receptor (Vetter et al. 2005a) and in mice lacking the SK2 channel (Vetter et al. 2005b).

To better reveal the onset time constant of the enhancement effect, DPOAE averaging time was reduced to 1 s. Based on these more densely sampled DPOAE measures obtained from the α9-null ears, we estimated the onset time constant to be 2 s.

To rule out the possibility that the slow post-shock enhancement seen in wildtype ears is caused by LOC activation rather than the MOC system, we re-examined data from a study in which the LOC was selectively destroyed by stereotaxic injection of neurotoxin (Darrow et al. 2007) and found clear examples of the enhancement phenomenon in ears with histologically verified lesions to the LOC cells of origin: one example is shown in Fig. 7B.

There is evidence for muscarinic ACh receptors in OHCs (Safieddine et al. 1996) in addition to the nicotinic subunits α9 and α10. In an ongoing study of cochlear phenotype in mouse...
lines with targeted deletion of each of the five muscarinic receptor subtypes, M1–M5 (Maison et al. 2007b), cochlear response to MOC shocks was studied: slow, post-shock enhancement was shown in each of the five mutant lines (data not shown).

MOC terminals in the mouse also express markers of GABAergic transmission (Maison et al. 2003). To examine whether GABAergic effects are involved in generation of the slow enhancement, we evaluated MOC-evoked effects in a number of mouse lines with targeted deletion of different GABA_A receptor subunits (Maison et al. 2006). That evaluation failed to reveal one in which post-shock enhancement was eliminated (Fig. 7A). Similarly, post-shock enhancement is robust in mice without GABA_B receptor signaling (data not shown), because of deletion of the gene for the B(1) subunit, a compulsory component of functional GABA_B receptors (Maison et al. 2007a).

DISCUSSION

α9-mediated effects versus α9-independent effects on hair cells and cochlear responses

The suppression of cochlear responses in vivo by stimulation of a cholinergic feedback pathway was first described 50 yr ago (Galambos 1956). Cochlear suppressive effects could be mimicked by ACh perfusion (Galley et al. 1973), but with an atypical pharmacology characterized by strychnine blockade (Bobbin and Konishi 1974). In vitro work suggested that ACh-induced hyperpolarization and conductance increase arise from coupling strychnine-sensitive Ca^{2+} entry through a cholinergic receptor to Ca^{2+} activation of an apamin-sensitive, small-conductance K^+ channel (Fuchs and Murrow 1992) identified as the SK2 channel (Dulon et al. 1998). A search for novel ACh receptors revealed first the α9 and subsequently the α10 subunits, both expressed in OHCs (Elgoyhen et al. 1994, 2001). Both α9 homomers and α9/α10 heteromers were shown to be extremely strychnine sensitive, with an IC_{50} of ~20 nM, and targeted deletion of the α9 subunit eliminated OC-mediated cochlear suppression (Vetter et al. 1999), consistent with the report that expression of α10 by itself does not yield any detectable ACh currents (Elgoyhen et al. 2001).

Later in vivo studies revealed that MOC activation evokes both a fast suppression of cochlear CAP, with onset time constant of ~100 ms, and a slower suppression with an onset
The mechanical counterparts of fast and slow suppression were also measured in basilar membrane motion (Cooper and Guinan 2003). Pharmacological studies suggested that both fast and slow suppression require ACh binding with an α9 receptor complex, followed by two distinct downstream effects of Ca\(^{2+}\) entry: 1) activation of an SK channel to give rise to the fast effect and 2) a wave of Ca\(^{2+}\)-induced Ca\(^{2+}\) release to evoke the slow effect (Murugasu and Russell 1996b; Sridhar et al. 1995, 1997). The fact that both fast and slow suppression involve changes in cochlear microphonics, the summed receptor potentials of OHCs, suggested that both produce changes in OHC K\(^{+}\) conductance (Sridhar et al. 1995). Pharmacological evidence suggested that the putative slow-effect Ca\(^{2+}\) wave leads to activation of a class of extrasynaptic K\(^{+}\) channels different from the apamin-sensitive synaptic K\(^{+}\) channels mediating the fast effect (Yoshida et al. 2001).

Previous studies of cochlear CAP or basilar membrane motion noted that MOC shocks sometimes elicited a slow-onset, slow-offset “overshoot” characterized by supranormal response amplitudes in the post-shock recovery to baseline (Cooper and Guinan 2003; Sridhar et al. 1995). However, it was assumed that this overshoot was part of the recovery from slow suppression. Because recovery from overshoot could be extremely slow, the phenomenon was difficult to study and was not systematically pursued. Here, we show, with strychnine administration or α9 deletion, that the “overshoot” can be dissected from both fast and slow suppression and thus must represent a separate, distinct, and α9-independent effect of efferent stimulation. The onset time constant of this novel slow enhancement seems to be \(\sim 2\) s, it tends to saturate after \(\sim 5\) s of continued MOC activation, and its offset time constant is \(\sim 100\) s. Previous study of α9-null mice failed to see the slow enhancement (Vetter et al. 1999), because a different OC stimulation paradigm was used (6-s shock trains were interleaved with 6-s control periods), which fails to evoke robust enhancements even in control mice (data not shown).

The discovery of a third robust and independent efferent effect on cochlear responses raises questions about the correspondence between in vivo and in vitro observations. It still
seems safe to assume that the Ca\(^{2+}\)-activated K\(^{+}\) conductance observed in vitro contributes to the fast suppressive effect observed in vivo. There has been no clear-cut dissection of fast and slow suppressive effects in the in vitro work with ACh-induced hair cell currents; thus there is also no clear in vitro correlate to be proposed for slow enhancement. However, in addition to conductance changes in isolated hair cells, ACh-induced increases in OHC electromotility have also been documented (Sziklai et al. 1996), which may involve Ca\(^{2+}\)-activated modification of cytoskeletal elements through intracellular GTPases (Zhang et al. 2003). The previous assumption that ACh-induced increase in OHC electromotility in vitro is intimately related to the slow suppression observed in vivo (Cooper and Guinan 2003) needs to be re-examined. Although the electromotility changes are strychnine sensitive (Dallos et al. 1997), we show here (Fig. 6) that both α9-mediated (suppressive) and non–α9-mediated (enhancing) in vivo effects are strychnine sensitive, albeit with a 20-fold difference in IC\(_{50}\).

Mechanisms underlying the slow enhancement

Ruling out non-MOC effects. Although electrical stimulation of the OC bundle at the floor of the IVth ventricle activates MOC fibers projecting to OHCs, there are other efferent pathways to the auditory periphery that could be activated by electrodes at this location and whose potential contribution to the novel shock-evoked effect must be considered.

Contribution of the lateral (L) component of the OC system is unlikely, because the lack of myelination of LOC fibers at the electrode site make them hard to stimulate (Groff and Liberman 2003), and because their peripheral targets are exclusively in the inner hair cell (IHC) area where it is hard to imagine eliciting an effect on DPOAEs (Liberman et al. 1997). Furthermore, we have shown that LOC destruction does not eliminate slow, post-shock enhancement in wild-type ears (Fig. 7B).

Contribution of the middle-ear muscles is also unlikely, given that activation of these pathways should only depress cochlear responses (Nuttall 1974), and we routinely paralyze our animals with curare, which eliminates all facial twitches otherwise elicited by the brain stem shocks (see also Rajan 1991). Middle-ear muscle activation increases the impedance of the ossicular chain and alters sound pressure in the ear canal, forming the basis for the noninvasive clinical test of middle-ear muscle function (Counter et al. 1989) and allowing us to evaluate individual runs of our assay for signs of middle-ear muscle contraction. Large post-shocks enhancements can routinely be seen without any significant changes in primary tone sound pressure, thus ruling out both middle-ear muscles and other conceivable changes in ear canal musculature that might alter sound transmission through the external and middle ears.

The cochlea’s sympathetic innervation arises from both the stellate and the superior cervical ganglia, giving rise, respectively, to a vascular innervation and a projection to the dendrites of cochlear afferent neurons as they exit the organ of Corti (Hultcrantz et al. 1982; Ren et al. 1993). Although both these ganglia are too distant from the electrode site to be directly stimulated, more indirect pathways are conceivable. Direct sympathetic effects on cochlear nerve fibers are poorly understood; however, it is difficult to imagine how a postsynaptic effect on the cochlea’s afferent neurons outside the organ of Corti could affect the DPOAE, which is known to be unaffected by loss of IHCs and/or cochlear afferents contacting them (Liberman et al. 1997). Depressing the resting level of stellate ganglion’s (vasoconstrictor) activity could theoretically enhance cochlear responses, given that cutting its peripheral projections can increase cochlear blood flow \(\approx 25\%\) (Laurekainen et al. 1997); however, existing literature suggests that changes in cochlear responses such as CAP are not associated with these relatively modest blood flow enhancements.
receptors in the ear: experiments, and there is evidence for muscarinic (m)ACh nine blockade (Verbitsky et al. 2000). However, effects of ionic currents are measurable in isolated hair cells after strych-nine blockade (Verbitsky et al. 2000). GABAA subunits did not eliminate the slow enhancement (M. C. Liberman et al. 1992). However, such putative muscarinic effects should not alter DPOAEs, given that this OHC-based response should not be sensitive to strychnine blockade (Fig. 6); more importantly, the spiral gradient of nAChRs (Bao et al. 2005), notably β2, α4, and α7 (Morley et al. 1998). In hippocampus, homomeric nAChRs containing α7 subunits or heteromeric receptors containing α4β2 subunits can be blocked by strychnine (Matsubayashi et al. 1998), although with a higher IC50 than reported for α9/α10 blockade. Thus, either of these pentameric receptor complexes could be present at the MOC/type II synapses and thus represent an interesting candidate link in the slow-enhancement phenomenon reported here.

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C. Liberman, unpublished data. The nature of the transmitter and receptors involved at the putative efferent component of the type II/OHC synapse is completely unknown. However, cochlear ganglion cells express a number of nAChRs (Bao et al. 2005), notably β2, α4, and α7 (Morley et al. 1998). In hippocampus, homomeric nAChRs containing α7 subunits or heteromeric receptors containing α4β2 subunits can be blocked by strychnine (Matsubayashi et al. 1998), although with a higher IC50 than reported for α9/α10 blockade. Thus, either of these pentameric receptor complexes could be present at the MOC/type II synapses and thus represent an interesting candidate link in the slow-enhancement phenomenon reported here.

**MOC terminals in the OHC area co-localize ACh and GABA (Maison et al. 2003); thus GABAergic effects on OHCs must be considered. There is immunohistochemical evidence for (ionotropic) GABA_A receptors on OHCs and/or ganglion cells, and strong phenotypes after targeted deletion suggest a cochlear role for α5, β2, and β3 subunits (Maison et al. 2006). Although GABA application affects OHC motility and stiffness in vitro (Batta et al. 2004; Sziklai et al. 1996), such effects are only seen in low-frequency cells, which does not match the high-frequency bias of slow enhancement (Fig. 4). Furthermore, we showed here that deletion of five different GABA_A subunits did not eliminate the slow enhancement phenomenon (Fig. 7). Not all subunits were assayed in this study; thus a role for GABA_A signaling cannot be conclusively ruled out. However, a role for (metabotropic) GABA_B signaling can be safely eliminated by the demonstration that slow enhancement can still be evoked in mice with targeted deletion of the gene for the B(1) subunit, a compulsory component of functional GABA_B receptors (Haller et al. 2004).

The complex synaptic circuitry in the OHC area (Fig. 8) suggests a number of additional pathways by which MOC activation could affect cochlear responses. Although large MOC synapses at the bases of OHCs are most prominent, MOC efferents also synapse along the sides of OHCs, and it is claimed that these supranuclear terminals have a distinct cytochemistry (Altschuler et al. 1984). However, the spiral gradient of supranuclear terminals (increasing numbers toward the low-frequency end; Liberman 1990) is opposite to that seen for slow enhancement (Fig. 4). MOC terminals also form synapses with type II “afferent” neurons, either at their cell body (Thiers et al. 2000) or on their terminals under the OHCs (Thiers et al. 2002); type II “afferents,” in turn, synapse with supporting cells (Fechner et al. 2001) that form the “flying buttress” at the outer border of the reticular lamina (Fig. 8). ACh-induced changes in supporting cell Ca2+ influx have been reported (Matsunobu et al. 2001); thus MOC activation could change DPOAEs and CAPs through cholinergic effects on cochlear mechanics that involve no change in OHC function per se. However, the apex-to-base gradient of these supporting cell synapses (Fechner et al. 1999) is also opposite to that of the slow-enhancement.

The final potential pathway arises from the discovery that synapses of type II neurons with OHCs seem to be reciprocal in nature (Nadol 1981), i.e., signals are transmitted in both directions. If true, MOC activation could affect OHCs indirectly through MOC synapses on type II terminals under the OHCs and the reciprocal synapses of type II terminals with OHCs. Interestingly, the apex-base gradient of type II innervation to OHCs in mouse seems to mirror that of the slow enhancement (M. C. Liberman, unpublished data). The nature of the transmitter and receptors involved at the putative efferent component of the type II/OHC synapse is completely unknown. However, cochlear ganglion cells express a number of nAChRs (Bao et al. 2005), notably β2, α4, and α7 (Morley et al. 1998). In hippocampus, homomeric nAChRs containing α7 subunits or heteromeric receptors containing α4β2 subunits can be blocked by strychnine (Matsubayashi et al. 1998), although with a higher IC50 than reported for α9/α10 blockade. Thus, either of these pentameric receptor complexes could be present at the MOC/type II synapses and thus represent an interesting candidate link in the slow-enhancement phenomenon reported here.


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