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Disruption of lateral olivocochlear neurons with a dopaminergic neurotoxin depresses spontaneous auditory nerve activity

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HIGHLIGHTS

- The neurotoxin MPTP disrupts lateral olivocochlear (LOC) neurons in the cochlea.
- Sound-evoked neural potentials were reduced in amplitude after MPTP.
- Spontaneous neural firing was also decreased by MPTP disruption of LOC neurons.
- The net effect of the LOC innervation on the auditory nerve is excitatory.
- Loss of LOC input decreases both spontaneous and sound-driven activity.

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ABSTRACT

Neurons of the lateral olivocochlear (LOC) system project from the auditory brainstem to the cochlea, where they synapse on radial dendrites of auditory nerve fibers. Selective LOC disruption depresses *sound-evoked* auditory nerve activity in the guinea pig, but enhances it in the mouse. Here, LOC disruption depressed *spontaneous* auditory nerve activity in the guinea pig. Recordings from single auditory nerve fibers revealed a significantly reduced proportion of fibers with the highest spontaneous firing rates (SRs) and an increased proportion of neurons with lower SRs. Ensemble activity, estimated using round window noise, also decreased after LOC disruption. Decreased spontaneous activity after LOC disruption may be a consequence of reduced tonic release of excitatory transmitters from the LOC terminals in guinea pigs.

Type I auditory nerve (AN) fibers innervate cochlear inner hair cells (IHCs) [14,42]. Fibers on the pillar side of the IHC have lower thresholds and higher spontaneous rates (SRs) than those on the modiolar side [23]. Pre-synaptic ribbon and post-synaptic receptor size may critically influence AN response properties, as synapses on the pillar side (lower threshold, higher SR) have smaller ribbons and larger receptor patches than those on the modiolar side (higher threshold, lower SR) [19]. The lateral olivocochlear (LOC) efferent system also influences AN response properties. LOC efferents originate in the lateral superior olive (LSO) and synapse primarily on radial dendrites of Type I AN fibers under the IHCs [22]. LOC innervation is biased toward the IHC modiolar side; there, low-SR fibers are innervated by twice as many LOC terminals as high-SR fibers [22]. A small number of LOC neurons synapse directly on IHCs [21,40,41]. LOC efferents contain multiple neurotransmitters, including dopamine (DA), dynorphin (dyn), enkepalin (enk), γ-aminobutyric acid (GABA), acetylcholine (ACh), and calcitonin-gene-related peptide (CGRP) (for reviews, see [1,7,12,16,18,34]). There is significant transmitter co-localization, although patterns vary across reports [4,26,36–38]. Taken together, LOC efferents are positioned to dynamically regulate AN fiber activity via release of excitatory and inhibitory neurotransmitters (for discussion, see [14]). Loss of LOC efferents amplifies noise-induced CAP amplitude depression [14], a finding that is consistent with observations that low-SR fibers are more vulnerable to noise than high-SR fibers [8,25].

Data from lesion [15,17,21,48,50], electrical stimulation [10], and gene knockout [27–30,45] studies suggest several potential functions for LOC neurons. LOC modulation of AN activity may support interaural level comparisons during sound localization

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[2,10], including relearning localization tasks after unilateral conductive hearing loss [11]. LOC modulation of AN activity may be involved in the protective phenomenon, "conditioning" [32,33]. We do not have a precise understanding of the role of LOC efferents in auditory function, however, as it is challenging to record from these small, unmyelinated neurons. Their close proximity to medial olivocochlear (MOC) efferents makes selective lesions difficult, and chemical diversity of LOC neurons further complicates systematic manipulation. Improved understanding of the role of LOC efferents in normal AN function, and in mediating AN vulnerability, is needed. Interestingly, recent mouse studies show decreased numbers of AN synapses after LSO damage [24], suggesting LOC neurons support AN survival and function.

Brainstem knife-cut studies provided some early insight; after cuts disrupting both MOC and LOC efferents, small but statistically significant AN threshold elevations have been consistently observed at some subset of test frequencies, in addition to decreased SRs [21,48,50]. However, MOC vs LOC effects could not be distinguished. Two approaches that selectively disrupt LOC efferents have since been used. First, small volumes of cytolytic melittin injected into the LSO selectively damaged LSO and *depressed* sound-driven AN activity in guinea pigs, measured as decreased whole-nerve compound action potential (CAP) amplitude [17]. With lesions largely centered in the lateral (low-frequency) limb of the LSO, responses evoked by low-frequency sounds were the most suppressed. In contrast, lesions of medial and lateral LSO limbs in mouse resulted in 30–50% *increases* in amplitude of wave-I of the sound-evoked auditory brainstem response (ABR) [3]. A second method for disrupting LOC efferents is application of the DA neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the round window membrane (RWM) [15]. MPTP is metabolized to MPP+ and taken up pre-synaptically by neurons containing DA; accumulation of MPP+ results in mitochondrial dysfunction, oxidative stress, and neuronal death [31,43]. Approximately 40% of the LOC innervation was disrupted 45-min after applying MPTP, and CAP amplitude reductions were equivalent to those measured after melittin-induced LSO lesions [15].

Across studies, the main effect of LOC lesions has been a change in sound-evoked auditory potential amplitude. In guinea pigs, CAP amplitude was depressed [15,17]. The net effect differed in mice, with ABR wave-I amplitude increasing post-lesion [3]. Although effect direction varied with species, it is clear that LOC disruption influenced sound-evoked AN activity. Both CAP and ABR are indirect measures of AN activity; a change in response amplitude indicates a different number of AN fibers discharging synchronously. Decreased CAP amplitude in guinea pigs after LSO lesions may reflect decreased numbers of AN fibers, or increased asynchronous activity, with fewer fibers responding synchronously to sound. This study specifically examined effects of selective LOC disruption via MPTP on spontaneous AN activity. If LOC transmitters are released tonically, then LOC disruption should alter spontaneous AN activity.

1. Materials and methods

1.1. Subjects

Subjects were 27 healthy, male and female adult pigmented guinea pigs (Elm Hill Breeding Labs, Chelmsford, MA) weighing 300–450 g. Animals were maintained with free access to food (Guinea Pig Chow, PMI Nutrition International Inc., Brentwood, MO) and water. The animal care program was AALAC accredited. All protocols were approved by the University Committee on Use and Care of Animals at the University of Michigan.

1.2. Surgical preparation

Animals were anesthetized [intramuscular ketamine (40 mg/kg) and xylazine (10 mg/kg) with supplemental anesthesia as needed]; rectal temperature was maintained at $38 \pm 0.5\,^{\circ}C$ via a thermostatically-controlled heating pad. Tympanic membranes were examined; then, the left bulla was exposed and opened via a post-auricular surgical approach. The cochlea was visualized and a silver ball electrode placed against the RWM. Electrodes were temporarily secured at the bulla using cyanoacrylate (VetBond), securely cemented to the bulla (Durelon), then used to measure CAP.

1.3. Whole-nerve sound-evoked CAP recordings

CAP input–output (IO) functions were determined for brief pure-tone stimuli (2–18 kHz, 2-kHz increments; 0–100 dB SPL in 5-dB increments; 5-ms duration, 0.5-ms rise-fall; 10/s). Stimuli were generated using Tucker-Davis Technology (TDT; Alachua, FL) System II/III hardware and SigGen 3.2 software. Signals were converted to analog (DA1), filtered (FT6-2, Fc = 40 kHz), attenuated (PA5), and presented using a 200-W transducer (Beyer Dynamic, Farmingdale, NY) coupled to the ear canal via ear bars. Evoked potentials were filtered (300–3000 Hz) and amplified (1000×) using in-house constructed equipment. BioSig 3.2 was used to average 25 presentations within each frequency/level combination. IO functions were measured immediately after securing the electrode and 30-min after RW application of test substances (6-μl volume); the middle ear was carefully dried prior to each CAP test using fine tip cotton points. In control subjects, an artificial perilymph (AP) solution was applied (145-mM NaCl, 2.7-mM KCl, 2.0–mM $MgSO_4$, 1.2-mM $CaCl_2$, 5.0-mM HEPES; pH = 7.40, osmolality = 280–285 mOsm). In experimental subjects, 50-mM MPTP dissolved in AP was applied (pH adjusted to 7.4 + 0.02). CAP threshold was defined using linear interpolation as the sound level that produced a 10-μV response.

1.4. Round window noise (RWN) ensemble activity recordings

In three preliminary subjects, the RW electrode was used to record ensemble spontaneous AN activity (following [5,39]). The recorded electrical noise was filtered (0.3–3.0 kHz) and transmitted to a spectrum analyzer (SR760, Stanford Research Systems, Sunnyvale, CA; sampling rate = 256-kHz, frequency span = 0–12.5-kHz, 32-ms time-domain records). Fast Fourier transform (FFT) was performed on each record, using Blackman–Harris (BMH) windowing (frequency resolution of resulting FFT = 31.25-Hz). 150 FFTs of records were acquired and averaged (linear, RMS averaging). In a normal animal, peak activity is around 900-Hz. A MATLAB™ script was used to retrieve the waveform from the SR760 and store data. For the first two subjects, the data were collected immediately after the bulla was opened and the electrode placed, and 30-min after MPTP was applied. In the third animal we measured RWN after artificial perilymph had been applied, at two tests times after MPTP had been applied (30-min and 60-min), and post-mortem. The RW data were collected prior to undertaking auditory nerve single-unit data collection.

1.5. Auditory nerve single-unit recordings

For single-unit recordings, a tracheal tube was inserted and subjects were held in a stereotaxic device (Kopf Model 1404) with hollow ear bars for sound delivery. A posterior fossa approach was used for AN fiber recordings [6,35,46]. In brief, the skin was incised and retracted, the bone over the cerebellum and posterior occipital cortex was removed, and a small portion of cerebellum aspirated to expose the AN. Spontaneous and sound-evoked potentials were