Suppression of putative tinnitus-related activity by extra-cochlear electrical stimulation

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Noreña AJ, Mulders WH, Robertson D. Suppression of putative tinnitus-related activity by extra-cochlear electrical stimulation. J Neurophysiol 113: 132–143, 2015. First published October 8, 2014; doi:10.1152/jn.00580.2014.—Studies on animals have shown that noise-induced hearing loss is followed by an increase of spontaneous firing at several stages of the central auditory system. This central hyperactivity has been suggested to underpin the perception of tinnitus. It was shown that decreasing cochlear activity can abolish the noise-induced central hyperactivity. This latter result further suggests that an approach consisting of reducing cochlear activity may provide a therapeutic avenue for tinnitus. In this context, extra-cochlear electric stimulation (ECES) may be a good candidate to modulate cochlear activity and suppress tinnitus. Indeed, it has been shown that a positive current applied at the round window reduces cochlear nerve activity and can suppress tinnitus reliably in tinnitus subjects. The present study investigates whether ECES with a positive current can abolish the noise-induced central hyperactivity, i.e., the putative tinnitus-related activity. Spontaneous and stimulus-evoked neural activity before, during, and after ECES was assessed from single-unit recordings in the inferior colliculus of anesthetized guinea pigs. We found that ECES with positive current significantly decreases the spontaneous firing rate of neurons with high characteristic frequencies, whereas negative current produces the opposite effect. The effects of the ECES are absent or even reversed for neurons with low characteristic frequencies. Importantly, ECES with positive current had only a marginal effect on thresholds and tone-evoked activity of collicular neurons, suggesting that the main action of positive current is to modulate the spontaneous firing. Overall, cochlear electrical stimulation may be a viable approach for suppressing some forms of (peripheral-dependent) tinnitus.

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Tinnitus, the auditory percept without any concomitant acoustic stimulation, is very prevalent in the general population and can dramatically impair the quality of life (McCormack et al. 2014). As tinnitus is associated with hearing loss in most, if not all, cases (Chung et al. 1984; Sindhusake et al. 2003), hearing loss has been suggested to act as a necessary cause or trigger of tinnitus. Many studies have shown that hearing loss is accompanied by both spontaneous and stimulus-induced hyperactivity at different levels of the central auditory system, i.e., the cochlear nucleus (Bledsoe et al. 2009; Kaltenbach and Afman 2000; Sumner et al. 2005; Vogler et al. 2011), the inferior colliculus (IC) (Bauer et al. 2008; Izquierdo et al. 2008; Mulders and Robertson 2009, 2011; Vogler et al. 2014) and the auditory cortex (Komiya and Eggermont 2000; Noreña et al. 2010; Noreña and Eggermont 2003, 2006; Seki and Eggermont 2003). This increase in central neural activity occurs despite the reduction of spontaneous and stimulus-evoked cochlear nerve activity after cochlear lesions (Hartmann et al. 1984; Heinz and Young 2004; Kujawa and Liberman 2009; Liberman and Dodds 1984; Shepherd and Javel 1997). The spontaneous central hyperactivity triggered by hearing loss has been suggested to represent tinnitus-related activity (Bauer et al. 2008; Brozoski et al. 2007; Kaltenbach et al. 2004; Mulders et al. 2014; Mulders and Robertson 2009; Noreña 2011; Schaette and Kempter 2012).

Interestingly, recent studies showed that the noise-induced hyperactivity recorded in IC within the first few weeks after noise trauma can be abolished by cochlear ablation or other treatments impairing cochlear function (Mulders and Robertson 2009, 2011) or by activation of the efferent system (Mulders et al. 2010). These results show that, in this time period after cochlear trauma, residual spontaneous activity in the cochlear nerve is necessary for the persistence of the noise-induced hyperactivity in IC.

In summary, the putative tinnitus-related activity in auditory centers (i.e., the noise-induced central hyperactivity) may result from the central amplification (through increase of neural sensitivity or gain due to hearing loss) of residual spontaneous activity provided by the cochlear nerve (Noreña 2011; Noreña and Farley 2013; Schaette and Kempter 2006).

Two different therapeutic approaches can be derived from this view. First, normalizing the averaged sensory inputs to a prehearing loss level may restore the prehearing loss neural sensitivity and then alleviate tinnitus by reducing the central gain or reversing some tinnitus-related central changes. This would be achieved by customized acoustic stimulation, hearing aids or cochlear implants (Noreña 2012; Noreña et al. 2002; Noreña and Eggermont 2005, 2006; Schaette and Kempter 2006). Second, reducing the cochlear source of spontaneous activity in the auditory system may also reduce tinnitus (Mulders et al. 2014; Mulders and Robertson 2009; Noreña 2011). A reduction of cochlear spontaneous activity can be achieved through extra- and intracochlear electrical stimulation with positive current (Evans and Borerwe 1982; Konishi et al. 1970; Schreiner et al. 1986) and could account for the suppression of tinnitus reported in earlier studies using extra-cochlear electric stimulation (ECES) with positive direct current (Cazals et al. 1978; Hatton et al. 1960; Portmann et al. 1979).

The present study is aimed at improving our understanding of the tinnitus mechanisms, in particular the coupling between the tinnitus-related activity and the spontaneous cochlear activity, and at gaining further insights into the possibility of
using cochlear electrical stimulation to suppress tinnitus in tinnitus subjects. The effects of electrical stimulation applied at the round window (RW) on both stimulus-induced and spontaneous single-unit activity in IC were investigated in anesthetized guinea pigs after noise trauma.

**MATERIALS AND METHODS**

**Animals**

Fifteen young adult pigmented guinea pigs of either sex were used. Animals weighed between 450 and 670 g at the time of electrophysiological recordings in the IC. The experimental protocols were approved by the Animal Ethics Committee of The University of Western Australia (03/100/1007) and were carried out in accordance with the Guidelines from the National Health and Medical Research Council of Australia regarding the care and use of animals for experimental procedures. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

**Recovery Surgery for Acoustic Trauma and Mechanical Lesion of the Cochlea**

Animals received a subcutaneous injection of 0.1 ml atropine sulfate (0.6 mg/ml), followed by an intraperitoneal injection of diazepam (5 mg/kg). Twenty minutes later animals were injected intramuscularly with Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; 1 ml/kg). When deep anesthesia was obtained, as determined by the absence of the foot withdrawal reflex, the area of incision was shaved, and animals were placed on a custom-made heating blanket in a soundproof room and mounted in hollow ear bars. A small opening of ~1 mm² was made in the bulla to expose the cochlea, and a plastic-insulated silver wire was placed on the RW. A compound action potential (CAP) audiogram (Johnstone et al. 1979) was recorded in the frequency range 4–24 kHz to assess the animals’ cochlear sensitivity. All sound stimuli were presented in a closed sound system through a 1/2-in. condenser microphone driven in reverse as a speaker (Bruel and Kjaer, type 4134). The system was calibrated using a 1/8-in. condensor microphone in place of the tympanic membrane and an absolute sound calibrator (Bruel and Kjaer type 4231). Pure tone stimuli (10-ms duration, 1-ms rise/fall times) were synthesized by a computer equipped with a DIGI 96 soundcard connected to an analog/digital interface (ADI-9 DS, RME Intelligent Audio Solution). Sample rate was 96 kHz. The interface was driven by a custom-made computer program (Neurosound, MI Lloyd), which was also used to collect single neuron data during the final experiments. CAP signals were amplified, filtered (100 Hz to 3 kHz band pass) and recorded with a second data acquisition system (Powerlab 4SP, AD Instruments).

When cochlear thresholds were within the normal range (Johnstone et al. 1979), animals received a unilateral acoustic trauma using the closed sound system described above. Acoustic trauma consisted of exposure to a continuous loud tone for 2 h (10 kHz, 124 dB sound pressure level for all animals, except one which was exposed to a continuous loud tone at 5 kHz, 124 dB sound pressure level for the same duration). During acoustic trauma, animals remained under anesthesia, and the contralateral ear was blocked with plasticine. Immediately after acoustic trauma, another CAP audiogram was measured to determine the magnitude of immediate hearing loss, and the incision was sutured. Survival time varied between 2 and 3 wk.

**Surgery for Acute Electrophysiological Recordings**

For the nonrecovery experiments, animals received a subcutaneous injection with 0.1 ml atropine followed by an intraperitoneal injection of Nembutal (pentobarbitone sodium, 30 mg/kg) and a 0.15 ml intramuscular injection of Hypnorm. Anesthesia was maintained with full Hypnorm doses every hour and half doses of Nembutal every 2 h. At the end of the experiment, animals were euthanized with an injection of 0.3 ml Lethabarb (pentobarbitone sodium 325 mg/ml; VIBRAC). When deep anesthesia was obtained, as determined by the absence of the foot withdrawal reflex, the areas of incision were shaved, and animals were placed on a custom-made heating blanket thermostatically controlled to maintain animal rectal temperature at (38°C) in a sound-proof room and artificially ventilated on carbogen (95% O₂ and 5% CO₂). Before single neuron recordings, paralysis was induced with 0.1 ml pancuronium bromide (2 mg/ml intramuscularly). The electrocardiogram was continuously monitored. Heart rate never increased over preparalysis levels at any stage of the experiments, and strong stimuli (foot pinch) did not alter ECG recordings. After the animals were mounted in hollow ear bars, the left and right cochleae were exposed by small openings in the bulla, and CAP audiograms were recorded on both sides with an insulated silver wire placed on the RW as for the recovery procedures.

To make extracellular single neuron recordings in the central nucleus of the IC (CNIC), a small craniotomy (~4 mm) overlying the visual cortex was performed. A glass-insulated tungsten microelectrode (Merrill and Ainsworth 1972) was then advanced using a stereotaxic motor microdrive (Rapidyn) and custom-made controller along the dorsoventral axis through the cortex into the CNIC contralateral to the cochlea subjected previously to acoustic trauma. Electrode placement in the CNIC (about 2.5 to 3 mm ventral to the cortical surface) was indicated by the presence of strong sound-driven activity with a short latency (cluster onset latencies <6.5 ms) and a systematic progression from low to high characteristic frequencies (CF) with increasing depth. We have previously confirmed histologically that these response properties correlate with location of the electrode in the CNIC (Malmierca et al. 1995, 2008; Mulders et al. 2011). The craniotomy was covered with 5% agar in saline to improve mechanical stability. When a single unit was isolated, its CF and threshold at CF were determined audio-visually, and depth from the cortical surface was recorded using methods described previously (Ingham et al. 2006; Mulders et al. 2010). In all neurons, the spontaneous firing rate was measured for a period of 10 s, as previously reported using an identical animal model (Mulders et al. 2010, 2011; Mulders and Robertson 2009). During this measurement, the polarization voltage of the speaker was turned off to avoid uncontrolled background noise from the sound delivery system.

For electrical stimulation of the RW of the cochlea, a plastic insulated and chlorided silver wire was placed on the RW using a micromanipulator. Another insulated chlorided wire was inserted in muscle next to the opening in the bulla as a current return electrode. Electrical stimulation to the electrodes was delivered via an isolated stimulator output (AM Systems Model 2100). When spontaneous activity of a CNIC neuron was determined, the effects of DC electrical stimulation of the RW on the neuron’s spontaneous rate was measured. For this purpose, histograms were constructed from 20 repetitions of 2-s epochs (unless otherwise specified), either without any electrical stimulation or with positive or negative DC current. Current intensity was varied between 25 and 100 μA in 25-μA steps. Electrical stimulation duration was 500 ms with a repetition rate of 2 s for all neurons, unless otherwise specified. We also measured the neural activity produced by tone stimuli (50-ms duration) during ECES (tone started 100 ms after the ECES onset) or without ECES.

To assess the effects of RW stimulation on cochlear function, CAP input/output functions were constructed in response to 10-ms tone bursts at either 4, 8 or 16 kHz over a range of sound intensities in 10-dB steps, repetition period 2 s. At each of the three frequencies, input/output functions were measured without any electrical stimulation or after positive DC current or negative DC current. The CAP recordings were obtained from a different electrode than that used for stimulation. Electrical stimulation duration was 500 ms as for single-neuron recordings and the tone burst was placed at either 20 or 105 ms.
after the end of stimulation. Ten stimulus repetitions were used, and the averaged CAP amplitude was expressed as the N1-P1 amplitude.

**Data Analysis**

Quantification of the ECES effect on “spontaneous” activity. If IC_{ECES} is the activity of IC during ECES (with negative or positive current), and IC_{ctrl} is the “control” activity of IC (without ECES), then the effects of the ECES (EFF_{ECES}) is calculated as follows:

\[
\text{EFF}_{\text{ECES}} = \frac{\text{IC}_{\text{ECES}} - \text{IC}_{\text{ctrl}}}{\text{IC}_{\text{ECES}} + \text{IC}_{\text{ctrl}}}
\]

IC_{ctrl} was derived from the 0-μV condition for a period of 40–60 s. There is no effect of stimulation when the index is equal to 0 (IC_{ECES} = IC_{ctrl}). If IC_{ECES} is 10 times the IC_{ctrl}, then the index is equal to 0.8. An index equal to −1 indicates complete suppression of neural activity produced by electrical stimulation. IC_{ctrl} was not obtained between stimulation periods, but from a different session of recording generally carried out before the sessions where electrical stimulation was provided. This was to avoid comparing neural activity during electrical stimulation to the activity after electrical stimulation, as the latter shows poststimulation changes in the pattern of firing. EFF_{ECES} will be displayed as a function of CF and averaged into partially overlapping 1-octave frequency bands.

Quantification of the ECES effect on stimulus-induced activity. The stimulus-induced activity of each unit was obtained by taking the maximum firing rate from the running average of the poststimulus time histograms using a 50-ms time window. This was similar to taking the average neural activity during the 50-ms acoustic stimulus; however, this method did not require knowing a priori the latency of the stimulus-induced responses. This activity was then averaged over all units to build the averaged rate-level functions. For most units, the level of the electrical current used to assess the effects of ECES on tone-induced activity was 25 or 50 μA (and −25 or −50 μA).

**Statistics**

The averaged effect of electric stimulation on spontaneous and stimulus-induced activity in IC was tested statistically using a Wilcoxon signed-rank test. Bonferroni’s correction was applied to adjust the \( P \) values for the number of comparisons being made.

**RESULTS**

Figure 1 shows the CAP threshold shift (difference between CAP thresholds obtained after and before noise trauma) in all animals (and the mean threshold). Some animals, but not all, have long-term hearing loss after noise trauma in mid (≈12 kHz) and/or high frequencies (16 kHz).

As the purpose of the study was to investigate the effects of ECES on central activity, especially on spontaneous activity, only units with spontaneous firing rate above zero were selected. A more complete description on the effects of noise trauma on the spontaneous firing in IC has been published elsewhere (Mulders and Robertson 2009, 2011, 2013; Vogler et al. 2014). Ninety-five percent of units in IC have a low spontaneous firing rate (<8 spikes/s) in normal animals (Mulders and Robertson 2009). Figure 2 shows the spontaneous firing rate of all the units reported in the present study as a function of their CF, as well as their spontaneous firing rate distribution. A total of 105 neurons were recorded. The distribution of units is biased toward CFs >3 kHz as the spontaneous activity of very low CF neurons was zero or too low for the purpose of this study. One notes that most recordings have been made in the CF region affected by the acoustic trauma frequency; the averaged spontaneous activity was the highest in this frequency region (~25 spikes/s) (Fig. 2).

**Effects of Cochlear Electric Stimulation on Spontaneous Collicular Activity**

Figure 3 shows raw recordings for a representative unit in IC (CF = 21.7 kHz) after trauma. This unit is hyperactive discharging with bursts of action potentials (first row). The effects of negative and positive currents are shown in the second and third rows, respectively. During current application, the negative current produces a strong increase of the discharges, whereas the positive current abolishes completely neural activity. The poststimulation periods were also dramatically modified: stimulations with negative current are followed by a strong suppression of neural activity, whereas a rebound of activity is observed after the stimulations with positive current. Figures 4 and 5 show the firing rate as a function of time in additional examples. These examples illustrate further the effects of ECES on neural activity in IC and also the important changes in the dynamic of the neural activity. Indeed, the ECES modulates central activity during and after the stimulation. These effects are particularly striking in the example shown in column 1 of Fig. 5. Note, however, that positive current suppresses the activity in some units with only a moderate poststimulation rebound of activity (Fig. 4, column 3). Some units (generally with low CF) showed a reversed pattern of effects, with negative current being suppressive and positive current being excitatory (Fig. 5, column 3). It is clear from the examples shown above that the poststimulation effects could be much longer than the 2-s interstimulation interval used in this study. Figure 6 shows an example of a unit discharging with bursts in which two different time intervals (2 and 5 s) between the electric stimulations were used. The negative stimulation had only a modest effect on the discharges when provided every 2 s, and triggered the burst of discharges when provided every 5 s. The positive stimulation had a strong suppressive effect for the two interstimulus intervals (at least neural activity was absent during stimulation). Although we did not investigate the effects of varying systematically the
interstimulation interval, it is interesting to note that the effects of electric stimulation seem to depend on a complex relationship between the discharge properties of neurons and the characteristics (polarity and temporal sequence) of electrical stimulation.

Figure 7, top, shows the effects of ECES as a function of CF for all units (see MATERIALS AND METHODS for the calculation of the ECES effect). Figure 7, bottom, shows the ECES effects averaged over partially overlapping 1-octave frequency bands (the center frequencies of the bands are indicated in the x-axis). The averaged responses were compared statistically to zero (Wilcoxon signed-rank test). Differences were considered significant at a level of 0.001 (Bonferroni’s correction, 6 frequency bands × 8 current conditions = 48 comparisons). For the negative current, the only differences that crossed the statistical threshold were those for the 25 A and the frequency bands centered on 14 and 20 kHz. For the positive current, the differences were statistically significant for the stimulation

![Graph showing spontaneous activity and ECES effects](image1)

**Fig. 2.** Bottom left: spontaneous activity (SA) (after noise trauma) as a function of the characteristic frequency (CF) for all recorded neurons in the present study. The gray line represents a running average of SA as a function of CF. A peak of SA is present slightly above the trauma frequency (10 kHz). Top: distribution of CF. Bottom right: distribution of SA.

Spontaneous activity

Negative current

Positive current

![Graph showing raw neural recordings](image2)

**Fig. 3.** Raw neural recordings (after filtering, see MATERIALS AND METHODS) as a function of time for a given neuron in three conditions. **Top:** SA without electrical stimulation. **Middle:** effects of an electric stimulation with a negative current. **Bottom:** effects of an electric stimulation with a positive current. The time windows of electrical stimulation (500-ms duration) are delimited by vertical dotted lines. Inserts in the middle and bottom panels indicate the pattern of the electric stimulation. Spontaneous firing is completely abolished during the stimulation with the positive current, whereas firing rate (FR) is greatly enhanced by the stimulation with the negative current. On that particular example, one also notes a poststimulation effect consisting of an enhanced FR or “activity rebound” after the stimulation with the positive current and a long-lasting suppression of the spontaneous firing after the stimulation with the negative current.
amplitude 25, 50 and 75 μA and the frequency bands centered on 10, 14 and 20 kHz. The difference was also significant for the stimulation amplitude 50 μA and the frequency band centered on 7 kHz.

Figure 8 shows the effects of electrical stimulation over time and averaged over units with a CF within 1 octave around 20 kHz (where the effects of electrical stimulation is maximal). One observes that the suppression of activity with positive current (bottom panel) is very rapid after the beginning of the stimulation and lasts as long as the stimulation is applied. When the stimulation is turned off, the activity increases over time and remains elevated (compared with the background activity) between the stimulation periods. The reversed pattern is observed for the negative current (top panel): neural activity is maintained at a high level as long as the negative current is provided (without any sign of neural adaptation) over the 500-ms period of stimulation. After the stimulation, the neural activity comes down to well below the background activity.

**Effects of Cochlear Electric Stimulation on Stimulus-induced Collicular Activity**

The results presented above show that positive current is able to reduce significantly the spontaneous activity in the auditory centers. While these results are very promising regarding the potential for therapeutic developments, this approach would be viable only if the positive current does not produce significant hearing loss. To investigate whether posi-
tive current abolished hearing at the current levels used in this study, stimulus-induced activity was recorded in IC, while electrical stimulation was provided at the RW (see MATERIALS AND METHODS). Figure 9 shows the poststimulus time histograms for four different units recorded with and without concomitant electrical stimulation. One notes that the negative current enhances the amplitude of stimulus-induced responses, whereas the positive current reduces it. Figure 10 shows the...
rate-level functions (top panel) and the ECES effects on the stimulus-induced activity (subtraction from the condition without electric stimulation) (bottom panel) averaged over units with CF above 8 kHz. The averaged differences were compared statistically to zero (Wilcoxon signed-rank test). Differences were considered significant if $P < 0.004$ (after Bonferroni’s correction, 12 comparisons). The negative current induced a significant increase of activity at 0-dB sound level (SL). The positive current induced a significant decrease of activity at 0-, 10- and 20-dB SL. One can observe that the rate-level function with positive current corresponds roughly to the rate-level function without stimulation shifted by 10 dB. The control neural activity at 0-dB SL was not significantly different from the neural activity at 10-dB SL during positive stimulation. This result indicates that the positive stimulation may produce an auditory threshold shift by about 10 dB, at most.

Effects of Cochlear Electric Stimulation on Cochlear Activity

To investigate the poststimulatory effects of ECES on tone-evoked activity in the periphery, CAPs obtained from different stimulus levels and at two delays after the end of ECES (20 and 105 ms) were recorded in two animals (Fig. 11). The results indicate that positive currents are followed by an increase of CAP amplitude. It is notable that at these short time delays, however, spontaneous single-unit activity is still reduced in IC (Fig. 8).

DISCUSSION

The present study was aimed at investigating whether cochlear electrical stimulation can reduce the spontaneous activity in the IC. The results show that positive direct current applied at the RW can dramatically reduce the noise-induced central hyperactivity, for units with high CF, while it generally does not change the firing (or produces the opposite effect) for neurons with low CF. Negative direct current applied at the RW produces the opposite. Moreover, cochlear electrical stimulation can marginally modulate tone-induced activity at low level of acoustic stimulation but not at moderate levels. This approach could lead to important developments to suppress tinnitus, provided that reduction of cochlear activity can be achieved from charge-balanced electrical stimulation.

Effects of ECES on the Spontaneous Firing of Cochlear Fibers

A stimulating electrode placed on the RW produces a current flow along the scala tympani that spreads over (and affects) a large cochlear region (from the base or high-CF region to the midcochlear or mid-CF region) (Clopton and Spelman 1982; Hartmann et al. 1984; Ho et al. 2004; Micco and Richter 2006; Spelman et al. 1982; Van den Honert and Stypulkowski 1984). Cochlear electrical stimulation can affect the spontaneous discharges of cochlear fibers through presynaptic and postsynaptic mechanisms.

Cochlear fibers have spontaneous firing in the absence of sound, which is related to the (presynaptic) spontaneous release of neurotransmitters by the inner hair cells (IHCs) (Liberman and Dodds 1984). The rate of the spontaneous release of neurotransmitters depends on the IHC membrane potential, which in turn controls the opening probability of the voltage-gated calcium channels (Hudspeth 1985; Pickles 1985). The
intracellular influx of calcium then catalyzes the fusion of synaptic ribbon to the plasma membrane, allowing exocytosis (Moser et al. 2006). ECES could affect the IHC membrane potential (and therefore the rate of spontaneous discharges) by 1) a direct effect of the injected current on the IHC membrane potential; and 2) an effect on the endocochlear potential and hence on the standing current through the IHCs (Nuttall 1985; Russell and Kössl 1991; Zeddies and Siegel 2004). A modification of the cochlear potential could be mediated by a direct effect of injected current on the scala media boundaries. Also, slow length changes of outer hair cells (OHCs) can affect the endocochlear potential through the opening probability of their mechano-electric channels. Namely, elongation of OHCs, for instance, is accompanied by a closure of the mechano-electric channels and a reduction of the current flow through the OHC. This may then result in an increase of the endocochlear potential (Javel and Shepherd 2000; O’Beirne and Patuzzi 2007). One notes that ECES may also affect the spontaneous firing of cochlear fibers through a change in the membrane potential of the efferent nerve terminals and a resulting hyperpolarization of OHCs (Guinan and Gifford 1988; Wiederhold and Kiang 1970).

It is also likely that ECES alters the spontaneous firing of cochlear fibers through postsynaptic effects of electrical stim-
ulation, i.e., direct modification of the membrane potential of cochlear fibers and the opening probability of voltage-dependent sodium channels present in the peripheral and central regions of the cochlear fibers (Hossain et al. 2005; Javel and Shepherd 2000).

When considering the possible mechanisms of the effects seen with positive and negative current, theoretical predictions are complicated. Nevertheless, it is reasonable to suggest that, for hair cells and cochlear fibers at the base of the cochlea i.e., near the RW electrode, a positive current, by producing a movement of negative charges away from the close environment of the cells, would result in a hyperpolarization of their membrane potentials. For IHCs and the cochlear nerve fibers, this would produce a reduction of the spontaneous firing. For OHCs, on the other hand, this would increase the length of the cells (Ashmore 1987; Brownell et al. 1985), closing the mecano-electric channels, reducing the shunt of strial current through OHCs and thereby increasing the endocochlear potential (O’Beirne and Patuzzi 2007). The increase of the endocochlear potential can produce an increase of the standing transducer current through the IHCs, depolarizing the IHCs, triggering glutamate release and thereby increasing the firing rate of cochlear fibers (Sewell 1984).

Overall, however, the net effect of ECES with positive current on cochlear nerve activity is a suppression (Evans and Borerwe 1982; Konishi et al. 1970; Miller et al. 2001; Schreiner et al. 1986; Tasaki and Fernandez 1952). All of the effects discussed above are expected to be reversed for negative currents (Evans and Borerwe 1982; Konishi et al. 1970; Schreiner et al. 1986).

**Effects of Extra-cochlear Electrical Stimulation on Auditory Centers**

The present study demonstrates that the spontaneous activity in the IC of animals that have been subjected to a prior cochlear trauma is tightly coupled to the activity in the cochlea. In other words, any modulation of the spontaneous activity in the cochlear nerve (including a reduction of activity) simply propagates through the central auditory system, at least up to the IC. This is consistent with an earlier study showing that decreasing the resting cochlear activity is accompanied by a reduction of the noise-induced hyperactivity in the IC (Mulders and Robertson 2009). All animals used in this study were exposed to noise trauma, as it was the sine qua non condition to get “enough” central spontaneous activity to test our hypothesis. In unexposed control animals, under our anesthesia regime, we have shown that spontaneous activity in IC neurons is very low (Mulders and Robertson 2009). As a consequence of this experimental design, we cannot say that ECES with positive current can suppress the central spontaneous activity in control (nonexposed) animals. Moreover, we cannot differentiate the neurons with elevated firing rate due to the noise trauma from neurons that had high firing rate before the trauma and that maintained high level of firing after trauma. This does not change the main conclusion of our study that ECES with positive current can suppress all central spontaneous activity, including the noise-induced central hyperactivity. We speculate that ECES with positive current can reduce the spontaneous firing of hyperactive and nonhyperactive neurons in IC as long as they receive strong inputs.
from the cochlear region affected by the ECES (from the base to the midregion of the cochlea corresponding to around 5 kHz).

The effects of ECES are stronger on high CF neurons than on low CF neurons, presumably due to the position of the stimulating electrode close to the base of the cochlea (corresponding to high CFs). One explanation could be that the current density may decrease as the current passes through the cochlea. The effects of ECES can be opposite for low-CF fibers compared with those for high-CF fibers. One notes, however, that, while this reversal can be very clear for some units, the changes for low-CF units are not statistically significant when the recordings from low-CF units are pooled. This indicates that ECES produces variable results for low-CF units. It is unknown whether this reversal for low-CF units results from an action of electrical stimulation directly on the cochlear apex or indirectly through cross-frequency interactions within the auditory centers. The large variability for low-CF units indicate that the reversal of effects may result from central mechanisms, i.e., lateral inhibition (Catz and Noreña 2013; LeBeau et al. 2001; Wu et al. 2008; Yang et al. 1992). Namely, a decrease of cochlear activity produced by ECES at the cochlear base may produce an increase of neural firing in auditory centers through a release from central inhibition for units with relatively low CF and, conversely, for ECES causing an increase in cochlear activity.

Regarding the modulation of tone-induced activity in IC produced by ECES, electrical stimulation of the cochlea has been shown to strongly affect the cochlear microphonic and tone-induced activity of the cochlear fibers (Tasaki and Fernandez 1952; Teas et al. 1970; Nuttall 1985). The direction of the changes (increase vs. decrease) depends on the current polarity (Konishi et al. 1970; Nuttall 1985; Tasaki and Fernandez 1952; Teas et al. 1970). Interestingly, while electrical stimulation changed the cochlear activity produced by relatively low-level tones, it did not change the maximum firing rate (Teas et al. 1970). This result is broadly consistent with our findings.

Finally, strong poststimulation effects are sometimes observed in the IC: on average, positive current is followed by a delayed rebound of activity, whereas negative current can produce a long-lasting reduction of activity. It is unclear whether these poststimulation effects result from the cochlea and/or the auditory centers. Prolonged direct stimulation can have long-lasting effects on the cochlea (Schreiner et al. 1986). Part of the poststimulation effects observed in IC may then result from the cochlea. Our CAP data obtained in two animals are, however, only partially consistent with the spontaneous activity recorded in IC. Indeed, while the collicular spontaneous activity remains decreased for up to 400 ms after stimulation with positive current, the CAP amplitude is increased shortly (20 and 105 ms) after the end of the stimulation. This suggests that some central mechanisms may be involved in the rebound of activity observed after stimulating with positive current.

**Implications for Understanding Tinnitus Mechanisms and Tinnitus Suppression**

ECES with positive current has been found to suppress the tinnitus percept in tinnitus subjects (Aran and Cazals 1981; Cazals et al. 1978; Hatton et al. 1960; Portmann et al. 1979). Our results suggest that tinnitus suppression produced by ECES with positive current is related to the unspecific suppression of the central spontaneous activity of central neurons, including hyperactive neurons (neurons supposed to propagate the tinnitus-related activity). In this context, one proposes that ECES can be a very promising approach for suppressing peripheral-dependent tinnitus, i.e., tinnitus linked to the residual spontaneous firing in the cochlea (Mulders and Robertson 2009; Norena 2011).

Since we did not try to reveal tinnitus in noise-exposed animals, the assumption that the tinnitus percept is related to the noise-induced hyperactivity is necessarily speculative (see, for instance, Coomber et al. 2014 and Kalappa et al. 2014, for conflicting views). One notes, however, that it would have been challenging to objectify tinnitus reduction or suppression while electrical stimulation was provided at the RW (i.e., which is the ultimate goal of the present study). In this context, we felt that we should first test our hypothesis (effects of ECES on central hyperactivity) without checking for tinnitus.

Peripheral-dependent tinnitus should be abolished by cochlear nerve section, and indeed cochlear nerve section can lead to tinnitus suppression (Barrs and Brackmann 1984; House and Brackmann 1981; Pulec 1995; Silverstein et al. 1986). As most tinnitus subjects have relatively good hearing (Pan et al. 2009; Sereda et al. 2011) with functional IIHCs and consequently residual spontaneous firing in the cochlear nerve, the peripheral-dependent tinnitus may be a relatively prevalent form of tinnitus. Any therapeutic approach aimed at suppressing tinnitus should not alter auditory thresholds, even temporarily. Here we show that ECES is able to reduce the putative tinnitus-related activity, while hearing thresholds are only modestly affected.

On the other hand, other forms of tinnitus, such as “centralized” tinnitus (forms of tinnitus that become independent of the residual spontaneous activity in the cochlear fibers), should not respond to this treatment (Mulders and Robertson 2011). Centralized tinnitus should be unaffected by cochlear nerve section, and indeed cochlear nerve section is sometimes inefficient to suppress tinnitus (Barrs and Brackmann 1984; House and Brackmann 1981; Pulec 1995; Silverstein et al. 1986). However, ECES with positive current has been shown to suppress tinnitus in subjects with severe hearing loss and chronic tinnitus (Cazals et al. 1978). This result suggests that some forms of chronic tinnitus may remain dependent on cochlear residual activity. Whether tinnitus is peripheral dependent or not may depend on the cochlear residual activity and therefore on the extent of cochlear damages. One notes that the susceptibility of the cochlea to various stressors can vary as a function of the context and among individuals and species (Canlon et al. 2007; Graham et al. 2011; Turner et al. 2005).

Electric stimulation used in cochlear implants is well known to reduce tinnitus (Baguley and Atlas 2007; Chang and Zeng 2012; Ito and Sakakihara 1994; Okusa et al. 1993; Quaranta et al. 2004; Rubinstein et al. 2003; Zeng 2004). The approach tested in this study is, however, very different from stimulating the cochlea with cochlear implants. Cochlear implants are meant to restore hearing and, therefore, to produce excitation of the cochlear fibers. The tinnitus reduction produced by cochlear implants could then be achieved through masking, residual inhibition or reduction of central gain (Norena 2011; Noreña 2012). Our approach, instead, is aimed at suppressing
the cochlear driver of tinnitus by hyperpolarizing IHCs and/or cochlear fibers.

Finally, while this study suggests that electrical stimulation can be a promising approach to suppress peripheral-dependent tinnitus, it is important to note that it cannot be used as such in tinnitus subjects. Indeed, direct current can be harmful for biological tissues by producing free radicals, changing the pH of the medium around the electrode and leading to electrode corrosion among other electrochemical reactions (Merrill et al. 2005; Shepherd et al. 1991, 1999). The main challenge faced by this approach is, therefore, to achieve robust suppression of cochlear activity from a charge-balanced electrical stimulation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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