TINNITUS

Auditory-somatosensory bimodal stimulation desynchronizes brain circuitry to reduce tinnitus in guinea pigs and humans

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The dorsal cochlear nucleus is the first site of multisensory convergence in mammalian auditory pathways. Principal output neurons, the fusiform cells, integrate auditory nerve inputs from the cochlea with somatosensory inputs from the head and neck. In previous work, we developed a guinea pig model of tinnitus induced by noise exposure and showed that the fusiform cells in these animals exhibited increased spontaneous activity and cross-unit synchrony, which are physiological correlates of tinnitus. We delivered repeated bimodal auditory-somatosensory stimulation to the dorsal cochlear nucleus of guinea pigs with tinnitus, choosing a stimulus interval known to induce long-term depression (LTD). Twenty minutes per day of LTD-inducing bimodal (but not unimodal) stimulation reduced physiological and behavioral evidence of tinnitus in the guinea pigs after 25 days. Next, we applied the same bimodal treatment to 20 human subjects with tinnitus using a double-blinded, sham-controlled, crossover study. Twenty-eight days of LTD-inducing bimodal stimulation reduced tinnitus loudness and intrusiveness. Unimodal auditory stimulation did not deliver either benefit. Bimodal auditory-somatosensory stimulation that induces LTD in the dorsal cochlear nucleus may hold promise for suppressing chronic tinnitus, which reduces quality of life for millions of tinnitus sufferers worldwide.

INTRODUCTION

Tinnitus, the phantom perception of sound in the absence of external stimuli, is a disorder that affects 15% of the population in the United States (*1*) and is the most prevalent service-connected disability for military personnel (*2*). Whereas some individuals are minimally disturbed by their tinnitus, about 10% are bothered by it, and about 2 million individuals are debilitated (*1*). Negative impacts of tinnitus include sleep disturbance, poor concentration, distress, depression, and anxiety (*1*, *3*). Current tinnitus therapies are more successful at managing a patient's reaction to their percept rather than addressing the tinnitus, and no one therapy is effective for all patients. Even when improving quality of life, none of the available tinnitus therapies treat the underlying pathology, and few have reported reductions in tinnitus loudness (*4*). A treatment that targets the underlying tinnitus mechanisms would greatly improve clinical outcomes for patients.

Whereas tinnitus is commonly associated with acoustic overexposure, many patients with tinnitus have clinically normal audiometric thresholds (*5*, *6*), and about 12% report a triggering event such as a tooth abscess or head and neck injury precipitating their tinnitus (*7*), indicating that events in addition to acoustic trauma can modify neural activity in auditory pathways. Indeed, 60 to 80% of tinnitus sufferers display a somatosensory component to their tinnitus, evident in their ability to modulate their tinnitus pitch or loudness by moving or applying pressure to their head or neck (*8*).

Tinnitus is thought to arise from dysregulated neural synchrony across neural ensembles along the auditory pathway (*9*), beginning in the dorsal cochlear nucleus (DCN) (*10*). The DCN is the first central site for multisensory integration, receiving input from the auditory nerve, auditory midbrain, auditory cortex, trigeminal and cervical ganglia, spinal trigeminal nucleus, and dorsal column nuclei (*11*–*13*). After noise exposure sufficient to temporarily elevate hearing thresholds, spontaneous activity and cross-neural synchrony of DCN output neurons, the fusiform cells, are increased in animals showing behavioral evidence of tinnitus. Animals without behavioral evidence of tinnitus do not show these neural correlates (*14*). Further, the tinnitus-related neural changes can occur even in the absence of permanent shifts in behavioral audiometric thresholds or electrophysiological measures of peripheral hearing status (*14*, *15*).

The DCN produces hypersynchronous output through its unique, cerebellar-like circuit (fig. S1). In this circuit, auditory nerve fibers from the cochlea form synapses with the fusiform cell basal dendrites, whereas the nonauditory (for example, somatosensory) inputs are relayed by granule cell axons that form synapses with the fusiform cell apical dendrites (*16*). The apical dendritic synapses display spike timing–dependent plasticity in which repeated elicitation of presynaptic excitatory postsynaptic potentials (EPSPs) followed by postsynaptic spikes produce long-term potentiation (LTP), whereas postsynaptic spikes followed by presynaptic EPSPs produce long-term depression (LTD) in vitro (*17*). In vivo, auditory (sound) stimulation can be used to evoke postsynaptic spikes, and somatosensory stimulation can be used to evoke presynaptic activity in fusiform cells, such that paired auditory-somatosensory stimulation produces long-term changes in fusiform cell firing rates. In vivo, the resulting long-term effects are termed "stimulus timing–dependent plasticity" (STDP). Whether LTP or LTD occurs depends on the precise order and timing between the bimodal stimuli (*15*). These "learning rules" are altered after noise exposure so that animals with tinnitus show a broader range of stimulus intervals that evoke LTP, have broader range OF intervals that evoke LTD (*18*). Theoretical models of feedforward networks predict that LTP-driven synaptic strengthening will increase circuit connectivity and result in hypersynchrony (*19*). Hypersynchrony can also be driven by inhibitory

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network components (*20*), such as the cartwheel cells in the DCN (fig. S1), which are also subjected to spike timing–dependent synaptic modulation (*17*). Thus, increased LTP in the fusiform cell circuit could contribute to the hypersynchrony and increased spontaneous activity that are considered neural correlates of tinnitus (*14*).

Here, using a guinea pig model, we determined whether enhanced LTP and reduced LTD in the fusiform cell circuit initiated hypersynchrony, resulting in behavioral evidence of tinnitus. We show, in vivo, that auditory-somatosensory stimulation strengthened or weakened neural synchrony between fusiform cells, depending on the bimodal stimulus order and timing. Furthermore, in animals with tinnitus, enhanced LTP correlated with increased synchrony and spontaneous activity in fusiform cells. To counteract tinnitus, we stimulated guinea pigs with repeated auditory-somatosensory bimodal stimulation for 20 min/day for 25 days, choosing a bimodal interval shown to produce LTD in the fusiform cell circuit. This noninvasive approach resulted in decreased synchrony and spontaneous activity in fusiform cells and reduced behavioral evidence of tinnitus. Furthermore, neither unimodal sound nor unimodal somatosensory stimulation reliably decreased behavioral or physiological evidence of tinnitus in these animals. These findings demonstrated that fusiform cell spike timing–dependent plasticity may play a fundamental role in regulating neural synchrony and perception and that LTD could be harnessed to reverse pathological hypersynchrony to reduce tinnitus.

Then, using stimulus protocols determined by the preclinical animal experiments, we conducted a similar study in 20 human participants with somatic tinnitus using a double-blinded, sham-controlled, crossover design. We reasoned that, because the human cochlear nucleus contains the cellular elements present in the DCN of rodents (*21*), similar learning rules should be present in humans and guinea pigs. We demonstrated that bimodal auditory-somatosensory stimulation, but not unimodal auditory stimulation, effectively reduced tinnitus loudness and intrusiveness cumulatively over the 4 weeks of treatment.

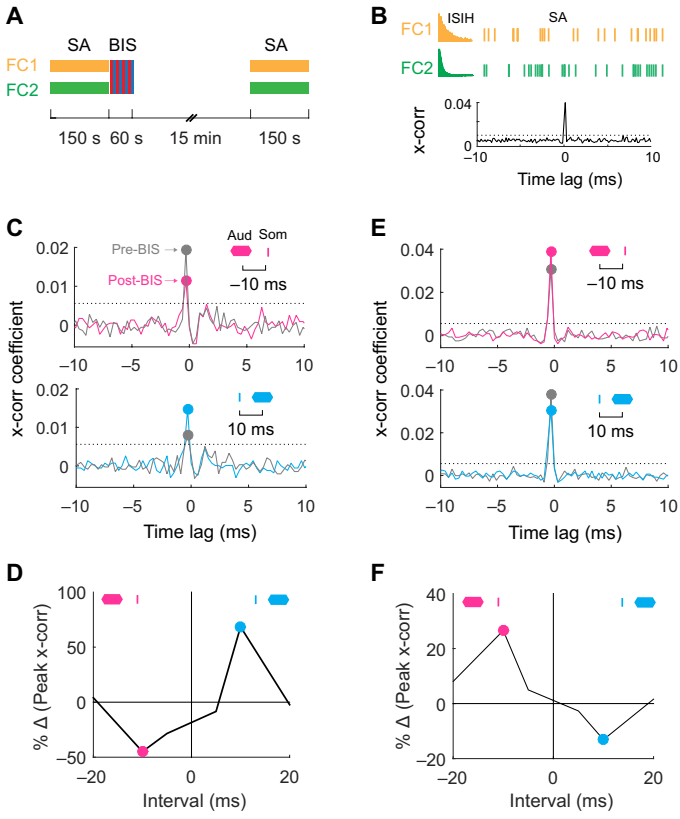

Fig. 1. STDP regulates synchrony in fusiform cells of the guinea pig DCN. (**A**) Spontaneous activity (SA) was recorded across the fusiform cell (FC) population in 25 guinea pigs for 150 s, followed by 60 s (5 Hz) of bimodal stimulation (BIS) with bimodal intervals (BI) from −20 to +20 ms. SA was recorded again 15 min after BIS for 150 s. (**B**) Synchrony was assessed by cross-correlations (x-corr) of spikes in FC pairs (FC1 and FC2). SA of FCs shows Poisson distributions in interspike interval histograms (ISIHs). Synchronous unit pairs are defined by threshold cross-correlation coefficients (x-corr coef) of 4 SD (dashed line). (**C**) In one representative FC unit pair, a BI of −10 ms (auditory preceding somatosensory stimulus by 10 ms; pink) reduced the peak x-corr coef (top), whereas a BI of 10 ms (somatosensory preceding auditory stimulus by 10 ms; blue) increased the peak x-corr coef 15 min after BIS (bottom). (**D**) Changes in peak x-corr coef for the FC unit pair in (C) are plotted as a function of BI (learning rule). (**E**) In a different FC unit pair, a BI of −10 ms increased peak x-corr coef (top), whereas a BI of 10 ms decreased peak x-corr coef 15 min after BIS (bottom). (**F**) For the FC unit pair in (E), changes in x-corr coef after BIS were opposite to that for the FC unit pair in (D).

RESULTS

STDP regulates synchrony among DCN fusiform cells in guinea pigs

To test the role of STDP in regulating synchronous firing among fusiform cells in the DCN, we recorded spontaneous spiking activity from single fusiform cells in anesthetized normal-hearing guinea pigs before and 15 min after bimodal stimulation (Fig. 1A). Bimodal stimulation consisted of sounds (tone bursts near the unit best frequency) and transcutaneous electrical stimulation of the neck, presented within a ±20-ms interstimulus window (Fig. 1A). Six bimodal intervals were studied (sound preceding electrical stimulus by 5, 10, or 20 ms or electrical stimulus preceding sound by 5, 10, or 20 ms) in a separate series in a randomized order; physiological measurements preceded and followed each series (see table S1 for STDP learning rule types across unit/unit pairs). To quantify synchronous firing, we measured peak cross-correlation coefficients between spontaneous spike trains from fusiform cell pairs (Fig. 1B). In one representative unit pair, the peak cross-correlation coefficient decreased (Fig. 1C, top) after auditory-preceding-somatosensory stimulation (−10-ms interval) but increased after somatosensory-preceding-auditory stimulation (10-ms interval; Fig. 1C, bottom). This unit pair exhibited a Hebbian-like learning rule (Fig. 1D) in which presynaptic, subthreshold activation of the parallel fibers by somatosensory stimulation followed by postsynaptic activation of the basal dendrites by auditory stimulation (sound) strengthened neural synchrony. In other unit pairs (for example, Fig. 1, E and F), the learning rule was anti–Hebbian-like, where the same bimodal interstimulus interval as Fig. 1C produced neural synchrony changes in the opposite direction. Other unit pairs exhibited LTP-only learning rules (where all bimodal intervals strengthened synchrony) or LTD-only learning rules (where all bimodal intervals weakened synchrony).

STDP regulates tinnitus-related increases in synchrony and spontaneous activity

Increased synchrony, bursting, and spontaneous activity are established neural correlates of tinnitus (*14*). To determine whether dysregulated

STDP contributes to tinnitus-related hypersynchrony, we induced tinnitus in guinea pigs using noise exposure and assessed tinnitus using gap-prepulse inhibition of the acoustic startle (GPIAS) response. GPIAS measures the acoustic startle response in the presence of a background narrow-band noise. When a gap is inserted into the background noise before the startle stimulus, the startle response is reduced in normal animals. However, in animals with tinnitus, the tinnitus obscures the gap, and there is no decrement in the startle response. By plotting the amplitude of the gap trials versus the no-gap trials, an estimate of the animals' tinnitus is obtained (see fig. S4) (*15*, *22*, *23*). Noise exposure produced only temporary hearing threshold elevations, which recovered after a few days (fig. S3), but resulted in chronic tinnitus in 16 of 22 (72.7%) guinea pigs after 8 weeks (fig. S4). Noise-exposed animals with tinnitus [exposed with tinnitus (ET)] exhibited significant increases in synchrony [one-way analysis of variance (ANOVA), $F_{(2)} = 14.9$, $P = 2.3 \times 10^{-6}$, post hoc $P < 0.05$; Fig. 2A] and spontaneous activity [$F_{(2)} = 17.5$, $P = 3.0 \times 10^{-7}$, post hoc $P < 0.05$; Fig. 2B) across the fusiform cell population compared to normal-hearing animals and the 27.3% of noise-exposed animals that did not develop tinnitus [exposed no tinnitus (ENT)]. STDP for synchrony was assessed and compared across the tinnitus, no-tinnitus, and normal-hearing groups. The tinnitus group exhibited a greater proportion of unit pairs with LTP-only learning rules, whereas the normal-hearing and the non-tinnitus groups exhibited greater proportions of anti-Hebbian–like and LTD-only learning rules (Fig. 2C and table S1) [$\chi^2_{(3)} = 15.8$, $P = 0.0013$]. STDP for spontaneous activity of single units followed a similar trend (Fig. 2D) [$\chi^2_{(3)} = 23.4$, $P = 3.3 \times 10^{-5}$]. To further quantify the learning rule distribution shift from LTD toward LTP, we compared the LTD-LTP index (Fig. 2E, inset), which sums all positive/LTP integration phases and all negative/LTD phases across all unit pairs or single units (see Fig. 2E for synchrony and Fig. 2F for spontaneous activity). The tinnitus group showed more LTPs across all learning-rule types, whereas the no-tinnitus group showed more LTD compared to the normal-hearing group [$F_{(2)} = 10.33$, $P = 5.3 \times 10^{-5}$ for synchrony; $F_{(2)} = 91.7$, $P = 1.6 \times 10^{-37}$ for spontaneous activity]. These findings indicated that the tinnitus-driven circuit had a high probability for LTP and strengthened neural synchrony.

Bimodal (but not unimodal) stimulation induces LTD to reduce fusiform cell synchrony and spontaneous activity

Given that increased synchrony and spontaneous activity correlated with an expansion of the LTP phase of the STDP learning rule, we hypothesized that inducing LTD would reduce synchrony and spontaneous activity. First, we determined the bimodal interval that produced the strongest LTD in the animals with tinnitus by quantifying LTD probability (overall proportion of units showing LTD at a given bimodal interval). We found that more units responded with decreased synchrony and spontaneous activity after bimodal stimulation intervals of −5 and −10 ms. Whereas ±20-ms intervals showed slight deviation from 0.5, they were not different from chance (Fig. 3A). Suppression of synchrony and spontaneous activity after −5-ms bimodal stimulation was significantly greater (due to less variance) than unimodal auditory or unimodal somatosensory stimulation, neither of which produced long-term effects [one-way ANOVA, $F_{(2)} = 11.3$, $P = 1.1 \times 10^{-6}$ for synchrony; $F_{(2)} = 142$, $P = 5.4 \times 10^{-66}$ for spontaneous activity; Fig. 3, B and C].

We next asked whether reducing synchrony in the fusiform cell circuit would affect the animal's tinnitus behavior. We hypothesized that repeated bimodal stimulation with an LTD-inducing interval (−5 ms) would reduce synchrony and spontaneous activity as well as behavioral evidence of tinnitus. Unimodal auditory stimulation, on the other hand, should not induce LTD because auditory synapses on the basal dendrites are not plastic; somatosensory input alone has been shown to induce LTP (*15*, *18*). To test this hypothesis, we treated guinea pigs with tinnitus with 20-min daily sessions of bimodal stimulation consisting of an 8-kHz tone burst (the frequency at which tinnitus was most prevalent; see fig. S4) paired with transcutaneous stimulation at the −5-ms interval for 25 days (ET-treat group). Three control groups were used, all expressing tinnitus after noise exposure. A sham group received a sedative but no bimodal or unimodal stimulation (ET-sham); an auditory-only group received the same 8-kHz tone but did not receive transcutaneous somatosensory stimulation (ET-audio); and a somatosensory-only group received only electrical stimulation (ET-som). After the 25-day treatment

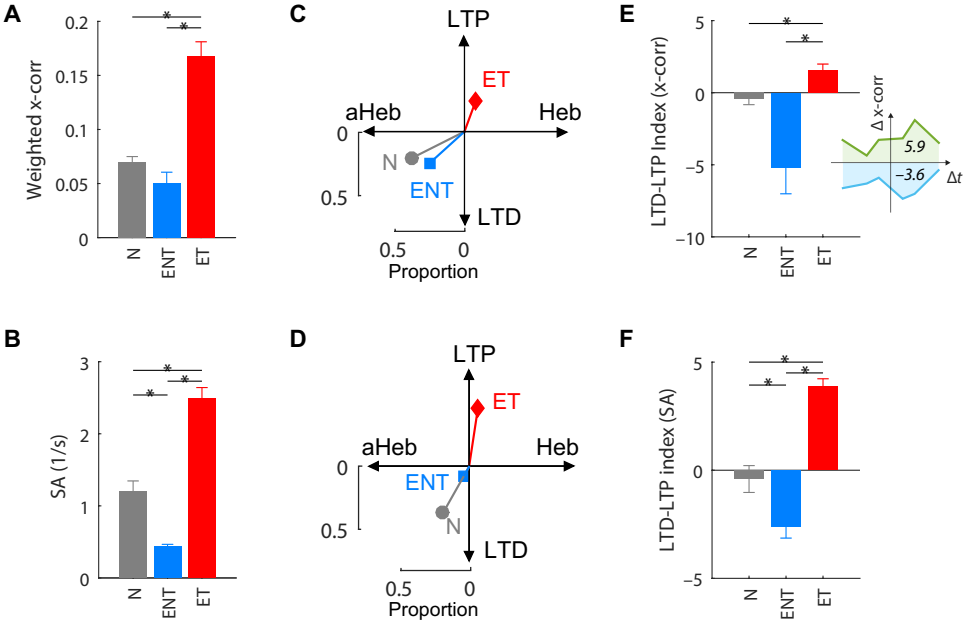

Fig. 2. STDP shifts toward LTP in guinea pigs with tinnitus. (**A**) Increased mean cross-correlation coefficient (x-corr; weighted by the proportion of synchronous unit pairs) and (**B**) increased mean spontaneous firing rate (SA) compared to the normal-hearing (N) and exposed-but-no-tinnitus (ENT) groups of animals. One-way analysis of variance (ANOVA), *$P < 0.05$; data are mean ± SEM. Spontaneous firing rates for the N, ENT, and ET (exposed tinnitus) groups were 116, 93, and 167 unit pairs for x-corr and 106, 387, and 478 units, respectively. (**C** and **D**) A shift in the proportion of learning rules toward Hebbian-like (Heb; *x* axis) and long-term potentiation (LTP) (*y* axis) in the ET group for (C) synchrony and (D) SA. aHeb, anti-Hebbian. (**E** and **F**) Long-term depression (LTD)–LTP index: total magnitude of LTP, that is, green area under the curve relative to total magnitude of LTD, that is, blue area above the curve of learning rules (E, inset), is increased in the ET group for (E) synchrony and (F) SA.

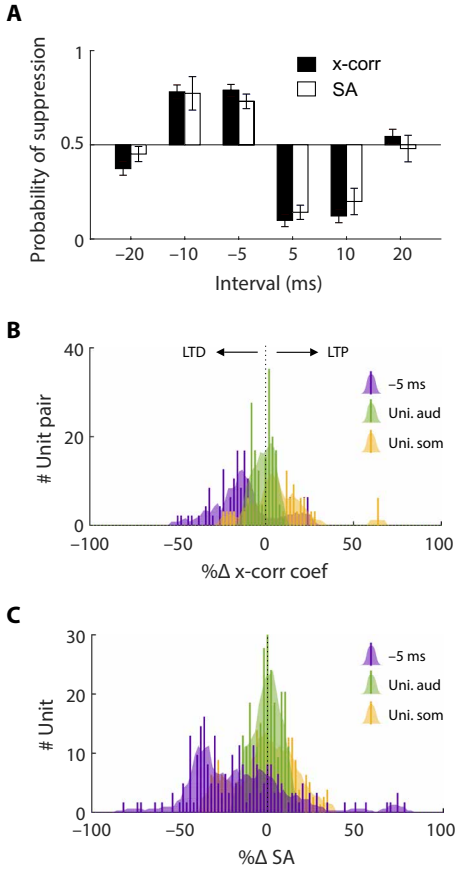

Fig. 3. Targeted bimodal stimulation suppresses synchrony and spontaneous activity in fusiform cells of guinea pigs. (**A**) Probability of synchrony (x-corr) or SA suppression as a function of bimodal interval. Probability is computed by proportion of unit pairs (total $n = 159$) or units ($n = 251$) showing decreased x-corr or SA at a given bimodal interval. A probability of 0.5 indicates an equal number of units showing increased or decreased x-corr or SA. The highest probability of suppression occurs for the −10- and −5-ms intervals [error bar, confidence interval (CI) for binomial proportion]. The −5-ms interval was chosen for the treatment. (**B**) The distributions of suppression versus enhancement of synchrony are compared for the −5-ms bimodal interval, unimodal somatosensory (uni. som), or unimodal auditory stimulation (uni. aud). The bimodal stimulus clearly suppressed synchrony, whereas the unimodal stimuli showed little deviation from zero. (**C**) Similar to synchrony, the bimodal stimulus suppressed SA, whereas the unimodal stimulus showed little deviation from zero (bar = 2% bin; shaded curve is fitted by Spline Interpolant).

period, we quantified tinnitus behavior in the four groups using the tinnitus index (TI), which compared gap startle responses normalized to the preexposure baseline before and after noise exposure (fig. S4). Representative findings presented in Fig. 4A (one animal per group) show increased normalized startle responses after noise exposure, indicating tinnitus, which was reduced after treatment only in the animal receiving bimodal stimulation (ET-treat). Group analysis presented in Fig. 4B showed that, compared to the pretreatment TI, animals receiving bimodal stimulation (ET-treat) exhibited a significant reduction in the TI at the treated frequency of 8 kHz and not at other tinnitus frequencies, whereas the sham group (ET-sham) and the auditory-only group (ET-audio) showed no changes [two-way ANOVA, $F_{(2,1)} = 3.70$, $P = 0.0069$ for frequency × group). The somatosensory-only group (ET-som) showed a small decrease in TI at one frequency and a large increase in TI in another frequency but no significant group mean change from control (post hoc $P > 0.05$). Tinnitus reduction correlated with lower neural synchrony [Pearson's linear correlation: $r_{(287)} = 0.15$, $P = 0.010$; correction of dependency using linear mixed-effect model: $P = 0.031$; Fig. 4C] and lower spontaneous activity [$r_{(1125)} = 0.20$, $P = 6.8 \times 10^{-12}$; correction of dependency using linear mixed-effect model: $P = 0.0011$; Fig. 4D]. Together, these results demonstrate that targeted LTD induction in guinea pigs reduced tinnitus produced by dysregulated STDP, increased neuronal synchrony, and spontaneous activity.

Bimodal (but not unimodal) auditory-somatosensory stimulation reduces tinnitus loudness in humans

The positive animal study outcomes prompted the investigation of bimodal treatment for humans suffering from tinnitus. A double-blinded, sham-controlled, crossover study was used to evaluate the effectiveness of bimodal auditory-somatosensory stimulation as a tinnitus treatment. All subjects and investigators were blinded as to whether subjects received an active (bimodal) or sham (unimodal-auditory) treatment for the duration of the study. Upon enrollment, participants were first assigned to either a sham group ($n = 10$, group 1) or an active bimodal treatment group ($n = 10$, group 2; Fig. 5). Assignment was by a random number list that was precomputed before the start of the study. Take-home devices were programmed to deliver the bimodal or unimodal treatment protocols by control software and data were encrypted to ensure blinding. The sound stimuli were delivered through calibrated insert earphones, and the electrical (somatosensory) stimuli were administered using Ag-AgCl cups placed on the skin of the cervical spine or the cheek. Participants used the devices for 30 min once a day for two 4-week sessions with a 4-week washout period after each session. After the washout period, subjects "crossed over" to receive the other treatment for the second 4-week period so that all subjects received both active and sham treatments. Participants returned to the laboratory weekly for monitoring and tinnitus assessment: loudness was assessed by matching tinnitus loudness to an external sound using TinnTester software, and intrusiveness was assessed using the Tinnitus Functional Index (TFI; see Material and Methods).

The auditory stimulus (the same for bimodal and sham) was derived from each individual's tinnitus spectrum and audiogram (fig. S5, see Materials and Methods). Devices provided either bimodal (auditory-electric) stimulation (bimodal active treatment) or unimodal (auditory alone) stimulation (sham treatment) for 30 min a day for 28 days. The bimodal interval was the same as that shown to be effective in the guinea pigs (−5 ms). Somatosensory stimulation alone was not provided because the animal study (Figs. 3B and 4, A and B) indicated that it could exacerbate the tinnitus.

The active bimodal treatment produced a significant ($P < 0.05$) cumulative decrease in tinnitus loudness assessed by TinnTester loudness matching each week of the active treatment (Fig. 6A). The greatest mean change in loudness occurred after the fourth and final week of treatment. In contrast, loudness was stable (unchanged) during sham treatment for both groups. There was no significant difference between groups 1 and 2 ($P = 0.88$), demonstrating that treatment order had no effect. Pooled groups showed a mean decrease of 8.035 ± 1.33 dB from a baseline of 54.42 ± 13.3 dB in loudness matches during the 4 weeks of active treatment [two-way ANOVA, $F_{(3,1)}$ 7.768, post hoc $P = 5.5 \times 10^{-5}$], significantly larger than the changes seen in the other conditions (sham, active washout, and sham washout) where changes from baseline were not significant (Fig. 6B). Tinnitus

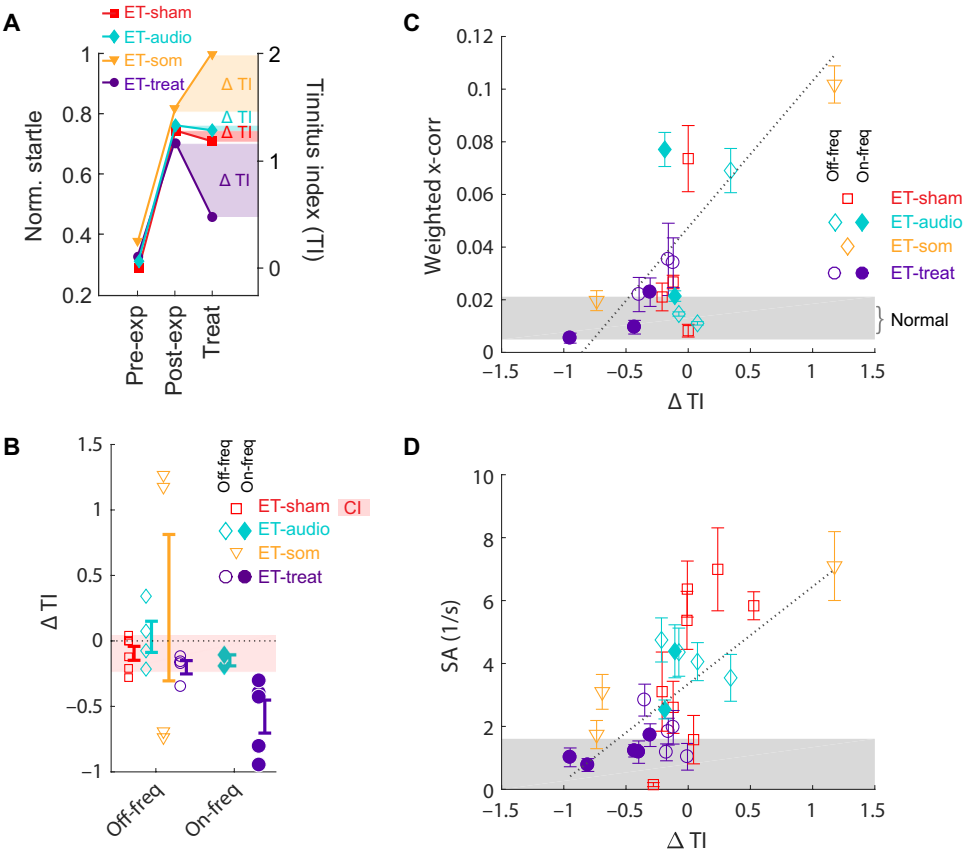

Fig. 4. LTD-induction reduces synchrony and spontaneous activity and reduces tinnitus in guinea pigs. (**A**) Four representative animals (one from each group) showed increased normalized startles (norm. startle) after noise exposure [from pre-exposure (pre-exp) to post-exposure (post-exp)] indicating tinnitus (left ordinate), quantified as the TI (right ordinate). After LTD induction by application of a bimodal auditory-somatosensory stimulus to the fusiform cells (ET-treat), there was a reduction in TI in the treated animal (ET-treat). Sham-treated (ET-sham; sedative only), auditory stimulus–only (ET-audio), and somatosensory stimulus–only (ET-som) animals showed either no reduction in TI or worsened TI. (**B**) Mean TI was significantly reduced in the ET-treat group at the treated frequency (on-freq; 8 kHz) but not at the untreated frequencies (off-freq; 12 and 16 kHz). TI was not significantly reduced in the ET-sham, ET-audio, or ET-som groups. Pink horizontal bar indicates the 95% CI for the ET-sham group. (**C**) The weighted mean cross-correlation coefficient (x-corr) for FCs (at best frequencies within the TI bandwidth) is plotted as a function of ΔTI (116, 36, 35, and 106 unit pairs for the ET-sham, ET-audio, ET-som, and ET-treat groups, respectively). Gray area indicates the range of x-corr for nonexposed animals. Reduction in synchrony significantly correlated with TI reduction. (**D**) SA plotted as a function of ΔTI (446, 204, 202, and 696 units). Reduction in SA significantly correlated with TI reduction. Data are mean ± SEM.

reduction reached an average of 12.2 dB in the fourth week of active treatment. Of the 20 participants tested, 2 reported complete elimination of their tinnitus toward the end of the active treatment period.

Bimodal (but not unimodal) stimulation improves TFI scores

Mean overall TFI scores decreased from baseline of 29.2 ± 2.6 to 22.9 ± 1.8 units during the active treatment but remained unchanged during the sham treatment (Fig. 6C). Improvements in TFI scores were sustained beyond the active treatment and into the washout period, unlike the changes in loudness matching. Because treatment order also had no significant effect on TFI scores [general linear mixed models (GLMM); $P = 0.819$], both groups were pooled for statistical analysis. The mean TFI scores across the different study periods (Fig. 6D) were significantly improved (that is, reduced relative to baseline) for both active and active washout periods (but not sham periods) [7.33 ± 0.956 TFI units; two-way ANOVA, $F_{(3,1)} = 7.712$, $P = 6.14 \times 10^{-5}$), indicating a diminished impact on daily life with mean reductions of 7.51 and 6.71 points, respectively. Eleven participants noted subjective changes in volume, pitch, or quality that resulted in their tinnitus becoming less "harsh" or "piercing" and more "mellow." Even participants who did not experience a complete elimination of their tinnitus reported anecdotally that their tinnitus was noticeably less obtrusive and easier to ignore.

Ten of the 20 subjects had a clinically significant reduction of at least 13 points in their TFI scores during active treatment, which is considered clinically meaningful for this questionnaire (*24*). There were no demographic differences across subjects showing significant TFI changes compared to stable subjects (table S2). Four participants had clinically significant drops during the sham treatment, but two of these also showed significant decreases in TFI during the active treatment. Further, both participants reported that their tinnitus improved more during the active treatment. The two participants who stated that the sham treatment was more effective also had the shortest tinnitus duration (less than 1 year).

Reductions in loudness relative to baseline correlated significantly with reductions in overall TFI scores (linear mixed-effects model: $\beta = 0.169 \pm 0.058$, $T = 2.94$, $P = 0.0035$; fig. S7). Furthermore, changes in loudness correlated with changes in TFI subscores: sense of control, intrusive, cognitive, and sleep (table S3).

DISCUSSION

Increases in synchrony, spontaneous activity and bursting (*14*), and altered STDP (*15*) are established neural correlates of tinnitus. In animal models of tinnitus, increased synchrony has been identified in the DCN (*14*), inferior colliculus (*25*), and auditory cortex (*26*). These studies suggest that the tinnitus percept emerges from increased spontaneous synchrony among neurons in cortical and subcortical regions that contribute to perceptual binding (the process of merging individual pieces of sensory information into coherent representations) (*27*). Here, we first examined the relationship between synchrony and STDP in normal-hearing guinea pigs, which exhibited Hebbian and anti-Hebbian learning rules as well as rules giving LTP or LTD. We then induced tinnitus in animals using noise exposure that produced only temporary threshold shifts and observed tinnitus-related increases in neural activity reflecting an overall dominance of LTP. Subsequently, we applied the optimal bimodal interval to induce LTD in sessions of 20-min duration for 25 days, which reversed hypersynchrony and behavioral evidence of

tinnitus at frequencies corresponding to the treatment frequency. None of the control stimuli (sedative alone, unimodal somatosensory, or unimodal auditory stimulation) had any effect on tinnitus behaviors or tinnitus correlates. On the basis of the outcome of the animal study, we used the same bimodal stimulus protocol to treat tinnitus in humans.

STDP is essential for shaping sensory perception through input-dependent learning. In the visual cortex, STDP modulates tuning of visual neurons to orientation and motion (*28*). Similar STDP processes shape map plasticity in the somatosensory and auditory cortices for frequency selectivity, pitch encoding, and discrimination (*29*–*31*). These context-dependent changes in sensory processing can alter connectivity and synchrony of neural ensembles (*32*, *33*). In the fusiform cell circuit, multimodal inputs induce context-dependent changes through STDP (*34*). Auditory-somatosensory integration in DCN constitutes an adaptive filtering process through which perception of behaviorally relevant sounds is amplified and internally generated sounds are attenuated (*17*, *35*, *36*). Fusiform cell synchrony regulation by STDP likely contributes to this perceptual task, whereas dysregulated multimodal STDP gives rise to phantom perception, as we show here.

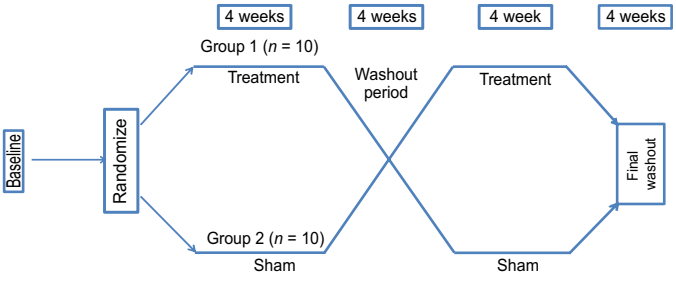

Fig. 5. Outline of crossover design for the human study. Subjects were randomly assigned to group 1, in which the bimodal treatment was presented first, or group 2, in which the sham treatment was presented first. After 4 weeks of 30 min/day of the treatment, there was a 4-week washout period. Thereafter, subjects crossed over to receive the treatment that they had not yet received for 4 more weeks. This was followed by a second, 4-week washout period. Loudness and Tinnitus Functional Index (TFI) assessments were done weekly in the clinic.

Synaptic plasticity has been suggested as a foundation for network-level homeostatic adaptation (*37*). In the fusiform cell circuit, glutamatergic inputs to the granule cell–parallel fiber circuit are up-regulated after hearing loss (*38*–*40*), resulting in increases in LTP (*41*). This homeostatic mechanism in response to altered input is not exclusive to the auditory pathway (*42*). After light deprivation, visual-cortical neurons exhibit expansion in STDP due to increased *N*-methyl-D-aspartate (NMDA) receptor activation (*43*). Blocking NMDA receptors in the fusiform cell circuit reduces neural synchrony (*44*). Muscarinic acetylcholine receptors, whose expression is up-regulated after noise exposure (*45*), also contribute to STDP (*46*, *47*).

STDP can affect intrinsic membrane excitability by altering ion channel conductance (*48*, *49*). Maladaptive changes to fusiform cell plasticity that decrease inhibition through reduced hyperpolarizing currents could also contribute to increased synchrony and spontaneous activity. Reduced potassium channel activation and reduced glycine and GABA (γ-aminobutyric acid) receptor activation of fusiform cells have been demonstrated in tinnitus models (*50*, *51*). A major source of GABA input and glycinergic input to fusiform cells arises in cartwheel cells (fig. S1). These DCN interneurons, which receive parallel-fiber synapses that exhibit STDP (*17*), provide recurrent inhibitory synapses onto fusiform cells. Cartwheel cells, therefore, may play an essential role in generating fusiform cell synchrony (*19*, *20*). Another potential player, the Golgi cell in the marginal region of the cochlear nucleus, provides feedback modulation of granule cell output, which may entrain parallel fibers into synchronized firing (*52*–*54*). These network components are likely to work together to increase synchrony in fusiform cells, thus potentially playing important roles in tinnitus.

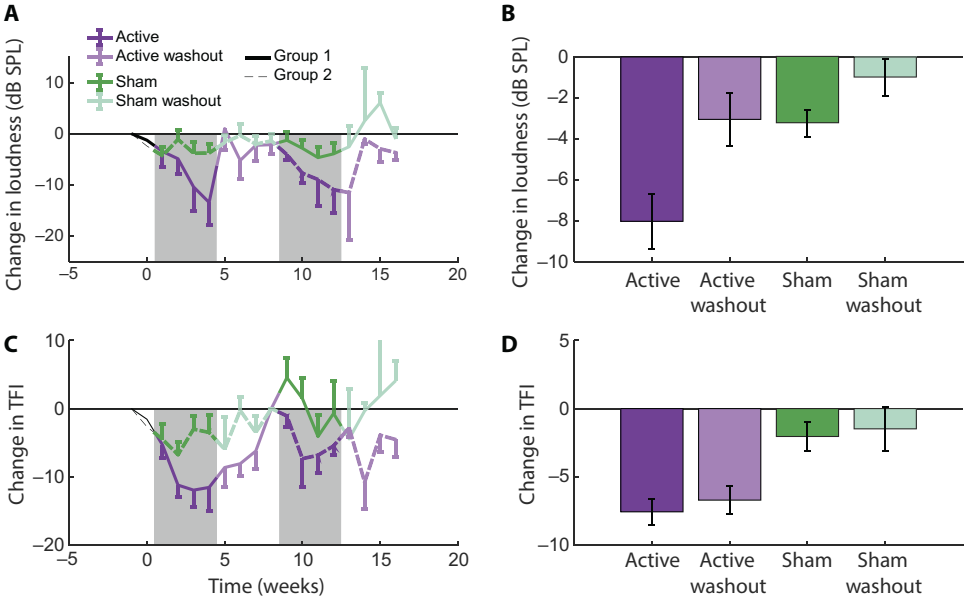

Fig. 6. Bimodal treatment results in reduced tinnitus loudness and reduced TFI scores in human patients. (**A**) Mean loudness by group. Group 1 ($n = 10$) received the active treatment first; group 2 ($n = 10$) received the sham treatment first. Loudness was assessed using the interactive software TinnTester, in which subjects match their tinnitus loudness and spectrum to an externally presented sound (see Materials and Methods). (**B**) Mean changes (normalized to baseline) in loudness matching for each condition. (**C**) Mean TFI changes (relative to baseline) for groups 1 and 2. (**D**) Mean changes (relative to baseline) in TFI scores. Error bars are SEM. dB SPL, decibels sound pressure level.

Because the human cochlear nucleus contains all of the cellular elements present in the DCN of rodents (*21*), we reasoned that the same bimodal protocol might suppress tinnitus in humans. In both the animal and the human studies, bimodal but not unimodal auditory stimulation effectively suppressed tinnitus. The documented failure of unimodal auditory stimulation to produce long-term changes in fusiform cell firing rates predicted that unimodal auditory stimulation would be inefficient at reducing tinnitus (*15*, *18*, *34*, *55*). The significant reduction in tinnitus in animals and tinnitus loudness and distress in humans suggests

that the bimodal treatment was successful at inducing frequency-specific LTD, reversing the pathological neural activity responsible for the generation of tinnitus.

Unimodal auditory treatment, in addition to being ineffective at reducing tinnitus during the sham treatment phase, tended to cause an increase in tinnitus loudness and TFI scores at the end of the sham treatment, possibly due to the increased attention paid to the tinnitus during the evaluation periods. Unimodal somatosensory stimulation, on the other hand, shown to cause LTP and not LTD in animal studies (*15*, *18*, *34*), predicted that unimodal somatosensory stimulation could exacerbate tinnitus. Unimodal somatosensory stimulation did exacerbate the tinnitus in some animals, preventing us from testing the electrical-only stimulation condition in humans.

Bimodal auditory-somatosensory stimulation in humans had no side effects, whereas invasive techniques such as deep brain stimulation and vagal nerve stimulation can have severe side effects. Our LTD induction approach is noninvasive, easy to implement, and presents minimal risk. Although reduced tinnitus loudness did not carry over into the washout period, this benefit was persistent enough to accumulate over several days of treatment. Improved adjustment to tinnitus, as reflected in the TFI scores, persisted during the washout period, for up to 3 weeks. Furthermore, reductions in tinnitus loudness correlated with TFI subscores regarding sense of control, intrusiveness, cognition, and sleep, suggesting that tinnitus loudness reduction during bimodal treatment conferred psychological benefits that outlasted the treatment.

Other approaches to treat tinnitus, such as the coordinated reset sound therapy or paired sound–vagal nerve stimulation also target putative aberrant neural activity but have not yet yielded positive results in the clinic. Paired vagal nerve stimulation, although showing promising results in an animal model, requires invasive surgery with accompanying risks and side effects, rendering it only suitable for the most debilitated patients. Sound therapies do not consistently reduce tinnitus loudness (*56*), perhaps because unimodal auditory stimulation has no effect on modulating long-term plasticity in the DCN (Fig. 3, B and C) (*15*, *18*).

There are some limitations to our study. Our study only tested one subgroup of tinnitus patients, those with somatic tinnitus; thus, it is unknown whether these results would translate to other subgroups. In addition, ethical considerations prevented us from testing some protocol conditions in the human patients, such as the somatosensory stimulation–alone condition, which was observed to exacerbate tinnitus in the guinea pig study. Nevertheless, the neural desynchronization strategy presented here offers a new and accessible treatment possibility for tinnitus sufferers.

MATERIALS AND METHODS

Study design

All animal procedures were performed per protocol established by the National Institutes of Health Publication No. 80-23 and approved by the University of Michigan's University Committee on Use and Care of Animals. First, noise-over exposure was used to induce tinnitus in guinea pigs (see fig. S2). Evidence of tinnitus was provided by a behavioral test (GPIAS) and confirmed with physiological signatures of increased spontaneous rates of firing and synchrony in DCN fusiform cells. Twelve guinea pigs were used for physiological assessment after noise exposure, and 13 were used for physiological assessment after treatment. To the latter group (all expressing tinnitus), we applied noninvasive, 20 min/day auditory-somatosensory stimulation (with three different controls) for 25 days and assessed behavioral and neurophysiological correlates of tinnitus. Second (Fig. 5), a double-blinded, sham-controlled, crossover study was performed to evaluate the effectiveness of the auditory-somatosensory stimulation in humans with tinnitus. The study was performed in accordance with the University of Michigan Institutional Review Board. Participants were randomly assigned to either sham ($n = 10$) or active treatment first ($n = 10$) groups. Participants were trained to use a small, customized take-home device that provided the active and sham treatments. Weekly tinnitus spectra estimation and self-reported questionnaires were obtained on site. All 20 participants who completed the study were included in the analysis.

Tinnitus assessment in guinea pigs

Tinnitus was assessed using GPIAS (fig. S4A) (*14*, *15*, *41*, *57*). A normalized startle ratio (NSR) was computed as the ratio of the mean startle amplitude for the gap/prepulse trials and the mean of the startle-only trials (fig. S4B). An animal was defined as having tinnitus in a frequency band if the postexposure mean NSR value for gap inhibition was significantly greater than the baseline value. Neural recordings to evaluate spontaneous activity and synchrony were performed after the completion of tinnitus assessments.

Human tinnitus assessment

A computerized procedure (TinnTester) (*58*) was used for weekly loudness matching in the laboratory throughout the trial. The TFI questionnaire was used to assess the impact of a subject's tinnitus on their quality of life (*24*).

Auditory-somatosensory treatment in guinea pigs and humans

The somatosensory stimulation was provided by transcutaneous active electrodes positioned on the skin overlying either the trigeminal ganglion or the cervical spinal cord in the region of C2 (with the ground electrode adjacent). In humans, electrode location depended on which maneuvers induced the strongest change in tinnitus. In guinea pigs, C2 was used throughout. Auditory stimulation was personalized according to each subject's tinnitus spectrum. In guinea pigs, 8 kHz (most prevalent tinnitus frequency) was used. For the active treatment, the auditory stimulus preceded the somatosensory stimulus by 5 ms.

Statistics

Two-tailed t test, χ^2 contingency tests, Pearson's linear correlation, one-way and two-way ANOVAs were used to determine statistical differences ($\alpha = 0.05$). Post hoc analyses for ANOVA were performed using the Tukey-Kramer test where indicated. For statistical significance evaluation of guinea pig's tinnitus behavioral versus neurophysiological results, patients' loudness versus TFI, and loudness matching measures, GLMM or linear mixed-effect models were used.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Development of STDP in the fusiform cell circuit.

Fig. S2. The experimental timeline for the animal study.

Fig. S3. Noise exposure produces only temporary threshold and suprathreshold shifts.

Fig. S4. GPIAS behavioral assessment of tinnitus in guinea pigs.

Fig. S5. Human treatment groups had similar hearing thresholds.

Fig. S6. Reduction in tinnitus loudness in humans correlates with reductions in TFI.
Fig. S7. Tinnitus modulation maneuver checklist.
Table S1. Distribution of STDP learning rule type across unit/unit pairs in guinea pigs.
Table S2. Subject demographics.
Table S3. Correlations between changes in loudness and changes in TFI subscore.
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Auditory-somatosensory bimodal stimulation desynchronizes brain circuitry to reduce tinnitus in guinea pigs and humans

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The sound of silence

Tinnitus reduces quality of life for millions of tinnitus sufferers worldwide. Using a guinea pig model of tinnitus induced by noise trauma, Marks *et al.* delivered precisely timed bimodal auditory-somatosensory stimulation designed to induce long-term depression (LTD) in the cochlear nucleus of these animals. Twenty minutes per day of bimodal stimulation to induce LTD in the cochlear nucleus reduced physiological and behavioral evidence of tinnitus in the animals. The same bimodal protocol reduced tinnitus loudness in human subjects in a double-blinded, sham-controlled, crossover clinical study. Unimodal stimulation did not reduce tinnitus in the animals or the humans. Bimodal auditory-somatosensory stimulation that induces LTD may hold promise for suppressing chronic tinnitus in patients.