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Project Summary

Tinnitus is characterized by an uncomfortable sensation of ringing in the ears in the absence of external sound. Approximately 12 million individuals in the United States have been diagnosed with tinnitus of which ~1 million have severe tinnitus interfering with their daily activities [35]. The central conclusion of this study is that pairing vagus nerve stimulation (VNS) with tones can generate neural plasticity that could be used to reverse the pathological neural activity associated with tinnitus (Supplementary Fig. 1). This method provides a safe and effective method of directing long lasting and highly specific changes in the central nervous system.

Determination and Confirmation of Vagus Nerve Stimulation Parameters

The VNS cuff electrodes used in this study were custom built in our lab based on the design of Clark and colleagues[10]. The cuff of the bipolar stimulating electrode was made from 4 mm of Micro-Renethane tubing (1.8 mm inner diameter). The electrodes were made of Teflon coated multi-stranded platinum iridium wire (0.006”). The region in contact with the nerve was stripped of insulation for 8 mm and attached to the inner surface of the cuff. The electrodes were separated by 2 mm. The cuff edges were closed using silk thread. Strain relief was provided by suturing the electrode leads to nearby fascia. The wires were tunneled under the skin and attached to a custom made skull cap. A 4 channel connector was used to make electrical contact with each of the VNS and EEG electrodes.

For each rat, we recorded the cortical EEG during daily training sessions. Our previous work and other studies have demonstrated that stimulation of the nucleus basalis (NB) reduces the amplitude of the cortical EEG [8]. Our results show that VNS with 0.8 mA intensity, 100 μs pulse width, 30 Hz stimulation rate for 0.5 seconds also reduces EEG amplitude (Supplementary Fig.

3). Supplementary Fig. 3b shows the average EEG power spectrum before and after VNS. These stimulation parameters are similar to the ones used for previous memory consolidation studies [10], with the main difference being a shorter pulse width (100 us vs. 500 us) and a brief duration of the 0.5 second pulse train compared to a prolonged 30 second stimulation period. To determine the range of parameters that desynchronize the EEG, we varied the intensity, pulse width, rate and duration of stimulation (data not shown). Our results suggest that using half as much current with the same parameters was not sufficient to reduce EEG amplitude. Decreasing the rate to 1 or 10 Hz was also not sufficient to reduce EEG amplitude. Therefore, our selection of parameters was based on the ability of VNS to significantly reduce EEG amplitude.

Acute effects of brief VNS

We observed that 30 seconds of VNS at 30 Hz and 0.8 mA transiently decreased the blood oxygen saturation (SPO_2) in 3 rats. The standard 0.5 second VNS used in this study had no measurable effect on either heart rate or oxygen saturation (data not shown). These results are consistent with visual observations that brief VNS causes no noticeable behavioral response. For example, rats did not stop grooming or awaken when brief VNS was delivered.

Our observations that brief VNS 1) caused no behavioral response, 2) caused no change in heart rate, and 3) caused no change in blood oxygen saturation, suggest that VNS induced plasticity is not equivalent to pairing tones with a painful or irritating stimulus (like footshock or air puff).

Temporal Specificity of VNS-Tone Pairing Induced Plasticity

Earlier studies using 30 second trains of VNS suggested that VNS enhances learning during a window lasting minutes to hours after stimulation [36, 37]. Based on the brevity of the EEG effect generated by the VNS used in this study, we expected that 0.5 seconds of VNS would generate plasticity that was specific to the paired auditory stimulus. To evaluate the temporal specificity

of VNS-tone pairing plasticity, we randomly interleaved the presentation of 4 and 19 kHz tones at 50 dB SPL every 15-45 seconds in 5 rats, but only paired the high tone with VNS. After 20 days of VNS-tone pairing, more A1 sites were tuned to frequencies near the paired tone (Fig. 1b). Seventy percent more A1 neurons became tuned to the high tone (CF from 12 to 23 kHz, 20±2% in naïve rats vs. 35±3% after VNS-tone pairing, $p<0.005$). The number of sites tuned to the unpaired low tone decreased from 41±4 to 22±5% (CF from 2 to 8 kHz, $p<0.01$). This result parallels our earlier findings using NB stimulation [8] and indicates that VNS tone pairing can be used in a temporally precise manner to drive highly specific map plasticity.

Pairing Tones with Trigeminal Nerve Stimulation Does Not Generate Plasticity

The mechanism by which VNS promotes plasticity is likely to be complex. To test whether the plasticity inducing effects of VNS is specific to the vagus nerve, we paired stimulation of another cranial nerve with a 19 kHz tone in two rats. We selected trigeminal nerve stimulation (TNS) because a previous report indicated that TNS can cause EEG deysnchronization [38]. The TNS tone pairing was delivered on the same schedule as VNS-tone pairing. We randomly interleaved the presentation of 4 and 19 kHz tones at 50 dB SPL every 15-45 seconds, but only paired the 19 kHz tone with TNS. The cuff electrode and electrical parameters of the TNS were also identical to those used for our VNS-tone pairing procedure. Unlike VNS-tone pairing, TNS-tone pairing caused no change in the A1 map of tone frequency. After 20 days of pairing, there was no significant difference in the percent of A1 sites tuned to the paired frequency in TNS paired rats compared to naïve controls (21.3±2.1% in naïve rats vs. 18.8±5.1% after TNS-tone pairing, $p=0.58$). The average bandwidth and latency of A1 neurons were also unaltered by the TNS tone pairing ($p > 0.05$). The result suggests the possibility that VNS may be uniquely suited to direct cortical plasticity due to its connections with the autonomic and central nervous systems [39]. VNS

is known to trigger release of acetylcholine, norepinephrine, serotonin, nerve growth factor, brain derived neurotrophic factor, and other neuromodulators [36, 40]. The similarity of map plasticity generated by pairing tones with vagus nerve or NB stimulation suggests the possibility that release of acetylcholine might play a role in VNS-directed plasticity. However, the cellular mechanisms of VNS-directed plasticity remain to be determined.

Previous studies have shown that transection of the vagus nerve blocks the memory enhancing effects of peripherally administered epinephrine, cholecystokinin, substance P, bombesin, gastrin-releasing peptide and glucose [41, 42, 43, 44]. Our results are consistent with the hypothesis that the vagus nerve is a key conduit by which the autonomic nervous system informs the central nervous system of important stimuli [45].

Auditory Brainstem Responses

The auditory brainstem response (ABR) was used to confirm that the high frequency noise exposure used in this study generates a large temporary threshold shift and a smaller but long lasting elevation in the ABR threshold as previously reported [18, 46]. We quantified ABR's under barbiturate anesthesia before and after noise exposure. We played 4, 10, 16 and 32 kHz tones from 0 to 85 dB SPL in 10 dB steps. ABR's were collected from a subset of rats (10 total) immediately before noise exposure, immediately after noise exposure, and 11 weeks after noise exposure (prior to intracortical recordings). Immediately after noise exposure there was no detectable ABR for 10 kHz and above even when presented at 85 dB SPL. The threshold for the ABR to a 4 kHz tone was elevated from ~30 to ~60 dB SPL. Eleven weeks after noise exposure both treated and sham rats had significantly higher ABR thresholds for all frequencies tested (Supplementary Figs. 4 & 5). Elevated ABR threshold does not necessary imply elevated

thresholds in the cortex. Our behavioral and cortical responses provide no evidence of hearing loss (i.e. elevated thresholds) in noise exposed rats at any frequency.

There was no significant difference in ABR threshold in noise exposed rats that received VNS-Multiple Tone pairing compared to noise exposed sham controls ($p>0.05$). Additionally, there was no significant correlation between ABR threshold and gap impairment ($R^2=0.22$, $p=0.4$). This result suggests that our therapy is unlikely to significantly affect the early auditory stages and that the modest ABR threshold elevation in both groups of noise exposed rats reflects a neural correlate of noise trauma (e.g. stereocilia damage or auditory nerve degeneration) [31, 47, 48].

Neural Correlates of Gap Impairment in Untreated Noise Exposed Rats

The degree of map distortion and broadening of frequency tuning were significantly correlated with the degree of gap impairment in untreated noise exposed rats. The fraction of A1 neurons responding to a 50 dB SPL 8 kHz tones was negatively correlated with startle suppression ($R= -0.72$, $p<0.05$, $n=8$ rats). The frequency bandwidth of multiunit activity at 10 dB above threshold was also negatively correlated with startle suppression ($R= -0.71$, $p<0.05$). The degree of spontaneous activity and synchronization in each rat was not significantly correlated with impaired gap detection. However, this negative result should not be viewed as definitive because spontaneous activity and synchronization could be more sensitive to variability in anesthesia levels compared to frequency tuning, which is known to be insensitive to small changes in the depth of anesthesia.

In principle, impaired ability to detect a gap in narrowband sounds of a limited frequency range could result if noise exposure broadened temporal or spectral integration windows in a frequency specific manner. Our analysis of frequency bandwidth and response latency revealed no evidence of a frequency specific change in either temporal or spectral integration

(Supplementary Fig. 14). This analysis lends support to the use of the Turner model as a behavioral correlate of tinnitus. Perhaps more importantly, broadened temporal or spectral integration are insufficient to explain the other behavioral correlates of tinnitus that have been observed in noise exposed animals [20, 21].

VNS-tone pairing reverses gap impairment for at least 10 weeks

Three weeks after the end of therapy, neural recordings were obtained from 13 of the 18 noise exposed rats. We continued to follow 4 rats (2 therapy and 2 sham rats) for an additional two months. Consistent with previous reports, the untreated rats continued to exhibit impaired gap detection 3.5 months after the noise exposure [31, 38]. Signs of impairment of gap detection did not return in either of the rats that received the VNS-multiple tone therapy. These results confirm that the VNS-multiple tone therapy causes a long lasting reversal of noise induced perceptual hearing abnormalities in rats.

Comparison of Awake EEG in Treated and Untreated Noise Exposed Rats

In an effort to document changes in cortical synchronization in unanesthetized rats, we looked for systematic effects of VNS-multiple tone pairing on EEG in noise exposed rats (7 VNS paired and 4 VNS alone rats). Although the data is variable because the animal's behavioral state was uncontrolled and the EEG electrode was located at the vertex and not over auditory cortex, we nevertheless observed statistically more gamma power relative to alpha in treated rats compared to untreated rats during the last week of therapy ($p<0.05$), but no difference between the EEG of therapy and VNS alone rats during the first week. These results are consistent with animal studies that tinnitus can be associated with abnormal cortical synchronization [49] and clinical reports of reduced alpha and increased gamma in tinnitus patients [50]. As in our anesthetized

recordings, the degree of EEG change was not significantly correlated with gap impairment in individual rats.

Neural correlates of hearing loss and hyperacusis are not correlated with gap impairment

Hearing loss, hyperacusis, and tinnitus often result from noise exposure [51] and any of the three could contribute to the gap impairments observed in this study. The noise exposure used in this study caused a dramatic temporary increase in the ABR threshold that largely recovers (Supplementary Fig. 5). Our high density microelectrode recordings in A1 allow for a more complete survey of minimum tone thresholds across the rat hearing range. These studies demonstrate that the noise exposure used in this study caused no lasting increase in minimum tone thresholds. In fact, noise exposure decreased the minimum cortical threshold observed for each rat across the hearing range (Supplementary Fig. 15), which is consistent with earlier reports. There was no significant difference between minimum thresholds in treated and untreated rats and no correlation between the threshold and gap impairment. There was also no correlation between gap impairment and ABR thresholds recorded prior to mapping (R^2=0.22, p=0.4). This finding suggests that the gap impairment is not a result of hearing loss in these rats. To determine whether hyperacusis contributes to noise induced impairment, we compared the rate level functions of rats in each group and measured the correlation between response strength and gap impairment. Compared with naïve controls, the rate level function was significantly elevated in noise exposed rats that received sham therapy (Supplementary Fig. 16). The scale of response enhancement caused by noise exposure is similar to MEG and fMRI studies in patients suffering from inner-ear hearing impairment with loudness recruitment [52, 53]. The VNS-multiple tone pairing therapy significantly reduced the cortical response to tones. These results suggested the possibility that increased cortical responses due to noise exposure could contribute to the gap

impairment. There was however no significant correlation between the degree of gap impairment and the size of the cortical evoked response. To further evaluate the possibility that our main findings (Figs. 2-4) could be explained by hyperacusis, we quantified the intensity of the startle response (i.e. downward force) in each group of rats. The response to the noise burst (when there was no gap to serve as a warning cue) was not significantly different between treated and untreated rats (Supplementary Fig. 17). Thus, it is reasonable to conclude that the gap impairments observed in this study are primarily related to tinnitus and unlikely to be primarily related to hearing loss or hyperacusis.

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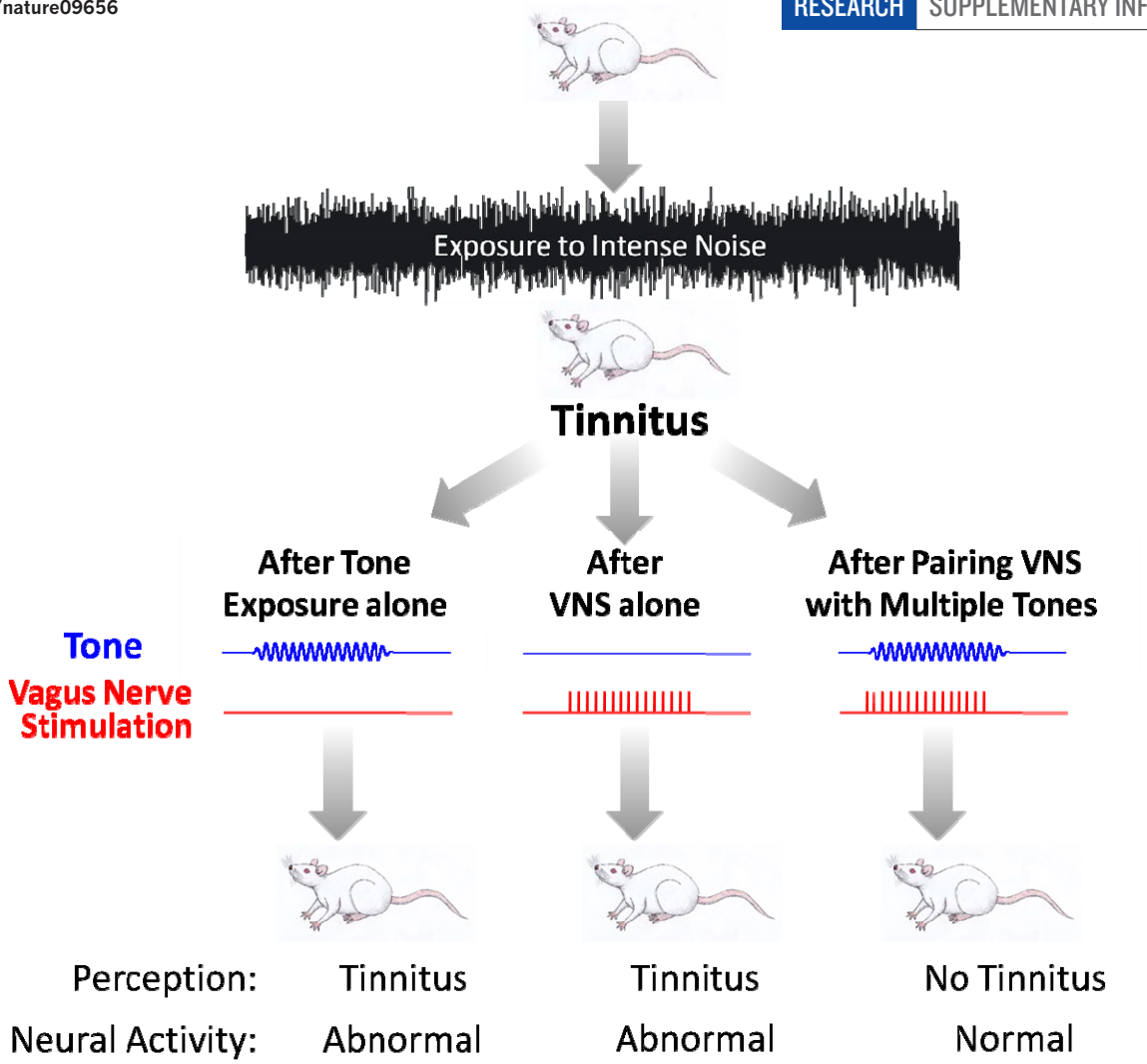
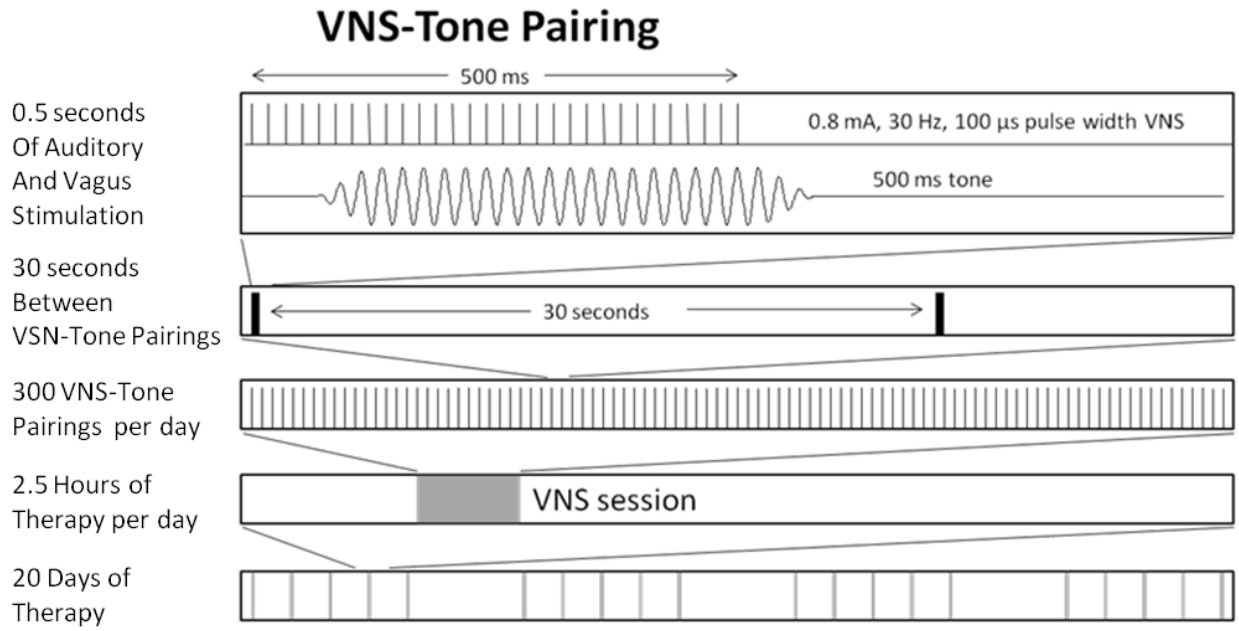
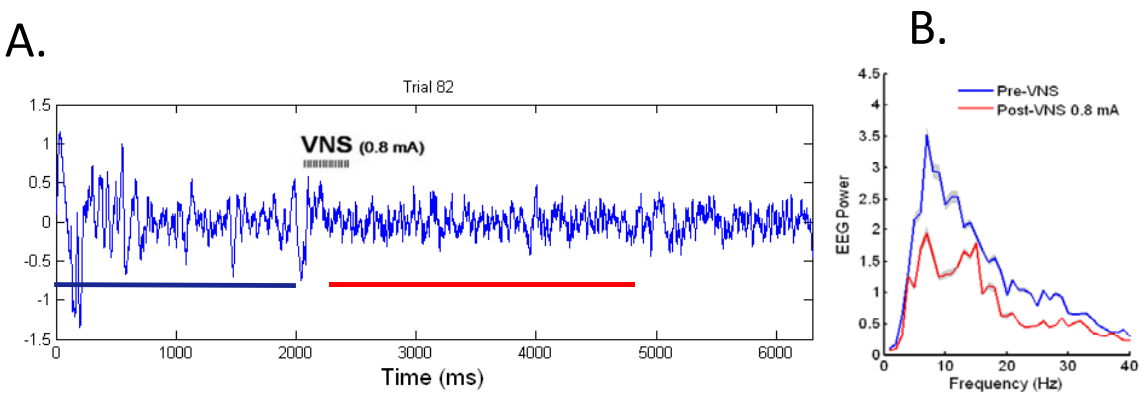


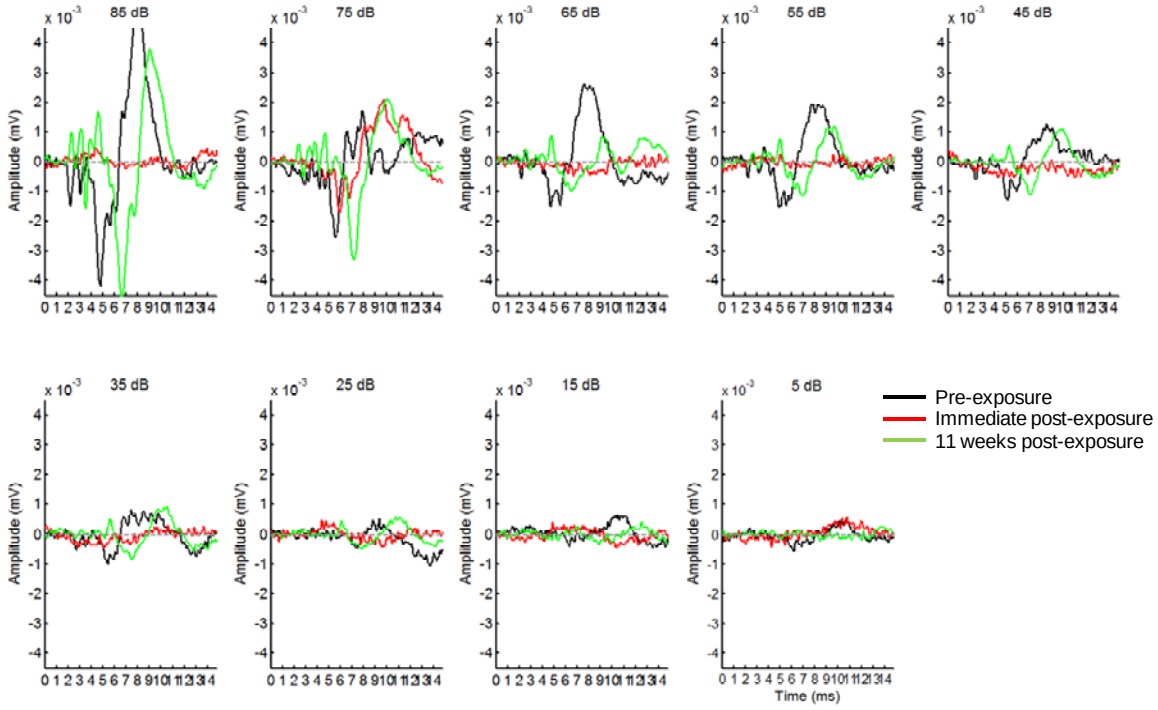
Supplementary Figure 1. Pairing vagus nerve stimulation (VNS) with tones reverses neural and behavioral correlates of tinnitus. In this study, tinnitus is defined as a frequency-specific impairment in the ability to detect a quiet gap in a continuous tone (apparently because ongoing tinnitus fills the gap). Abnormal neural activity in noise exposed rats included reduced frequency selectivity, distorted frequency map organization, increased excitability, and greater neural synchronization. After exposure to intense noise, rats were treated with 1) tone exposure, 2) VNS, or 3) VNS paired with tones. Only the rats that received VNS paired with tones showed reversal of the abnormal neural activity and tinnitus.



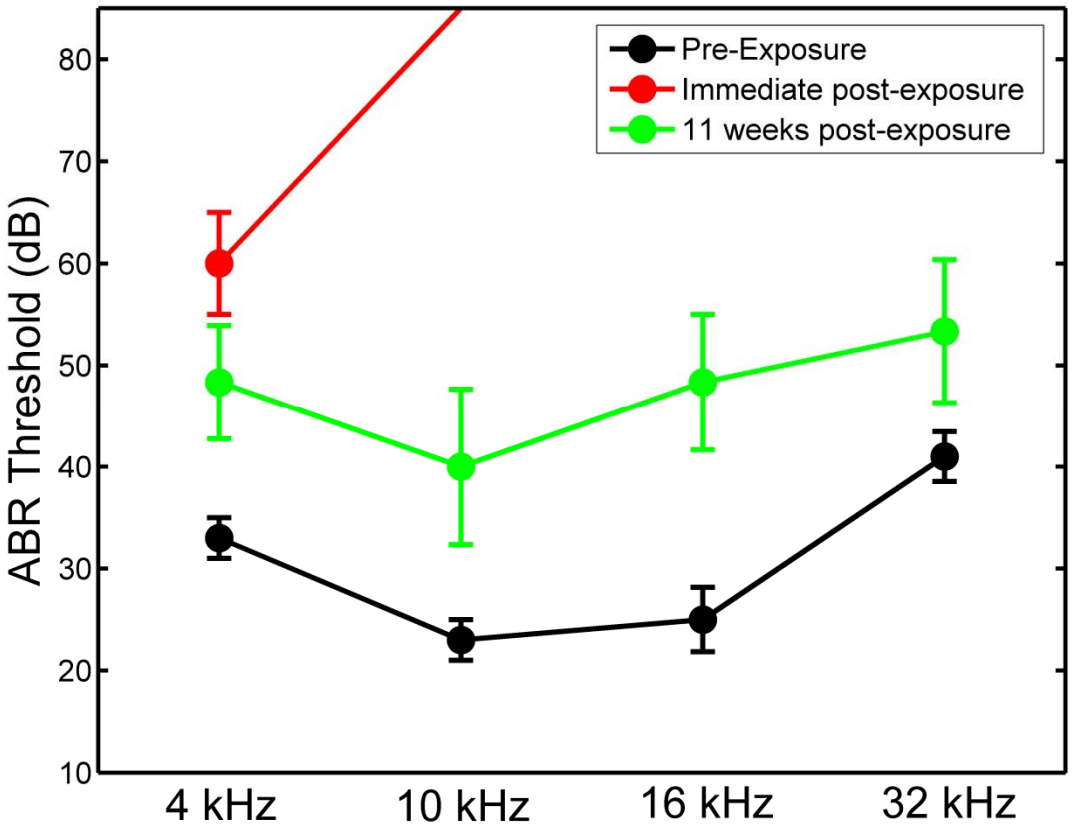
Supplementary Figure 2**.** Schematic of the timing of VNS-tone pairing. VNS parameters were identical for every rat in this study. A 0.5 second 30 Hz train of 0.8 mA 100 µs pulse width was delivered to the left vagus nerve via a platinum-iridium bipolar cuff electrode. When VNS was paired with tones, the tones were 0.5 seconds long and began 150 ms after VNS onset. Rats received VNS on average every 30 seconds, 300 times during each 2.5 hour session. VNS was only delivered on weekdays. A total of 50 minutes of 30 Hz vagus nerve stimulation was delivered during the month of therapy, which is 1% of current therapies for epilepsy and depression. Since the pulse width we used was 5 fives shorter than current therapies, the total charge delivered was 0.2% of current therapies.



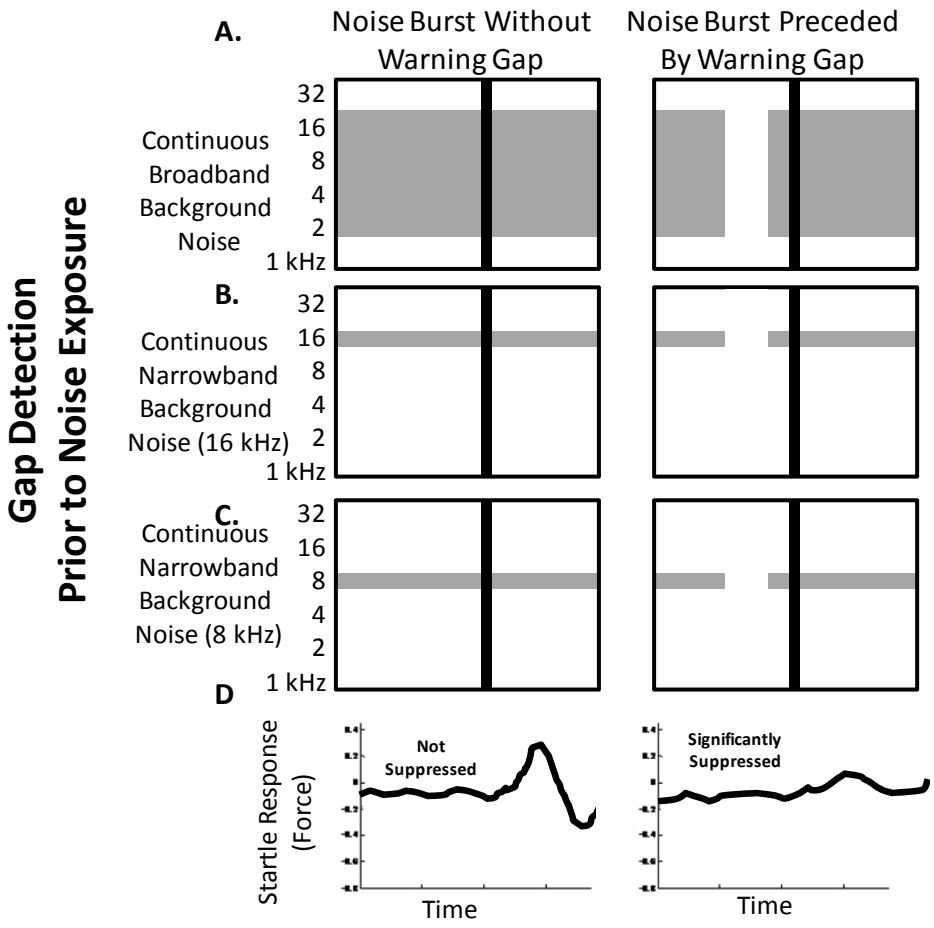
Supplementary Figure 3.VNS induced EEG deysnchronization in a sleeping rat (A). Average power spectrum of the same session (B) shows that VNS decreased the EEG power at all frequencies for several seconds after VNS (thick red line), compared to baseline (blue line). Grey shading indicates standard error of the mean.



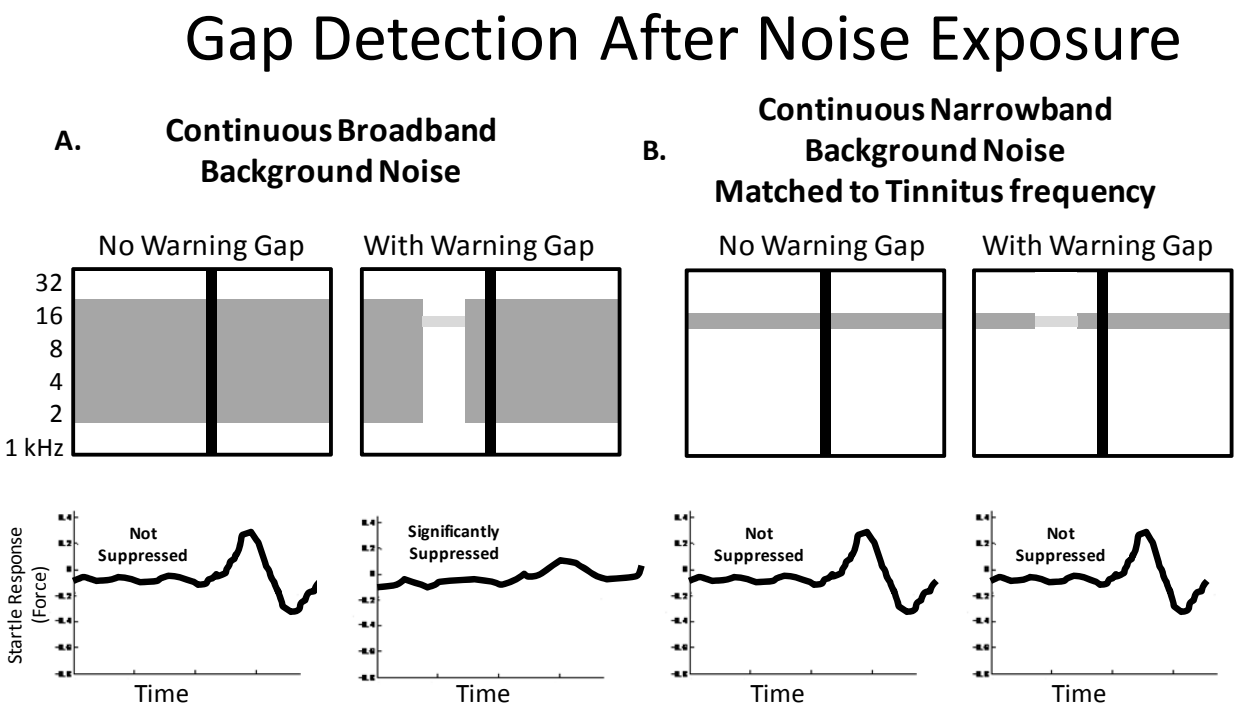
Supplementary Figure 4. Tone evoked auditory evoked potentials in response to a 10 kHz pure tone in a representative rat before (black) and after noise exposure (red and green). In this example from a VNS tone paired rat, ABR's were completely suppressed immediately after noise exposure (red) but returned to pre-exposure levels when tested at 11 weeks after exposure (green). For each rat, tone pips (2.5 ms rise/fall; 10 ms long) were delivered at 4, 10, 16 and 32 kHz. For each frequency, the intensity ranged from 85 dB SPL to 5 dB SPL in 10 dB steps. Each frequency intensity combination (4 tones X 9 intensities) was randomly interleaved and repeated 1500 times. Threshold was defined as the lowest 10 dB step at which an ABR could be recognized. In this example, the pre-exposure threshold (black) was 15 dB SPL and 11 week post-exposure threshold was 35 dB SPL.



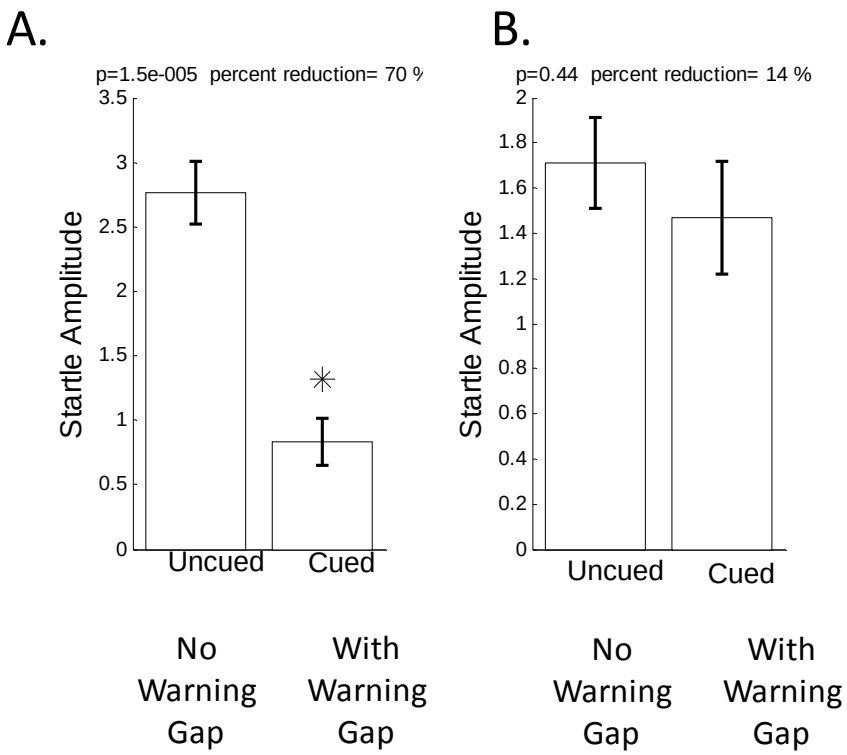
Supplementary Figure 5. Average ABR thresholds before, immediately after and 11 weeks after noise exposure at 4, 10, 16 and 32 kHz. No ABR was evoked by 10, 16, and 32 kHz immediately after noise exposure. Error bars represent standard error of the mean.



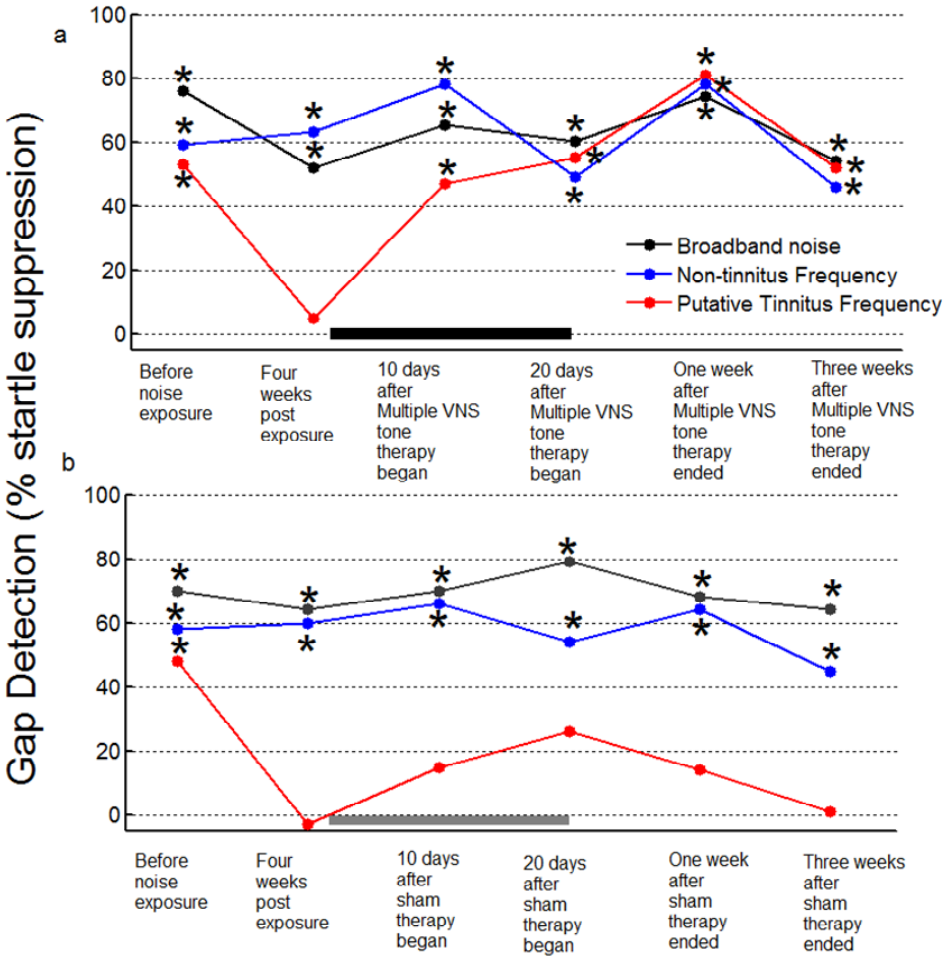
Supplementary Figure 6. Schematic of the startle gap detection task. Each panel (A, B and C) represents a broadband sound, a narrowband sound centered at 16 kHz and a narrowband sound centered at 8 kHz, respectively. The left panels depict a 20 ms 100 dB SPL broadband startle burst (vertical black line) in continuous background sound while in the right panels, the startle burst is preceded by a 50 ms gap. In trials without the gap, rats are not warned of a forthcoming startle burst resulting in a strong startle response (D, left panel). In gap trials, the preceding gap serves as an effective warning, resulting in a weak startle response (D, right panel).



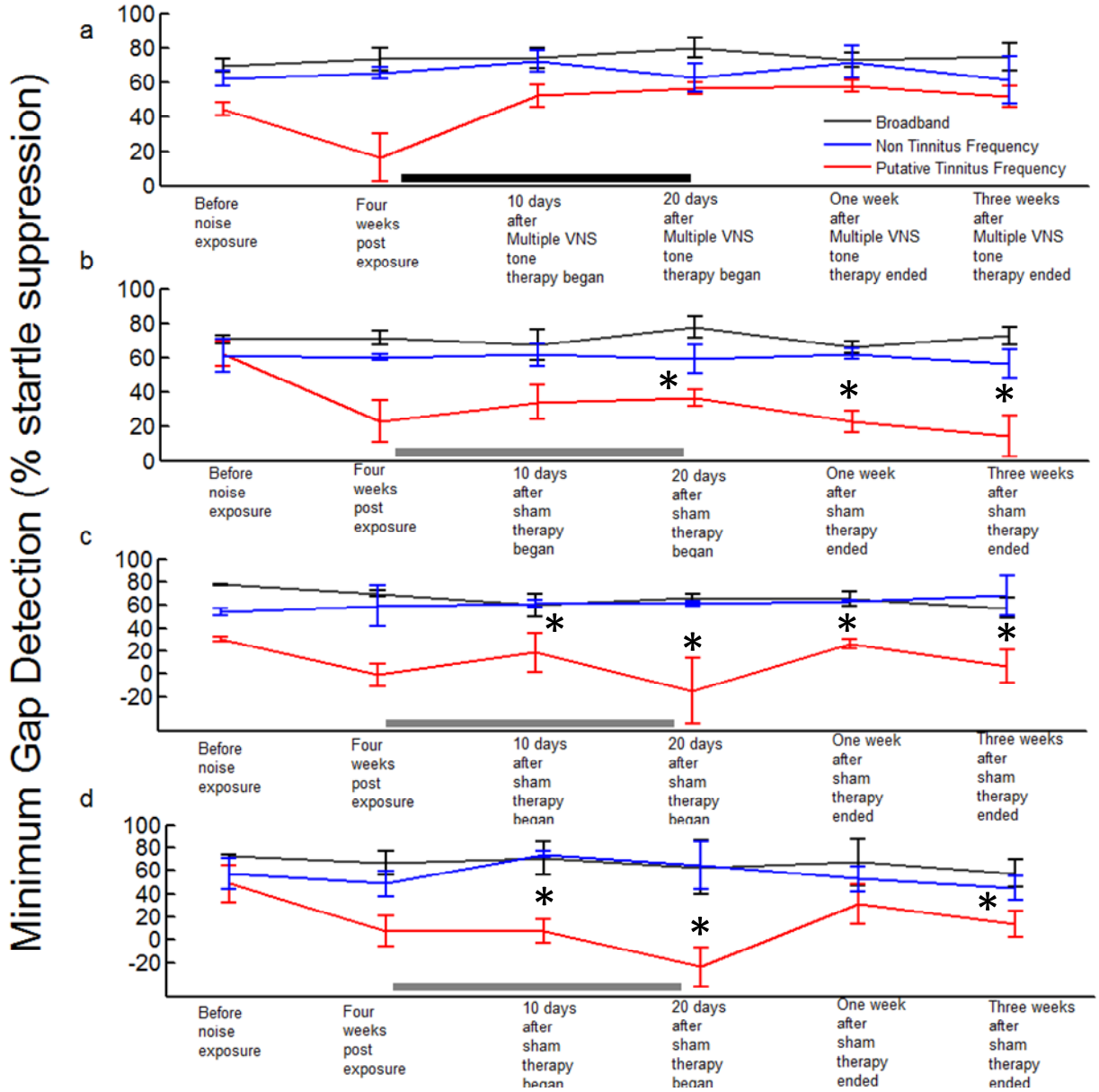
Supplementary Figure 7. Gap detection after noise exposure. (A) After noise exposure, rats are able to detect a gap in broadband sound because the putative tinnitus frequency is not matched to the background broadband sound. (B) After noise exposure, gaps in a specific narrowband sound (usually 8 or 10 kHz) do not serve as an effective warning, apparently because the ongoing tinnitus percept prevents the rats from detecting the silent gap.



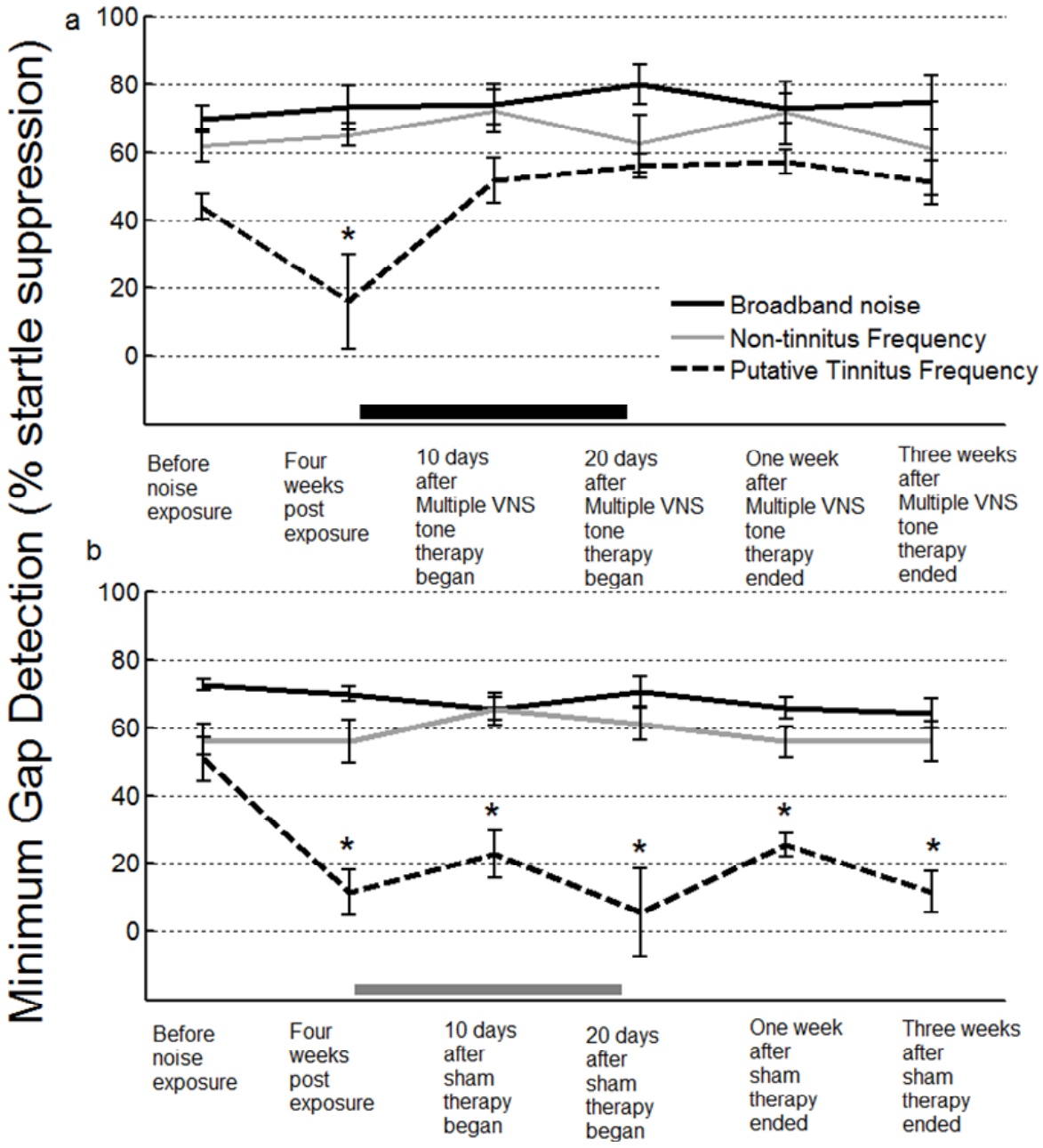
Supplementary Figure 8. Representative example from two startle gap detection sessions in a noise exposed rat. (A) The rat was able to detect a gap in broadband background sound as shown by the significant decrease in startle amplitude ($p < 0.000001$). The startle response was 70% less when the warning gap was present. (B) After noise trauma, the rat was not able to detect a gap in an 8 kHz narrowband sound ($p > 0.05$). Trials with and without gaps were randomly interleaved. Error bars indicate standard error of the mean.



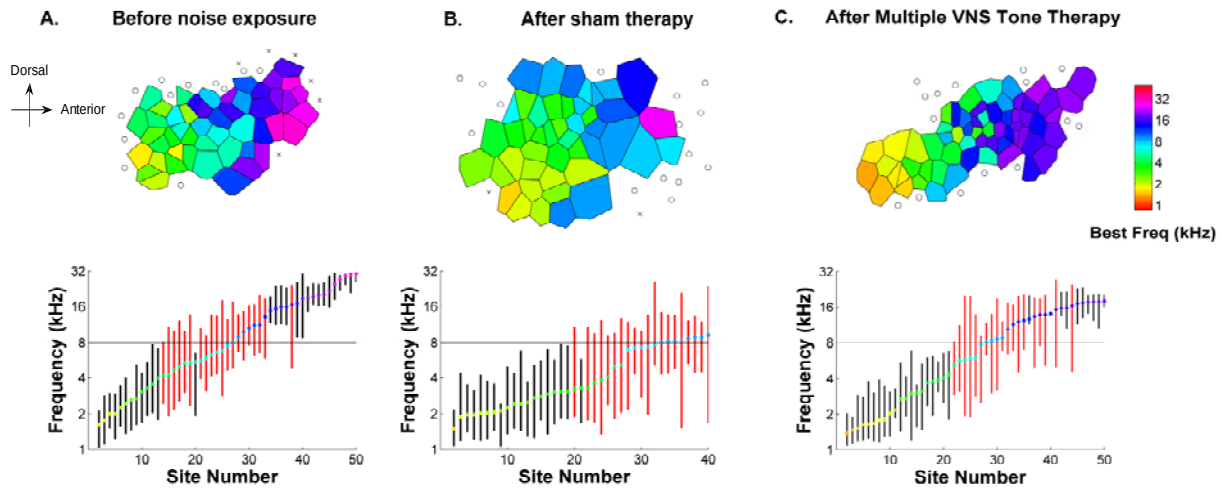
Supplementary Figure 9. Representative example of gap detection for a noise exposed VNS-multiple tone paired rat (a) and a noise exposed sham rat (b). Asterisks indicate that the rat was able to detect a gap in each of the sounds on the cued trials (significant decrease in startle amplitude, Supplementary Fig. 8a). Four weeks after noise exposure, rats in both groups were unable to detect a gap at the putative tinnitus frequency (Supplementary Fig. 8b). After VNS tone pairing, VNS tone paired rats were able to detect a gap at the putative tinnitus frequency and continued to do so many weeks after the end of therapy. Sham control rats showed no significant improvement in gap detection at the putative tinnitus frequency (Fig. 2).


Minimum Gap Detection (% startle suppression)
a
b
c
d
Broadband
Non Tinnitus Frequency
Putative Tinnitus Frequency
Before noise exposure
Four weeks post exposure
10 days after Multiple VNS tone therapy began
20 days after Multiple VNS tone therapy began
One week after Multiple VNS tone therapy ended
Three weeks after Multiple VNS tone therapy ended
10 days after sham therapy began
20 days after sham therapy began
One week after sham therapy ended
Three weeks after sham therapy ended

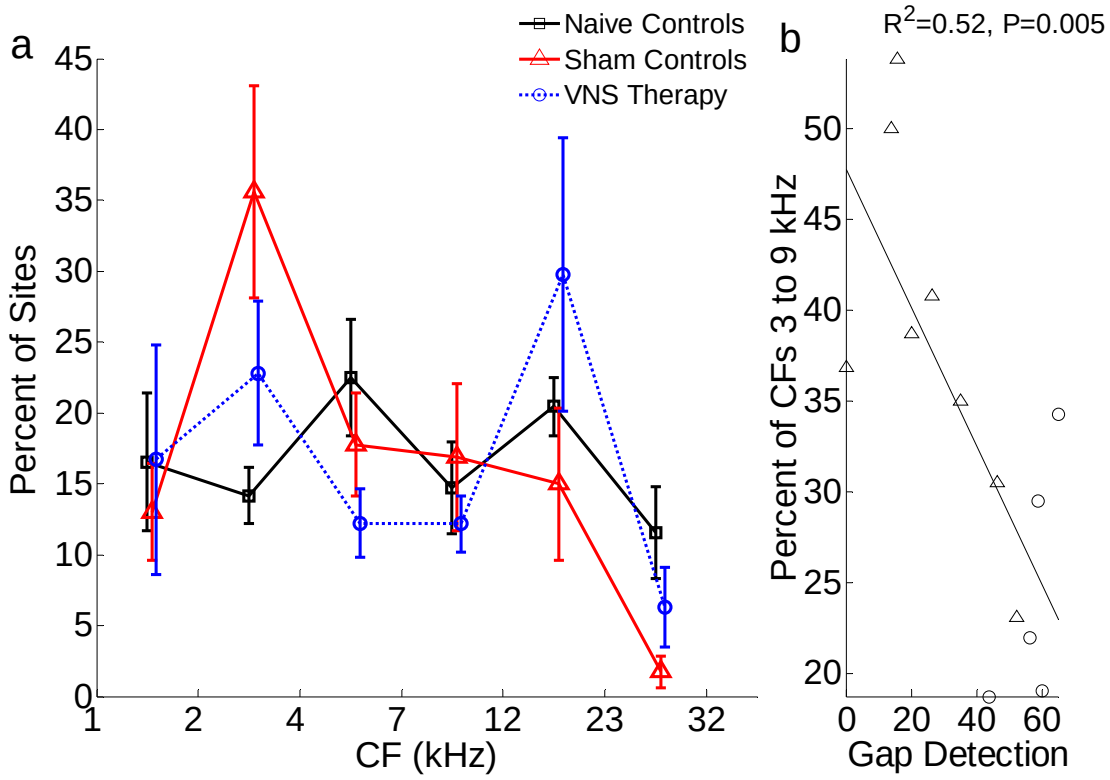
Supplementary Figure 10. Gap detection task in VNS tone paired rats (a) compared to three groups of control rats (b-d). Four weeks after noise exposure, rats are unable to detect a gap in a narrowband tone presumably because of ongoing tinnitus. (a) In the VNS-tone paired group, rats were able to detect a gap in the narrowband frequency (red) after 10 days of VNS tone pairing and the improvement persisted until the acute physiology experiment (11 weeks after noise exposure). (b) In the tones alone group, rats were exposed to the same sounds but no VNS was delivered. (c) In the no therapy groups (n=3), rats did not receive VNS or heard tones. (d). In the VNS alone group, rats received VNS without any tone presentations. In the 2 control groups with either tones or VNS, rats received 300 tones alone or VNS alone trials per day and were exposed or stimulated for 18 days, similar to the VNS tone paired group. Each of the control groups continued to be impaired (* $p < 0.05$) until the acute physiology experiment. For the 16 kHz narrowband sound and broadband sound (blue and black lines respectively), rats in all groups were able to detect a gap in each of the sounds on the cued trials. This indicates that rats were not impaired at these frequencies. Asterisks represent significant differences in gap detection at the putative tinnitus frequency between VNS therapy and sham therapy rats ($p < 0.05$). Error bars represent standard error of the mean.


a
b
Minimum Gap Detection (% startle suppression)
100
80
60
40
20
0
*
Broadband noise
Non-tinnitus Frequency
Putative Tinnitus Frequency
Before noise exposure
Four weeks post exposure
10 days after Multiple VNS tone therapy began
20 days after Multiple VNS tone therapy began
One week after Multiple VNS tone therapy ended
Three weeks after Multiple VNS tone therapy ended
Before noise exposure
Four weeks post exposure
10 days after sham therapy began
20 days after sham therapy began
One week after sham therapy ended
Three weeks after sham therapy ended

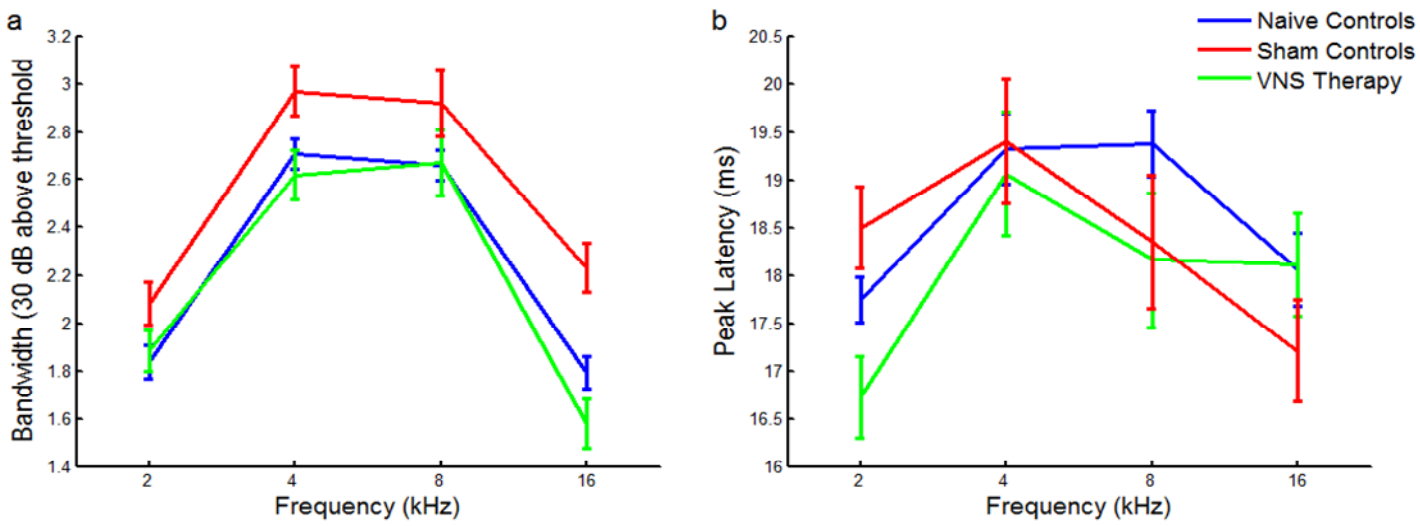
Supplementary Figure 11. Behavioral correlate of tinnitus in treated and untreated rats. This figure is identical to Figure 2, except that this analysis does not restrict analysis to a single frequencies based on the minimum startle suppression on a single day (i.e. four weeks post exposure). In this figure, gap detection was quantified based on the worst performance for each rat (i.e. lowest % startle suppression) for any of the three narrowband sounds (8, 10, and 16 kHz) on any given day. This analysis relaxes the assumption that the tinnitus frequency must be a single tone frequency and must not change over time. This analysis (like the analysis in Figure 2) reveals that VNS-tone pairing reverses the gap detection impairment the follows noise trauma. As expected the gap detection impairment is greater when the worst performance for each day was used. This analysis indicates that rats treated with VNS-tone pairing do not show evidence of tinnitus at any of the frequencies tested. Four of the seven VNS-tone pairing rats were also tested for gap detection impairments after therapy using one or two additional frequencies (2, 4, 20, or 24 kHz) of narrowband noise (data not shown). We observed significant startle suppression (i.e. normal gap detection performance) for each of these rats at every frequency tested. These results suggest that it is unlikely that VNS-tone pairing shifts rather than reverses gap detection impairments. Asterisks represent significant differences in gap detection at the putative tinnitus frequency compared to the non-tinnitus frequency or broadband noise ($p < 0.05$). Error bars represent standard error of the mean.



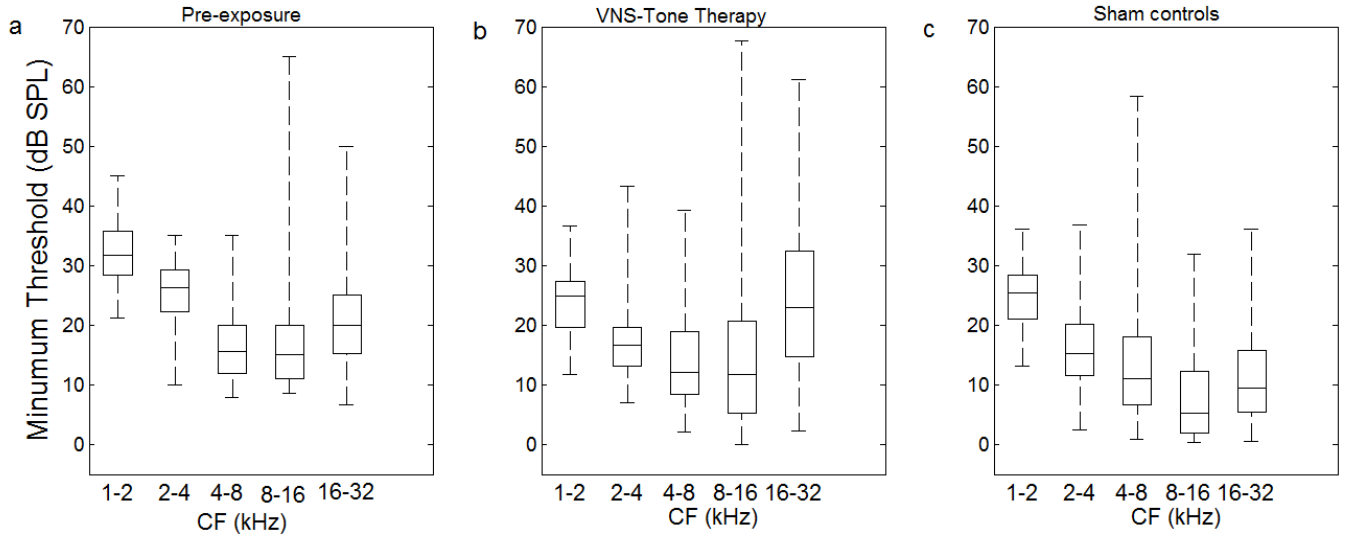
Supplementary Figure 12. A1 maps of tone frequency from a representative experimentally naïve rat (A), a rat that had been exposed to one hour of intense high frequency noise 11 weeks earlier (B), a rat that had been exposed to one hour of intense high frequency noise 11 weeks earlier but received VNS-multiple tone therapy from the fourth to the eighth week post noise exposure (C). Each polygon represents a single microelectrode penetration. The color of each polygon indicates the characteristic frequency (CF) of each recording site. Penetrations that were either not responsive to tones (O) or did not meet the criteria of A1 responses (X) were used to determine the A1 borders. A1 was defined on the basis of its short latency (8- to 20-ms) responses and continuous tonotopy (BF increases from posterior to anterior). Responsive sites that exhibited clearly discontinuous BFs and either long latency responses, an unusually high threshold, or very broad tuning were considered to be non-A1 sites. The lines below each map indicate the frequency bandwidth of every multiunit recording site. Each site is ordered from low to high frequency based on the CF of the site. The CF of each site is also shown using a circle colored as in the color bar in panel C. Note the broadening of receptive fields in the sham rat (B) and the increased selectivity in the treated rat (C).

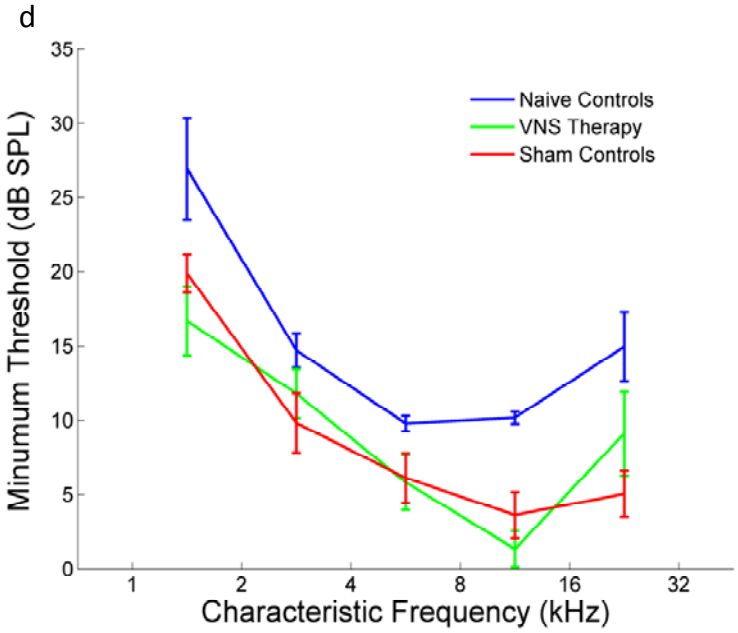


Supplementary Figure 13. a) After noise exposure, twice as many A1 recording sites were tuned to frequencies between 2 and 4 kHz compared to naïve controls (35±7% vs. 14±2%, $p<0.05$) b) The fraction of A1 neurons tuned to mid frequencies (3-9 kHz) is significantly negatively correlated with the ability to detect a gap in a mid frequency tone. Triangles and circles represent rats from the sham and VNS-tone pairing group, respectively. Error bars represent standard error of the mean.

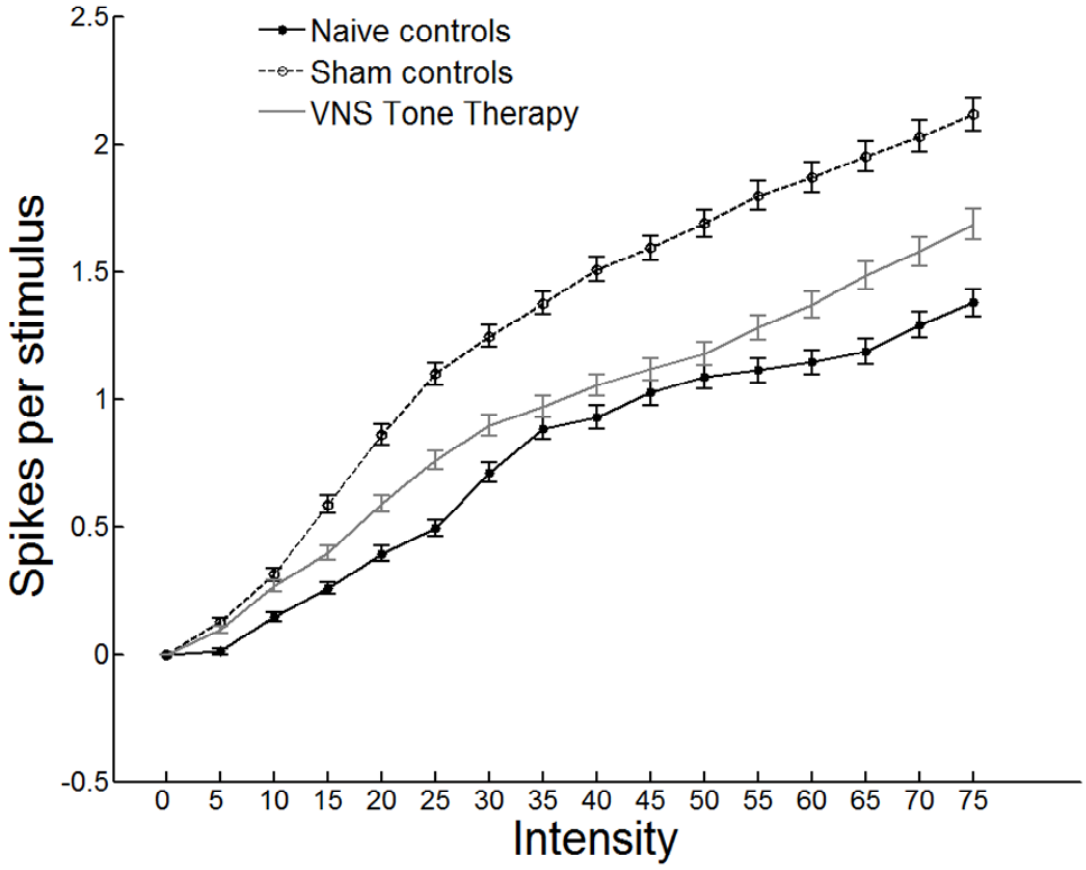


Supplementary Figure 14. Spectral and temporal precision of A1 sites in all three groups. a) Noise exposure significantly decreases spectral selectivity of A1 sites across the frequency map ($p < 0.05$). VNS-multiple tone pairing reverses the decrease in selectivity. b) Noise exposure has little effect on peak latency (or onset latency [not shown]). Error bars represent standard error of the mean.

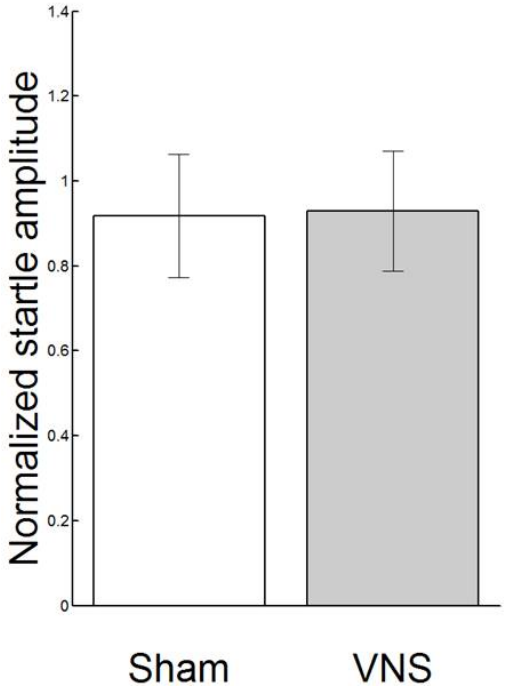

a
Pre-exposure
b
VNS-Tone Therapy
c
Sham controls
Minumum Threshold (dB SPL)
0
10
20
30
40
50
60
70
1-2
2-4
4-8
8-16
16-32
CF (kHz)


d
Naive Controls
VNS Therapy
Sham Controls
Minumum Threshold (dB SPL)
0
5
10
15
20
25
30
35
1
2
4
8
16
32
Characteristic Frequency (kHz)

Supplementary Figure 15. Tone thresholds of A1 sites. a-c) Box plots of tone thresholds from naïve control rats, VNS therapy rats, and sham control rats. The line across each box shows the median of all A1 sites with a CF in the defined ranges. The edges are the 25th and 75th percentiles. The whiskers extend to the most extreme data points. d) Minimum tone thresholds for naïve, sham, and treated rats. Minimum threshold was quantified for each rat within each octave band (i.e. 1-2 kHz, 2-4 kHz, etc.) based on the threshold of the most sensitive A1 site observed. Error bars represent standard error of the mean.



Supplementary Figure 16. Rate level function for naïve (n= 424 sites), sham (n= 321 sites) and therapy (n= 220 sites) rats. The y-axis plots the number of action potentials evoked by pure tones of 0-75 dB SPL (with spontaneous activity subtracted). Responses to tones of frequencies from 1 to 32 kHz were averaged together. Error bars represent standard error of the mean.



Supplementary Figure 17. Treated and sham rats exhibited indistinguishable startle response to a 100 db SPL 20 ms noise burst. To control for differences in the size of different rats, the startle response (downward force) was divided by the startle response of each rat prior to noise exposure. Consistent with earlier results there was a small but insignificant reduction in the startle response of both groups due to repeated startle testing. Error bars represent standard error of the mean.