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RESEARCH ARTICLE | *Sensory Processing*

Pairing vagus nerve stimulation with tones drives plasticity across the auditory pathway

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Borland MS, Vrana WA, Moreno NA, Fogarty EA, Buell EP, Vanneste S, Kilgard MP, Engineer CT. Pairing vagus nerve stimulation with tones drives plasticity across the auditory pathway. *J Neurophysiol* 122: 659–671, 2019. First published June 19, 2019; doi:10.1152/jn.00832.2018.—Previous studies have demonstrated that pairing vagus nerve stimulation (VNS) with sounds can enhance the primary auditory cortex (A1) response to the paired sound. The neural response to sounds following VNS-sound pairing in other subcortical and cortical auditory fields has not been documented. We predicted that VNS-tone pairing would increase neural responses to the paired tone frequency across the auditory pathway. In this study, we paired VNS with the presentation of a 9-kHz tone 300 times a day for 20 days. We recorded neural responses to tones from 2,950 sites in the inferior colliculus (IC), A1, anterior auditory field (AAF), and posterior auditory field (PAF) 24 h after the last pairing session in anesthetized rats. We found that VNS-tone pairing increased the percentage of IC, A1, AAF, and PAF that responds to the paired tone frequency. Across all tested auditory fields, the response strength to tones was strengthened in VNS-tone paired rats compared with control rats. VNS-tone pairing reduced spontaneous activity, frequency selectivity, and response threshold across the auditory pathway. This is the first study to document both cortical and subcortical plasticity following VNS-sound pairing. Our findings suggest that VNS paired with sound presentation is an effective method to enhance auditory processing.

NEW & NOTEWORTHY Previous studies have reported primary auditory cortex plasticity following vagus nerve stimulation (VNS) paired with a sound. This study extends previous findings by documenting that fields across the auditory pathway are altered by VNS-tone pairing. VNS-tone pairing increases the percentage of each field that responds to the paired tone frequency. This is the first study to document both cortical and subcortical plasticity following VNS-sound pairing.

auditory processing; central auditory pathway; plasticity; vagal nerve stimulation

INTRODUCTION

Multiple auditory fields process sounds, and there are differences in the neural response pattern to sounds across fields (Carrasco and Lomber 2011; Perez et al. 2013; Polley et al. 2007; Walker et al. 2011). Neural responses closely resemble the acoustics of the sound early in the auditory pathway, while at higher levels in the auditory pathway, responses better reflect the perceptual characteristics of the sound (Perez et al. 2013; Ranasinghe et al. 2013; Steadman and Sumner 2018). Extensive auditory training can alter the neural response to the trained sound throughout the auditory pathway. For example, training on a tone-frequency discrimination task expands the percentage of primary auditory cortex that responds to the trained tone (Polley et al. 2006; Recanzone et al. 1993; Reed et al. 2011). Interestingly, training-induced plasticity differs between the different auditory fields (Atiani et al. 2014; Engineer et al. 2015b; Takahashi et al. 2011). Previous studies have observed either a stronger response or a weaker response to trained songs in songbirds, depending on the auditory field (Gentner and Margoliash 2003; Thompson and Gentner 2010).

Pairing the presentation of a tone with neuromodulator release results in an expansion of the auditory region that responds to the paired tone (Bakin and Weinberger 1996; Borland et al. 2016; Edeline et al. 2011; Engineer et al. 2011; Kilgard and Merzenich 1998; Martins and Froemke 2015). The nucleus basalis (NB) has cholinergic projections to the auditory cortex (Mesulam et al. 1983), and numerous studies have documented primary auditory cortex (A1) plasticity following NB stimulation paired with the presentation of a tone (Bakin and Weinberger 1996; Kilgard and Merzenich 1998; Reed et al. 2011). NB-tone pairing also results in inferior colliculus (IC) plasticity that is specific to the paired tone frequency, although the amount of collicular plasticity is smaller and recovers faster than A1 plasticity (Ma and Suga 2003; Zhang et al. 2005). Interestingly, NB stimulation paired with a tone yields plasticity in posterior auditory field (PAF) that is distinct from the plasticity observed in the A1 (Puckett et al. 2007). In A1, there is a well-established expansion of the region of A1 responding to the paired tone frequency. However in PAF, there is a significant contraction of the low-frequency region of PAF following NB stimulation paired with a high-frequency tone. This low-frequency contraction is accompanied by increased frequency selectivity (decreased bandwidths) in the high-frequency region of PAF, which is not observed in A1 following NB-tone pairing. NB-tone paring results in distinct forms and time courses of plasticity through the auditory pathway.

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The locus coeruleus (LC) has noradrenergic projections to auditory subcortical and cortical structures (Klepper and Herbert 1991; Sara 2009). Previous studies have documented auditory thalamus and cortical plasticity following LC stimulation paired with the presentation of a tone (Edeline et al. 2011; Martins and Froemke 2015). Unlike NB-tone pairing, LC-tone pairing results in an initial increase in the A1 response to all sounds before refining the increased response specific to the paired frequency (Martins and Froemke 2015). Additionally, LC-tone pairing yields plasticity in the thalamus that is distinct from the plasticity observed in the A1 (Edeline et al. 2011). Compared with NB-tone pairing, LC-tone pairing results in longer-lasting changes in the A1 response (Martins and Froemke 2015), although changes in the thalamus are shorter lasting (Edeline et al. 2011).

While both NB-tone pairing and LC-tone pairing result in plasticity, there are distinct differences in both the specificity and timing of the plasticity to the paired sound. Stimulation of the vagus nerve results in activation of both the NB and the LC through the nucleus tractus solitarius (Dorr and Debonnel 2006; Hulsey et al. 2016, 2017). Vagus nerve stimulation (VNS)-directed plasticity requires the release of modulatory neurotransmitters, including acetylcholine, norepinephrine, and serotonin (Hulsey et al. 2016, 2019). While it is well-documented that VNS-tone pairing significantly enhances A1 responses to the paired tone (Borland et al. 2016, 2018; Engineer et al. 2011; Shetake et al. 2012), it is unknown whether VNS-tone pairing alters other cortical and subcortical auditory fields.

Similar to the previous NB- and LC-tone-pairing studies, we hypothesize that VNS-tone pairing will alter neural responses across multiple fields in the auditory pathway. Previous NB- and LC-tone-pairing studies have documented subcortical plasticity that is smaller and shorter lasting compared with cortical plasticity (Edeline et al. 2011; Froemke et al. 2007, 2013; Ma and Suga 2003; Zhang et al. 2005; Zhang and Yan 2008). Based on this previous literature, it is likely that our recordings in IC that occur more than 24 h after the last VNS-tone-pairing session will not reveal IC plasticity. However, multiple studies have documented long-lasting A1 plasticity following NB-, LC-, and VNS-tone pairing that is specific to the paired tone frequency, so we expect plasticity in each of the cortical fields following VNS-tone pairing.

Additionally, it is unknown what receptive field changes, if any, will be observed in each field following VNS-tone pairing. Previous studies examining A1 responses following VNS-tone pairing have found no alterations in response threshold, spontaneous firing rate, response bandwidth, or response latency (Borland et al. 2018; Buell et al. 2018). Previous studies examining A1 responses following NB- or LC- tone pairing, however, have documented significant alterations in response bandwidth and response latency (Kilgard et al. 2001; Martins and Froemke 2015). In addition, multiple studies have documented alterations in response threshold following NB-tone pairing in both the IC and the ventral division of the medial geniculate body of the thalamus (Zhang et al. 2005; Zhang and Yan 2008). It is likely that any receptive field changes following VNS-tone pairing will be field specific.

MATERIALS AND METHODS

Neural responses were recorded from 15 experimentally naïve control rats and 18 rats that experienced VNS paired with a 9-kHz tone for 20 days. Responses were recorded from the IC, A1, anterior auditory field (AAF), and PAF in adult female Sprague-Dawley rats between 4 to 6 mo of age. The University of Texas at Dallas Institutional Animal Care and Use Committee approved all surgical protocols and recording procedures.

Vagus nerve surgery. Rats were initially anesthetized with ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg) and received supplemental doses to maintain areflexia as needed. Ringer's lactate solution was given subcutaneously throughout the surgery and recovery to prevent dehydration. An antibiotic (cefotaxime sodium, 10 mg) was given subcutaneously before and after surgery to prevent infection. As in earlier studies, rats were implanted with a skull mounted connector attached to four cranial bone screws with acrylic and implanted with a cuff electrode around the left vagus nerve (Borland et al. 2016; Engineer et al. 2011, 2015a; Porter et al. 2012; Shetake et al. 2012). A local anesthetic (lidocaine, 0.5 ml) was subcutaneously injected at the neck incision site. The bipolar cuff electrode comprised of two Teflon-coated multistranded platinum iridium wires attached to Micro Renethane tubing (4-mm length) (Rios et al. 2019). The portion of the wire lining the inside circumference of the tubing was stripped of insulation. The platinum iridium wires were spaced 1.5 mm apart along the length of the tubing. A lengthwise cut along the tubing allowed the vagus nerve to be placed inside the cuff electrode. The cuff electrode impedance for each rat was between 1 and 10 kΩ. Leads from the cuff electrode were tunneled subcutaneously to the head and connected to the skull mounted connector. The vagus nerve was stimulated using an A-M Systems isolated pulse stimulator (model no. 2100), and an oxygen saturation drop was observed to ensure that the cuff electrode was functional. All rats were given amoxicillin (5 mg) and carprofen (1 mg) for 2 days after surgery, and a topical antibiotic cream was applied to the incision sites to prevent infection and facilitate recovery. The experimentally naïve control rats in the current study did not undergo sham surgery. This decision was based on multiple previous studies showing no difference between experimentally naïve rats and rats that had implants that were not activated (sham stimulation) or rats that received VNS that was not paired with a sensory or motor event (Engineer et al. 2011; Khodaparast et al. 2014; Porter et al. 2012; Shetake et al. 2012). Rats were individually housed and maintained on a reverse 12-h light-dark cycle.

VNS paired with tones. VNS was paired with the presentation of a 9-kHz tone 300 times per day for a period of 20 days, as in previous studies. This VNS-sound pairing paradigm has been successfully used both preclinically in rats (Borland et al. 2016, 2018; Buell et al. 2018, 2019; Engineer et al. 2011, 2015a; Loerwald et al. 2018; Shetake et al. 2012) as well as clinically in patients with tinnitus (De Ridder et al. 2014; Tyler et al. 2017; Vanneste et al. 2017). The number of stimulations and number of days of pairing were chosen based on previous studies. Previous experiments have documented that 50 VNS-tone-pairing stimulations per day for 20 days was not sufficient to drive A1 plasticity when recorded 24 h after the completion of the last day of pairing (Borland et al. 2018). Similarly, 5 days of NB stimulation paired with tone presentation indicates that A1 plasticity generated by NB stimulation is progressive in nature (Kilgard and Merzenich 1998). Five days of NB-tone pairing generates less than half of the plasticity documented following 20 days of pairing (18% increase in the response to the paired tone after 5 days of pairing versus 44% increase after 20 days of pairing).

Rats were placed in a 25 × 25 × 25 cm cage located in a 50 × 60 × 70 cm chamber lined with acoustical foam. The stimulation consisted of a 500-ms train of 15 pulses presented at 30 Hz (Buell et al. 2018). The 100-μs biphasic pulse-width VNS train was delivered at a current intensity of 0.8 mA (Borland et al. 2016). The onset of the

VNS train began 150 ms before tone onset. The 9-kHz tone was 500 ms in duration and was presented at an intensity of 50 dB SPL. There was an average of ~30 s in between each VNS-tone-pairing presentation, and each daily session lasted 2.5 h (Borland et al. 2018). The impedance of the cuff electrode was tested daily to ensure that each VNS implant remained functional (≤10 kΩ). We videomonitored all pairing sessions for each animal, and VNS did not evoke any behavioral response from the animals. The 0.5-s train of VNS did not wake sleeping rats or interfere with ongoing behaviors in any detectable way (Hulsey et al. 2016; Porter et al. 2012). Additionally, a 0.5-s train of VNS had no detectable effect on cardiac or pulmonary function.

Neural recordings. Following 20 days of VNS-tone pairing, multiunit responses were recorded from the IC, A1, AAF, and PAF. Twenty-four hours after the last pairing session, rats were anesthetized with sodium pentobarbital (50 mg/kg), and supplemental doses of pentobarbital (0.2–0.4 ml, 8 mg/ml) were administered as needed throughout the procedure to maintain anesthesia depth. A 1:1 ratio of dextrose (5%) and standard Ringer's lactate solution was regularly administered to prevent dehydration. A tracheotomy was performed to minimize breathing problems, and a cisternal drain was performed to minimize cerebral edema. The skull section over the temporal ridge was removed to expose the right auditory cortex. The dura was removed, and the cortex was maintained throughout the experiment under a thin film of silicone oil to prevent desiccation. For cortical recording sites, four parylene-coated tungsten microelectrodes (1.5–2.5 MΩ; FHC) were lowered simultaneously to layer IV/V (600–700 μm below the surface of the cortex), as in previous studies (Borland et al. 2018; Engineer et al. 2008, 2011). The recording site locations were chosen to generate a detailed, evenly spaced map while avoiding damage to the blood vessels on the cortical surface. For IC recording sites, two microelectrodes were lowered through a hole located 9 mm posterior and 1.5 mm lateral to bregma at 200-μm intervals along the dorsal ventral axis to a depth between 1,000 and 5,000 μm, as in previous studies (Perez et al. 2013; Ranasinghe et al. 2013). All recordings took place in a soundproof recording booth using a speaker positioned 10 cm from the left ear. Multiunit neural activity was captured using Brainware software (Tucker-Davis Technologies), and the location of each recording site was documented on a detailed digitized photo of the exposed auditory cortex. Auditory frequency tuning curves were obtained at each recording site by presenting tones at 81 frequencies between 1 and 32 kHz in 0.0625-octave steps at 16 intensities from 0 to 75 dB SPL in 5-dB steps. Tones were presented every 500 ms and were randomly interleaved. Following the completion of neural recordings, the vagus nerve was stimulated for 10 s and an oxygen saturation drop was observed to ensure that the cuff electrode was functional. If VNS failed to elicit a drop in blood oxygen saturation, the recordings were excluded from analysis.

Data analysis. All data analysis was performed blind to the experimental group using SPSS version 25 and custom MATLAB software. Tonotopy, response selectivity, and response latency were used to assign individual recording sites to each auditory field, as in previous studies (Centanni et al. 2013; Engineer et al. 2015b; Polley et al. 2007) (Figs. 1 and 2). The Voronoi tessellation procedure was used to transform the discretely sampled cortical surface into a continuous map (Engineer et al. 2015a; Kilgard and Merzenich 1998; Polley et al. 2007). This method adjusts for any small differences in sampling spacing, such that any regions with high-sampling density generate smaller tessellations, which does not bias cortical response measures.

For each tone-frequency tone-intensity combination, the percentage of each field responding to each tone was calculated (Engineer et al. 2011, 2014). For the percentage of each field responding to each tone-frequency, tone-intensity combination (Figs. 4 and 5), a Benjamini-Hochberg correction was used to control the false discovery rate (Benjamini and Hochberg 1995). For the percentage of each field responding to the paired tone frequency (Fig. 6) and the percentage of recording sites tuned to the paired tone frequency (Fig. 3), mixed-effects models were used to account for the different fields recorded from each rat. The experimental group (naïve control versus VNS-tone paired) and auditory field (the IC, A1, AAF, and PAF) were evaluated as fixed factors, and the individual rats were evaluated as a random factor. A simple contrast analysis was used for the post hoc

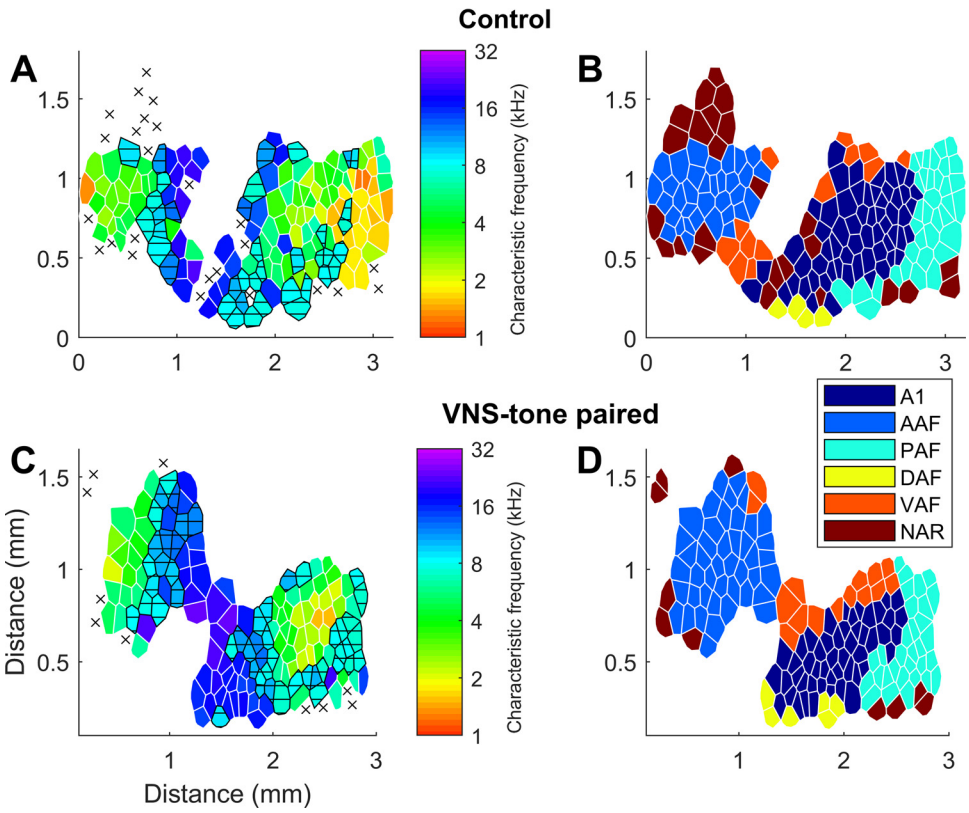

Fig. 1. Auditory cortex maps in an example control rat and vagus nerve stimulation (VNS)-tone paired rat. Each colored polygon represents a single electrode penetration. *A*: the characteristic frequency (CF) map for all auditory cortex recording sites is shown in a representative control rat. Hatched polygons indicate recording sites with CFs between 8 and 16 kHz. Color indicates the CF of the recording site, from low frequency (1 kHz, red) to high frequency (32 kHz, purple). Anterior is depicted to the left, and ventral is depicted at the top. The "x" symbols indicate recording sites with no auditory response. *B*: the positioning of the auditory cortex fields is shown for the same control rat. *C*: the CF map for all auditory cortex recording sites is shown in a representative VNS-tone paired rat. *D*: the positioning of the auditory cortex fields is shown for the same VNS-tone paired rat. A1, primary auditory cortex; AAF, anterior auditory field; PAF, posterior auditory field; NAR, no auditory response; DAF, dorsal auditory field; VAF, ventral auditory field.

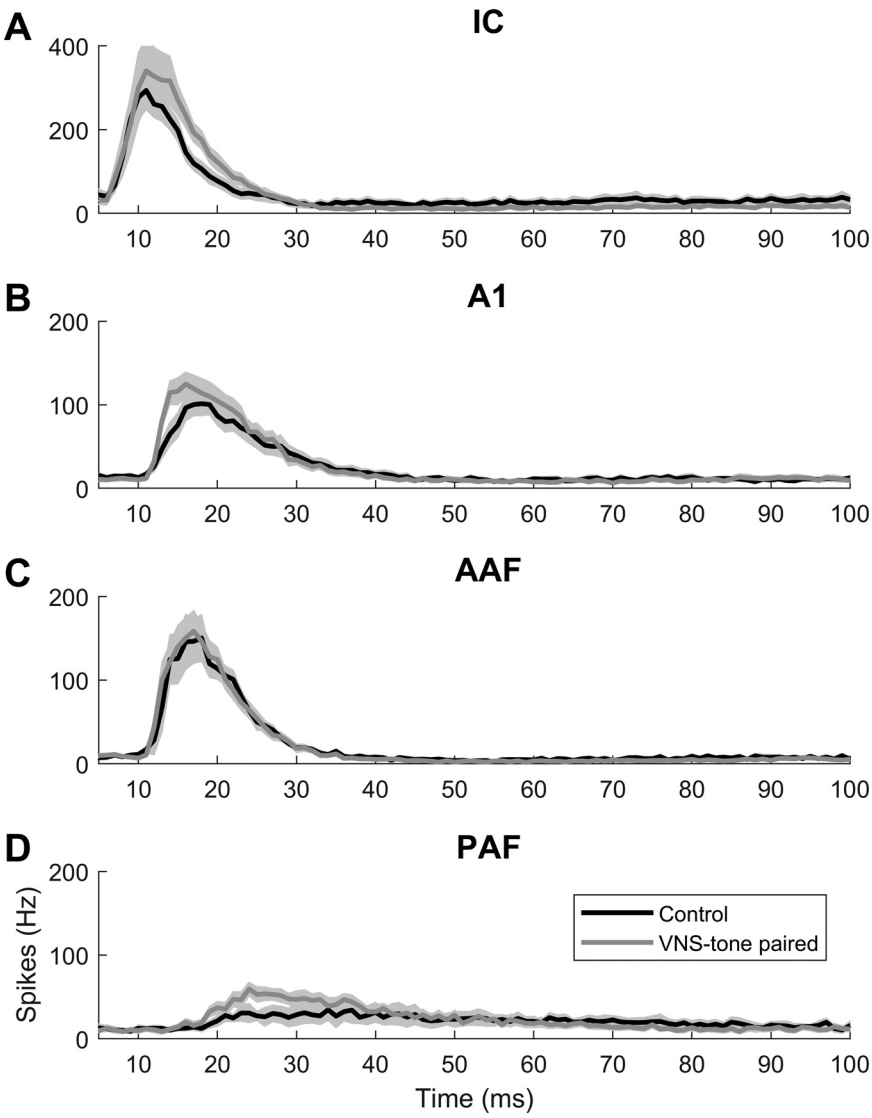

Fig. 2. The poststimulus time histogram (PSTH) response to 8- to 16-kHz tones presented at 50 dB in each of the fields. *A–D*: the average PSTH response to 8- to 16-kHz tones in the inferior colliculus (IC; *A*), primary auditory cortex (A1; *B*), anterior auditory field (AAF; *C*), and posterior auditory field (PAF; *D*) in control and vagus nerve stimulation (VNS)-tone paired rats. Gray shading indicates SE across rats.

comparisons between the two groups and was corrected to account for multiple comparisons using Holm-Bonferroni correction.

For the receptive field analysis (Table 1), a generalized linear mixed-effects model was used to account for the different fields recorded from each rat and the different number of recording sites obtained in each rat. The auditory field was nested within each experimental group (naïve versus VNS-tone paired), and the experimental group and auditory field were evaluated as a fixed factor. An analysis weight was used to correct for the number of sites. Post hoc comparisons between experimental groups used a pairwise comparison and were corrected to account for multiple comparisons using Holm-Bonferroni correction. The threshold was defined by a blinded expert reviewer as the lowest intensity that evoked a reliable neural response, as in previous studies (Anomal et al. 2015; Buell et al. 2018; Carrasco and Lomber 2009; Centanni et al. 2013; Engineer et al. 2008; Recanzone et al. 1993; Hernández et al. 2005; Polley et al. 2007; Puckett et al. 2007; Ranasinghe et al. 2013). The characteristic frequency was defined as the frequency at which the threshold occurred. The characteristic frequency range obtained in each rat was defined as the difference between the maximum characteristic frequency and the minimum characteristic frequency and was used to confirm that the complete extent of auditory responses was collected from each field. The range of frequencies that evoked responses 10, 20, 30, and 40 dB above threshold was defined as the bandwidth. The spontaneous firing rate was defined as the spike rate observed during silence. The spontaneous rate was calculated across the 400-ms duration of a trial, across all 81 tone frequencies, when presented at an amplitude of 0 dB. A poststimulus time histogram with 1-ms bin widths was constructed from the responses to tone-intensity combinations within the receptive field. The peak latency was quantified as the time point of the maximum number of spikes for each site. Partial correlations were used to determine the relationship between percent responding and each receptive field property while controlling for the other receptive field properties (Fig. 8). Holm-Bonferroni correction was used to account for the multiple comparisons.

For each tone-frequency, tone-intensity combination, the number of spikes evoked by each tone was calculated (Engineer et al. 2011, 2014). For the response strength analysis (Figs. 9 and 10), generalized linear mixed-effects models were used to account for the different fields recorded from each rat and the different number of recording sites obtained in each rat. The auditory field was nested within each experimental group (naïve versus VNS-tone paired). The experimental group, field, and tone frequency (Fig. 9) or tone intensity (Fig. 10) were evaluated as fixed factors. An analysis weight was used to correct for the number of sites. Post hoc comparisons between experimental groups were conducted using pairwise contrasts corrected to account for multiple comparisons using sequential Bonferroni correction.

Table 1. *Receptive field properties were altered in VNS-tone paired rats compared with control rats for sites with a CF between 8 and 16 kHz*

Field and Group	Number of Sites	Threshold, dB	Spontaneous Rate, Hz	Bandwidth, octaves	Peak Latency, ms
All					
Control	1,491	15.0 ± 0.9	22.8 ± 1.7	1.17 ± 0.06	23.1 ± 1.1
VNS-tone	1,459	10.2 ± 0.4*	16.2 ± 0.8*	1.43 ± 0.03*	21.9 ± 0.5
IC					
Control	304	5.7 ± 2.0	51.1 ± 3.8	0.97 ± 0.14	13.3 ± 2.4
VNS-tone	270	2.3 ± 0.95	33.9 ± 1.7*	1.33 ± 0.06*	14.0 ± 1.1
A1					
Control	463	8.7 ± 2.1	15.9 ± 3.8	1.25 ± 0.14	20.4 ± 2.4
VNS-tone	478	5.9 ± 0.8	11.4 ± 1.5	1.42 ± 0.05	18.7 ± 0.9
AAF					
Control	419	14.2 ± 1.5	12.3 ± 2.7	1.36 ± 0.10	18.6 ± 1.7
VNS-tone	481	8.7 ± 0.7*	10.8 ± 1.4	1.54 ± 0.05	18.4 ± 0.9
PAF					
Control	305	31.3 ± 1.7	12.1 ± 3.2	1.11 ± 0.12	40.1 ± 2.1
VNS-tone	230	23.6 ± 0.9*	8.9 ± 1.7	1.44 ± 0.06*	36.5 ± 1.1

All values are presented as the means ± SE. PAF, posterior auditory field; A1, primary auditory cortex; AAF, anterior auditory field; IC, inferior colliculus; VNS, vagus nerve stimulation. *Significantly different compared with control rats (Holm-Bonferroni corrected).

RESULTS

Following the completion of 20 days of VNS-tone pairing, multiunit responses were recorded from four auditory fields: the IC, A1, AAF, and PAF (Figs. 1 and 2). A total of 2,950 recording sites with driven activity were collected across 33 animals (Table 1). At each recording site, responses were recorded to 1,296 tones varying in frequency and intensity. To ensure that there was equivalent sampling of the extreme ends of the maps in each rat, the difference between the maximum characteristic frequency and the minimum characteristic frequency was quantified. There was no significant difference in the range of characteristic frequencies obtained between VNS-tone paired and control rats in any field, which indicates that recordings spanned the frequency range of each field equivalently between the experimental groups (IC: $U = 43$, $z = -0.16$, $P = 0.91$ Mann-Whitney U-test; A1: U = 33.5, z = −1.78, $P = 0.08$; AAF: $U = 88$, $z = 0.92$, $P = 0.38$; PAF: $U = 47$, $z = 0.16$, $P = 0.91$).

VNS-tone pairing increases the response to the paired tone frequency. VNS-tone pairing significantly increased the percentage of recording sites tuned to frequencies near the paired tone frequency. The characteristic frequency-tuning percentage varied across both tone frequency and auditory field [auditory field × tone frequency × experimental group interaction: $F(39, 380) = 11.21$, $P < 0.00001$; Fig. 3]. All four auditory fields exhibited an increase in the percentage of sites tuned to

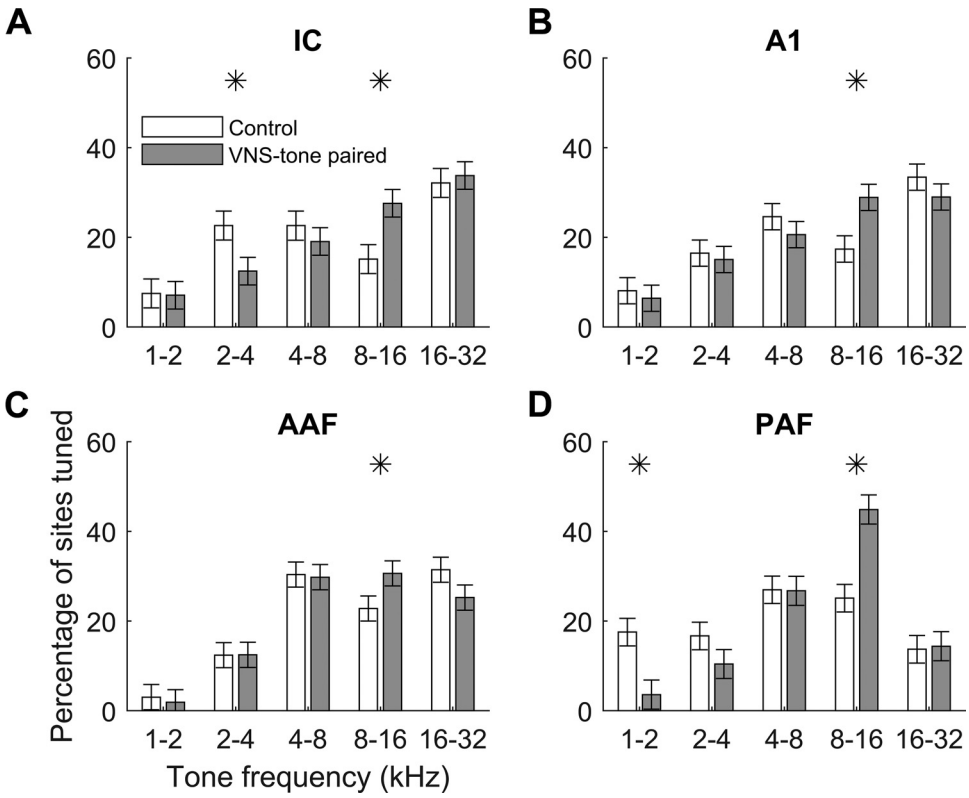

Fig. 3. *A–D*: the percentage of neurons with a characteristic frequency tuned between 8 and 16 kHz was increased in vagus nerve stimulation (VNS)-tone paired rats compared with control rats in the inferior colliculus (IC; *A*), primary auditory cortex (A1; *B*), anterior auditory field (AAF; *C*), and posterior auditory field (PAF; *D*). Error bars indicate SE. *Fields that were significantly different between VNS-tone paired and control rats (Holm-Bonferroni corrected).

frequencies within the paired 8- to 16-kHz octave. VNS-tone pairing increased the percentage of sites with a characteristic frequency tuned within the paired tone-frequency octave by 82% in the IC [$t(380) = -2.79$, $P = 0.006$], 66% in A1 [$t(380) = -2.78$, $P = 0.006$], 34% in AAF [$t(380) = -1.98$, $P = 0.049$], and 79% in PAF [$t(380) = -4.43$, $P = 0.00001$; Fig. 3]. In some fields, this increase in tuning in the paired frequency octave was accompanied by a decrease in tuning in lower tone-frequency octaves (Fig. 3).

Previous studies have consistently documented that following 20 days of VNS-tone pairing, a larger percentage of A1 responds to the specific tone frequency paired with VNS (Borland et al. 2016, 2018; Buell et al. 2018; Engineer et al. 2011). For each field, the mean percentage of the field that responds to a tone of each frequency-intensity combination was calculated for control (Fig. 4*A*) and VNS-tone paired rats (Fig. 4*B*). For example, in A1, subtracting the percentage of A1 responding in control rats from VNS-tone paired rats indicates that VNS-tone pairing resulted in an increase in the percentage of A1 responding to high-frequency tones (Fig. 4*C*).

VNS-tone pairing significantly increased the proportion of recording sites that respond to frequencies near the paired tone frequency. The magnitude of the percentage responding varied across both tone frequency and auditory field [auditory field × tone frequency × experimental group interaction: $F(39, 380) = 9.56$, $P < 0.00001$; Figs. 5 and 6]. All four auditory fields exhibited an increase in the percentage of sites responding to frequencies within the paired 8- to 16-kHz octave. VNS-tone pairing increased the percentage of sites responding to tones within the paired tone-frequency octave by 47% in the IC [$t(380) = -2.86$, $P = 0.005$], 39% in A1 [$t(380) = -2.93$, $P = 0.004$], 25% in AAF [$t(380) = -2.36$, $P = 0.02$], and 81% in PAF [$t(380) = -4.57$, $P < 0.00001$; Fig. 6]. In some fields, this increase in responding to the paired frequency

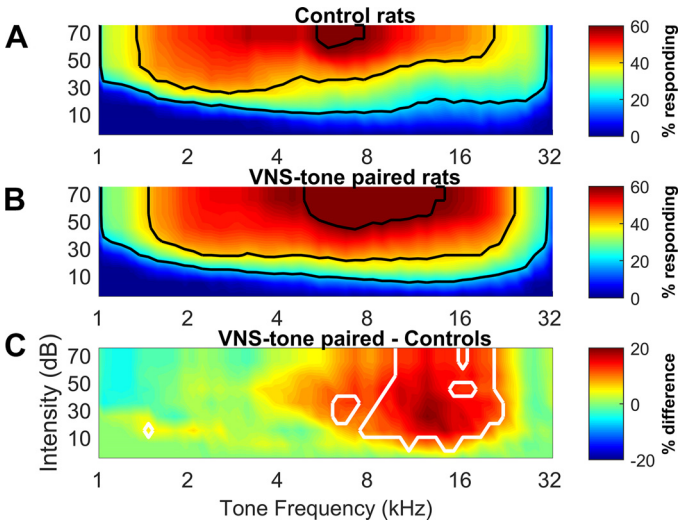

Fig. 4. The percentage of primary auditory cortex responding to high-frequency tones increased in vagus nerve stimulation (VNS)-tone paired rats. *A*: the percentage of primary auditory cortex (A1) neurons that respond to each presented tone frequency-intensity combination in control rats. Black contour lines indicate 20, 40, and 60% of A1 responding. *B*: the percentage of A1 neurons that respond to each tone in VNS-tone paired rats that experienced a paired tone frequency of 9 kHz. *C*: the difference in the percentage of A1 that responds between VNS-tone paired and control rats. White contour lines indicate the region of tones that was significantly increased in VNS-tone paired rats. False discovery rate was used to account for multiple comparisons.

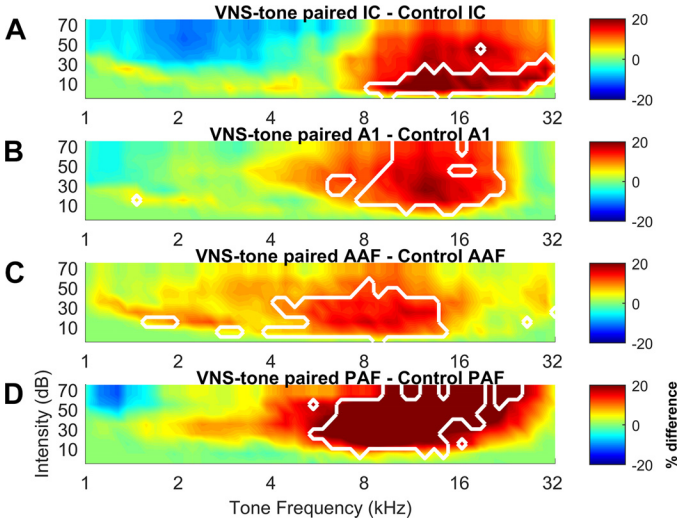

Fig. 5. *A–D*: the percentage of each field responding to high-frequency tones was increased in vagus nerve stimulation (VNS)-tone paired rats compared with control rats in the inferior colliculus (IC; *A*), primary auditory cortex (A1; *B*), anterior auditory field (AAF; *C*), and posterior auditory field (PAF; *D*). White contour lines indicate the region of tones that was significantly increased in VNS-tone paired rats compared with control rats. False discovery rate was used to account for multiple comparisons.

octave (8–16 kHz) was also accompanied by increased responding in neighboring tone-frequency octaves (Fig. 6). Both the IC and PAF exhibited an increased percentage of sites responding to tones in the 16- to 32-kHz octave, while both AAF and PAF exhibited an increased percentage of sites responding to tones in the 4- to 8-kHz octave.

Neural recordings were made in multiple auditory structures in individual VNS-tone paired and control rats. Within individual rats, the plasticity observed in each auditory field is often correlated with the plasticity in the other auditory fields, particularly for the cortical fields. The percentage of sites responding to 8–16 kHz in PAF is correlated with the percentage of sites responding to 8–16 kHz in IC, A1, and AAF (Fig. 7). The percent responding in A1 is also correlated with AAF. Interestingly, the percent responding in IC is not correlated with A1 or AAF.

VNS-tone pairing alters receptive field properties. Similarly, VNS-tone pairing altered a number of receptive field properties across the auditory pathway. Across all auditory fields, VNS-tone pairing decreased response threshold by 4.8 dB compared with control rats [$F(1,758) = 22.68$, $P = 0.000002$; Table 1] and decreased spontaneous firing rate by 7 Hz [$F(1,758) = 12.47$, $P = 0.0004$; Table 1]. VNS-tone pairing also increased the bandwidth 10 dB above threshold by 0.3 octaves [$F(1,758) = 14.38$, $P = 0.0002$; Table 1] but did not alter the peak firing response latency [$F(1,758) = 1.06$, $P = 0.30$; Table 1]. Within individual fields, IC exhibited a significant decrease in spontaneous rate and a significant increase in response bandwidth. A1, on the other hand, did not exhibit significant alterations in any receptive field properties. AAF exhibited a statistically significant decrease in the response threshold. PAF also exhibited a significant decrease in response threshold, as well as a significant increase in response bandwidth. Together, these receptive field changes indicate that VNS-tone paired rats were more responsive to tones within the paired tone-frequency octave compared with control rats.

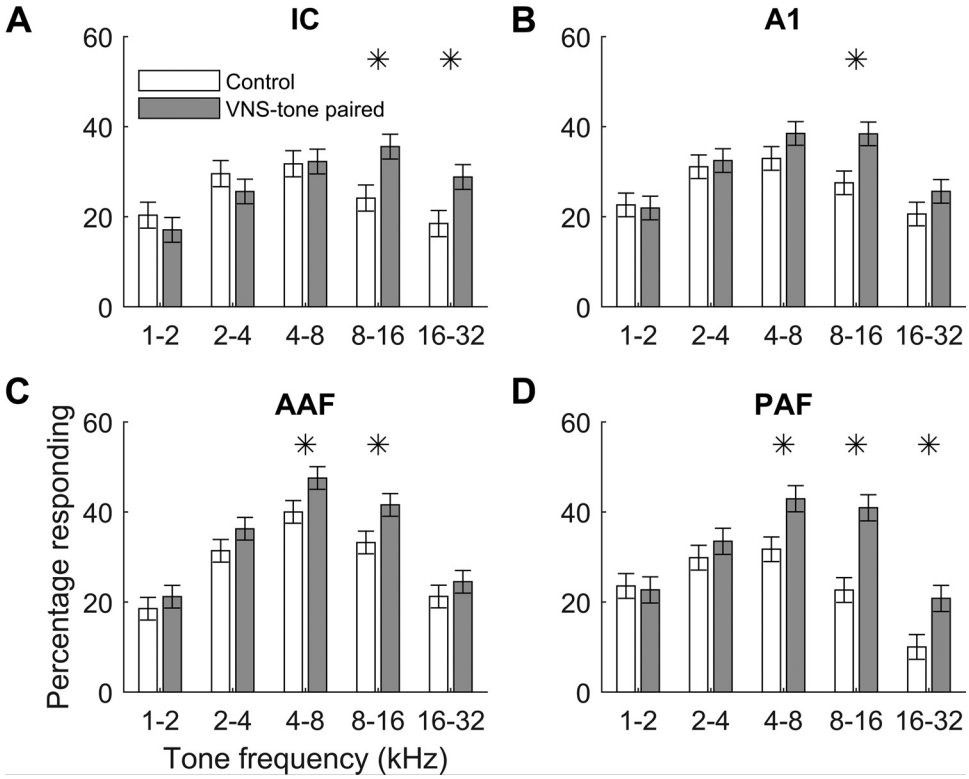

Fig. 6. *A–D*: the percentage of each field responding to tones within the paired tone-frequency octave was increased in vagus nerve stimulation-tone paired rats compared with control rats across all tested fields in the inferior colliculus (IC; *A*), primary auditory cortex (A1; *B*), anterior auditory field (AAF; *C*), and posterior auditory field (PAF; *D*). Error bars indicate SE. *Fields that were significantly different between VNS-tone paired and control rats (Holm-Bonferroni corrected).

Additionally, there was a relationship between the percentage of recording sites responding to the paired tone-frequency octave (8–16 kHz) and the receptive field properties. Across all fields, shifts in CF to the paired tone-frequency octave were associated with an increased percentage of sites responding (Fig. 8). Additionally, in the IC, increases in response bandwidth were associated with an increased percentage of IC sites responding (Fig. 8). Response threshold, response latency, and spontaneous firing rate could not be used to predict changes in the percentage of recording sites responding.

Within individual rats, the CF was weakly correlated between auditory fields. The percentage of recording sites tuned to 8–16 kHz in A1 was significantly correlated with the percentage of recording sites tuned to 8–16 kHz in AAF ($R^2 = 0.41$, $P = 0.005$). Between all other fields, however, the correlation did not survive correction for multiple comparisons

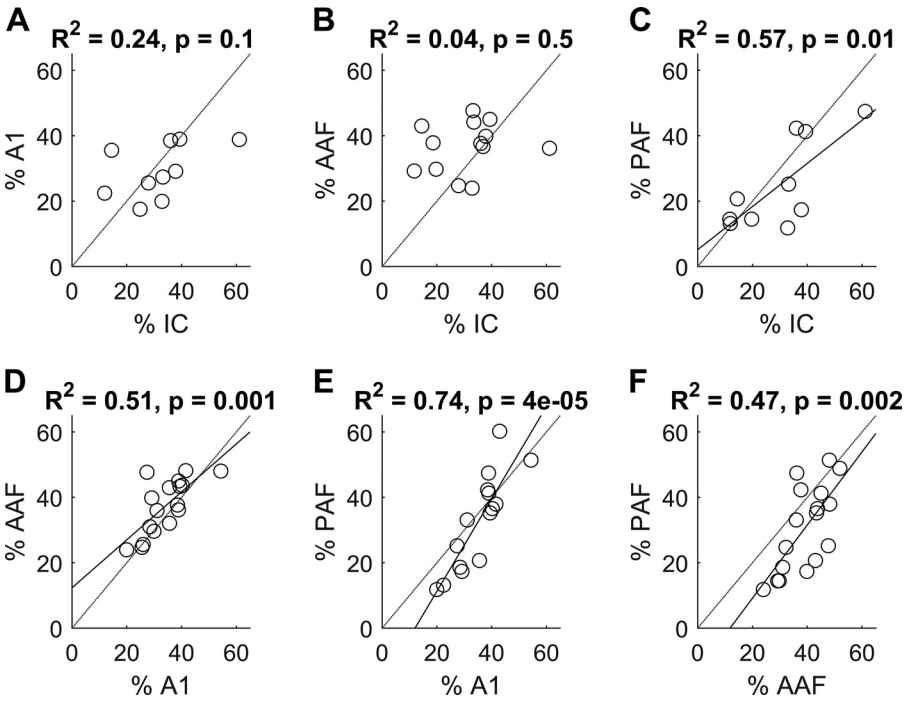

Fig. 7. *A–F*: correlations between the percentage of sites responding to 8–16 kHz in each auditory field in individual vagus nerve stimulation-tone paired and control rats. Within individual rats, the plasticity observed in each auditory field is often correlated with the plasticity in the other auditory fields. Each dot represents an individual rat. Black lines indicate significant correlations after Holm-Bonferroni correction for multiple comparisons. A1, primary auditory cortex; IC, inferior colliculus; AAF, anterior auditory field; PAF, posterior auditory field.

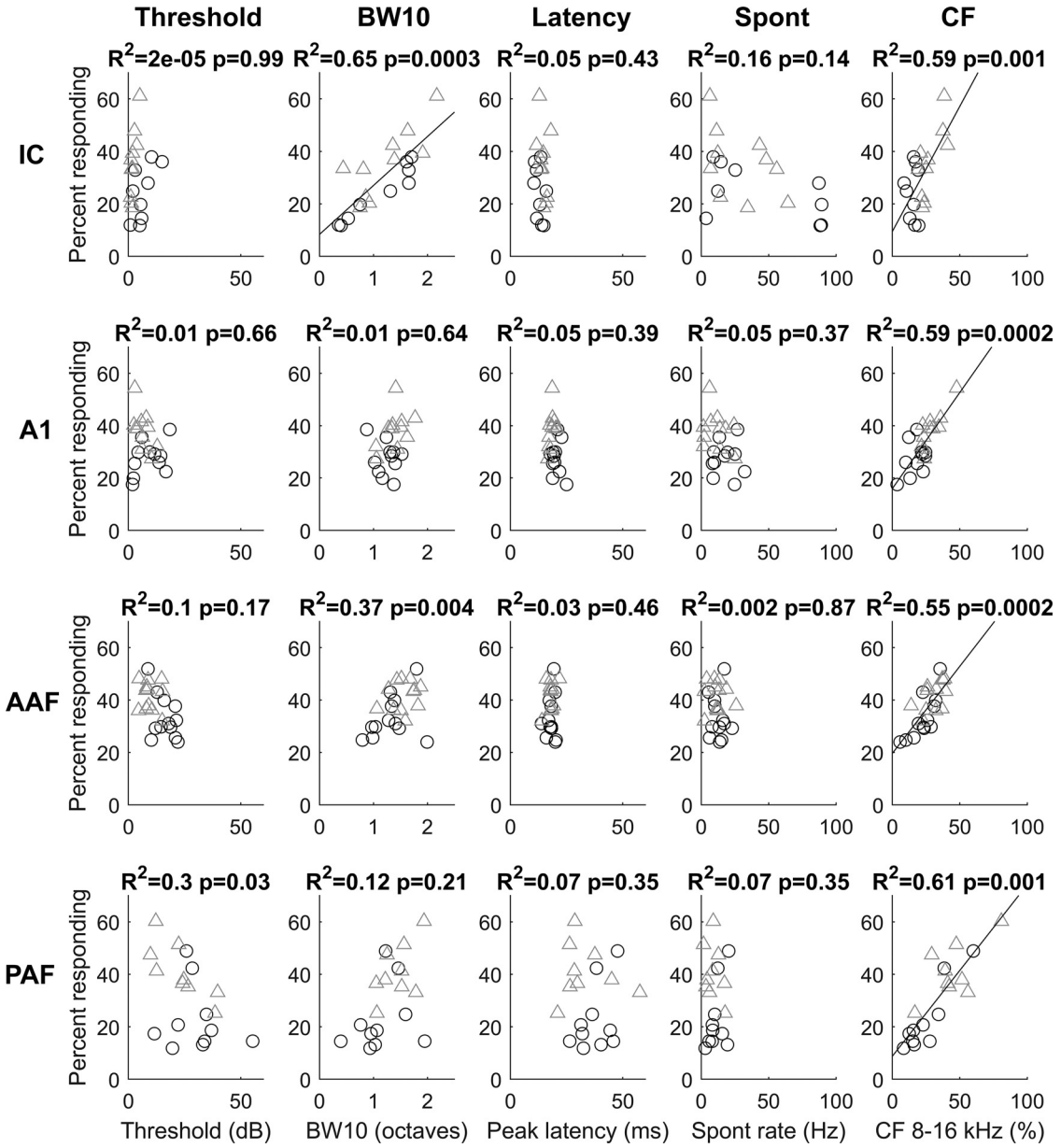

Fig. 8. Correlations between the percentage of recording sites responding to 8–16 kHz and each of the tested receptive field properties. Each circle represents 1 control rat, while each triangle represents 1 vagus nerve stimulation (VNS)-tone paired rat. Partial correlation was used to measure the relationship between percent responding and each receptive field property while controlling for the other receptive field properties. Black lines indicate significant correlations after Holm-Bonferroni correction for multiple comparisons. A1, primary auditory cortex; BW10, bandwidth 10 dB above the response threshold; IC, inferior colliculus; AAF, anterior auditory field; PAF, posterior auditory field; CF, characteristic frequency.

(IC and A1: $R^2 = 0.45$, $P = 0.03$; IC and AAF: $R^2 = 0.07$, $P = 0.4$; IC and PAF: $R^2 = 0.09$, $P = 0.4$; A1 and PAF: $R^2 = 0.32$, $P = 0.03$; AAF and PAF: $R^2 = 0.33$, $P = 0.01$).

VNS-tone pairing strengthens responses to tones. VNS-tone pairing also significantly increased the response strength to tones. Tones evoked 25% more spikes in VNS-tone paired rats compared with control rats (1.25 ± 0.02 spikes in VNS-tone paired rats; 1.00 ± 0.02 spikes in control rats). The magnitude of the response strength increase to tones varied across both tone frequency and auditory field [auditory field $\times$ tone frequency $\times$ experimental group interaction: $F(31, 14710) = 6.76$, $P < 0.00001$; Fig. 9]. Both IC and A1 exhibited an increase in the response strength to tones in the paired 8- to 16-kHz octave. Following VNS-tone pairing, responses in IC were increased for all tone frequencies >2 kHz, while the response strength increase in A1 was specific to the paired 8- to 16-kHz octave (Fig. 9). AAF did not exhibit a significant increase in response strength following VNS-tone pairing in any tone-frequency bins, while PAF exhibited a significant increase in response strength in the 4- to 8-kHz tone-frequency range (Fig. 9). Similarly, there was also a significant interaction between auditory field, tone intensity, and experimental group [$F(108, 47072) = 92.88$, $P < 0.00001$; Fig. 10]. Following VNS-tone pairing, IC exhibited a generalized increase in the response strength to tone intensities between 10 and 75 dB. The response strength increase in the cortical fields, however, was restricted to louder sound intensities in A1 (65–70 dB), AAF (65–75 dB), and PAF (50–75 dB; Fig. 10).

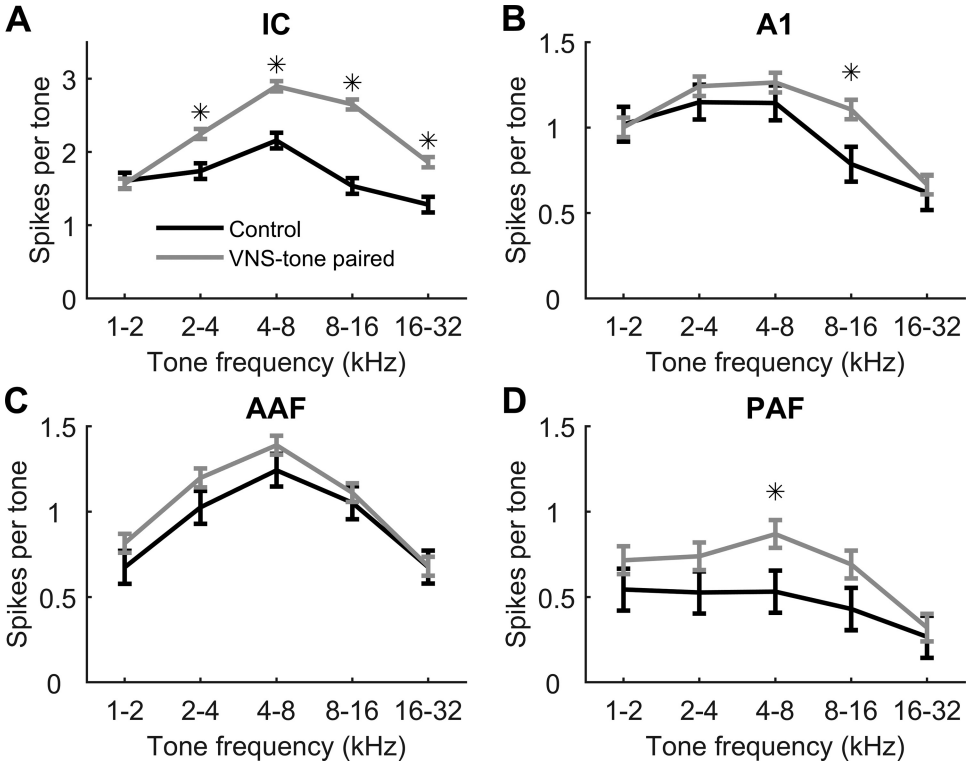

Fig. 9. The response strength to tones was significantly stronger in vagus nerve stimulation (VNS)-tone paired rats compared with control rats. *A*: VNS-tone paired rats evoked more spikes per tone at nearly all frequency bins tested in inferior colliculus (IC) compared with control rats. *B–D*: VNS-tone pairing increased the response strength to 8- to 16-kHz tones in primary auditory cortex (A1; *B*), did not alter response strength in anterior auditory field (AAF; *C*), and increased the response strength to 4- to 8-kHz tones in posterior auditory field (PAF; *D*). Responses are the average spikes evoked per tone for tones within five 1-octave bins (1–2, 2–4, 4–8, 8–16, and 16–32 kHz). *Frequencies that evoke a stronger response in VNS-tone paired rats compared with control rats ($P < 0.0025$, Bonferroni corrected). Error bars indicate SE.

DISCUSSION

Previous studies have reported A1 plasticity following VNS-sound pairing (Borland et al. 2016; Engineer et al. 2011, 2015a; Loerwald et al. 2018). Here, we extend the previous findings by documenting that VNS drives coordinated, robust changes in multiple fields across the auditory pathway. Pairing VNS with the presentation of a 9-kHz tone significantly increases the percentage of IC, A1, AAF, and PAF that responds to the paired frequency. Across all fields, the response strength to tones is strengthened in VNS-tone paired rats compared with control rats. Alterations to receptive field properties across the auditory pathway include decreased response threshold, decreased spontaneous firing rate, and increased response bandwidth following VNS-tone pairing. This is the first study to

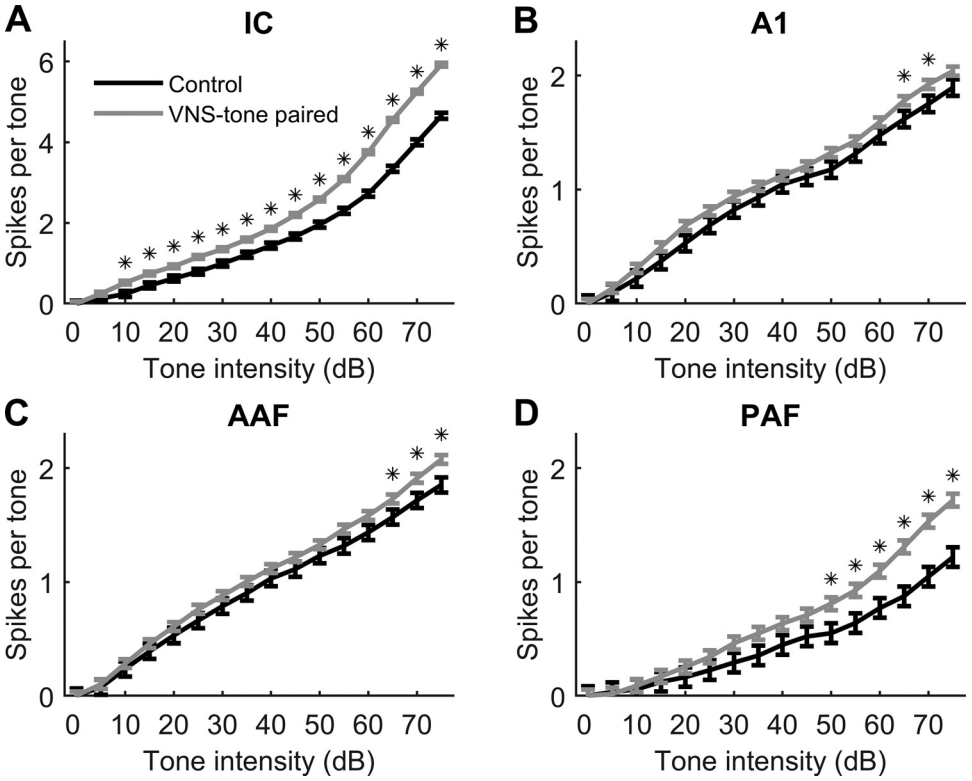

Fig. 10. *A–D*: the response strength to tones was significantly stronger in vagus nerve stimulation (VNS)-tone paired rats compared with control rats, plotted as a function of tone intensity in the inferior colliculus (IC; *A*), primary auditory cortex (A1; *B*), anterior auditory field (AAF; *C*), and posterior auditory field (PAF; *D*). VNS-tone paired rats evoked more spikes per tone in each field compared with control rats. Responses are the average spikes evoked per tone at each tested intensity (0–75 dB in 5-dB steps). *Intensities that evoke a stronger response in VNS-tone paired rats compared with control rats ($P < 0.00078$, Bonferroni corrected). Error bars indicate SE.

document both cortical and subcortical plasticity following VNS-sound pairing. These coordinated changes spanning multiple stations in the auditory pathway may underlie the preclinical and clinical findings of VNS-dependent benefits in the context of tinnitus. The present study provides a framework for future studies in both humans and animals to address the contribution of these changes to the therapeutic effects of VNS.

Relation to previous sound pairing with neuromodulator release literature. This study is consistent with many previous findings that document plasticity in the auditory pathway in response to pairing presentation of a tone with concurrent release of neuromodulators via neurostimulation (Borland et al. 2016; Edeline et al. 2011; Engineer et al. 2011; Froemke et al. 2013; Kilgard and Merzenich 1998; Martins and Froemke 2015; Zhang et al. 2005; Zhang and Yan 2008). Many previous studies have documented stimulus-specific A1 plasticity following VNS-sound pairing (Borland et al. 2016, 2018; Buell et al. 2018; Engineer et al. 2011, 2015a). It was previously unknown whether other auditory fields exhibit response alterations following VNS-sound pairing. In this study, VNS paired with a 9-kHz tone for 20 days appears to alter both subcortical and cortical auditory fields to a similar degree. Similarly, VNS paired with motor training drives plasticity in both cortical and subcortical motor networks, which is consistent with the current results (Ganzer et al. 2018).

The receptive field changes observed in the current study match the receptive field changes observed in previous studies following NB-tone or LC-tone pairing. VNS-tone pairing resulted in a response threshold decrease that was statistically significant in AAF and PAF and trending toward significance in IC and A1. Decreased response thresholds have also been observed in the IC following NB-tone pairing (Zhang et al. 2005). Similarly, VNS-tone pairing resulted in a statistically significant increase in response bandwidth in IC and PAF and a trending increase in A1 and AAF. Broader receptive fields were observed in A1 immediately following LC-tone pairing, before responses were refined over hours toward the paired tone (Martins and Froemke 2015). Overall, the receptive field changes observed in the current study indicate that VNS-tone paired rats were more responsive to tones within the paired tone frequency octave compared with control rats.

In terms of the frequency specificity of the plasticity, previous studies also document plasticity in frequencies neighboring, but not necessarily specific to, the paired frequency (Bieszczad et al. 2013; Borland et al. 2016, 2018; Buell et al. 2018; Edeline et al. 2011; Engineer et al. 2011; Kilgard and Merzenich 1998; Kilgard et al. 2001). The precise network dynamics responsible for these changes are not clear. It is possible that these changes are more pronounced in the higher frequency region due to the basic response characteristics observed in experimentally naïve rats, where a greater percentage of cortex responds to the frequencies immediately below 9 kHz compared with the frequencies immediately above 9 kHz (Fig. 4*A*). This could create a ceiling effect for plasticity in response to frequencies immediately below 9 kHz. Interestingly, following LC-tone pairing, both paired tone frequency-specific effects and general effects were documented (Edeline et al. 2011; Martins and Froemke 2015). The frequency-specific effects were limited to within one-fourth of an octave on each side of the paired frequency, while the general effects occurred for more than an octave on each side of the paired frequency (Edeline et al. 2011). These frequency-specific and general changes were present in both auditory cortex, which exhibited both increases and decreases, and auditory thalamus, which only exhibited response increases.

Potential role for neuromodulators in regional differences. Stimulation of the vagus nerve results in activation of both the NB and the LC through the nucleus tractus solitarius (Dorr and Debonnel 2006; Hulsey et al. 2016, 2017). VNS-directed plasticity requires the release of modulatory neurotransmitters, including acetylcholine, norepinephrine, and serotonin (Hulsey et al. 2016, 2019). Differences in the neuromodulatory inputs to auditory areas may account for differences in the specificity of plasticity between auditory fields (Chavez and Zaborszky 2017). In particular, there are substantial differences in the proportion of cholinergic and noncholinergic projections to A1 versus nonprimary auditory fields (Chavez and Zaborszky 2017). The magnitude and polarity of plasticity are highly sensitive to the levels of neuromodulators (Seol et al. 2007); thus differences in VNS-dependent plasticity across auditory stations may arise from variations in the proportion of neuromodulatory input specific to each area.

It is likely that the plasticity in the different regions of the auditory pathway works together to affect the overall system through both local and long-range changes. From the NB-tone-pairing literature, studies documenting plasticity in IC and the medial geniculate nucleus have documented that subcortical plasticity is abolished when the auditory cortex is inactivated (Zhang et al. 2005; Zhang and Yan 2008). As there is no evidence documenting NB projections to the IC, corticofugal projections are believed to be essential for the IC plasticity following NB-tone pairing. On the other hand, the LC projects to both auditory subcortical and cortical areas. Subcortical plasticity resulting from LC-tone pairing could result both locally and from input from other auditory areas. Compared with the frequency-specific response strength increases observed in the cortical regions, the IC exhibited a nonspecific response strength increase to all tone frequencies above 2 kHz following VNS-tone pairing (Fig. 9*A*). This generalized response increase closely mirrors previous LC-tone-pairing studies, which document an initial greatly increased response to all sounds (Edeline et al. 2011; Martins and Froemke 2015). The differences in the NB and LC subcortical and cortical projections may account for the nonspecific increase in response strength in the IC compared with the frequency specific cortical increase in response strength.

Interestingly, all previous NB-tone and LC-tone-pairing studies have documented smaller and shorter lasting subcortical plasticity compared with cortical plasticity (Edeline et al. 2011; Froemke et al. 2013; Ma and Suga 2003; Zhang et al. 2005; Zhang and Yan 2008). In the current study, subcortical IC plasticity was large and long-lasting, which could be due to differences in the stimulation paradigms used between studies. Future research is necessary to determine the relative timing of VNS-dependent changes in these fields (Takahashi et al. 2011), as well as the role of the corticofugal system in VNS-sound-pairing plasticity (Suga 2012). The anatomical projections of the neuromodulatory systems and their interplay likely produces differential effects on plasticity.

Functional consequences. Previous literature hints at the potential behavioral outcomes associated with an increased representation of the paired tone frequency. Auditory cortex

map plasticity is associated with improved perceptual discrimination ability (Bieszczad and Weinberger 2010; Polley et al. 2006; Recanzone et al. 1993). For example, NB-tone pairing induces A1 map plasticity that enhances tone frequency discrimination learning (Froemke et al. 2013; Reed et al. 2011). Similarly, LC-tone pairing also induces A1 plasticity that improves auditory perception (Glennon et al. 2019; Martins and Froemke 2015). In addition to A1 plasticity, the current study documented plasticity in IC, AAF, and PAF. This enhanced neural representation of the paired frequency across multiple levels of the auditory pathway will likely impact behavioral discrimination accuracy. It is important to note that the specific task demands also play a role. For example, in rats trained to identify tone frequency or tone intensity using an identical set of sounds, behavioral performance on the trained task, but not the untrained task, is correlated with the percentage of A1 and suprarhinal auditory field recording sites tuned to the task's target sound (Polley et al. 2006). The behavioral consequences of VNS-sound pairing will likely depend on the specifics of the neuromodulator release, sounds, and task demands. While the present study provides a thorough characterization of plasticity across auditory fields, future studies are necessary to directly assess the functional consequences of this plasticity.

Other considerations. VNS-dependent enhancement of plasticity requires the central action of neuromodulatory networks, and peripheral actions of VNS, while numerous, are unlikely to contribute. VNS can induce peripheral changes under certain conditions; however, the parameters used in the present study are insufficient to drive changes in cardiovascular function, which requires activation of B-fibers. Additionally, while reduction of oxygen saturation is a diagnostic for effective stimulation of the vagus nerve, this oxygen saturation drop only occurs when rats are deeply anesthetized and VNS trains are used that are at least 10 times longer than the train duration used during VNS-tone pairing (McAllen et al. 2018; Paintal 1973). It is important to note that increasing the VNS intensity to levels above the 0.8 mA used in this study prevents auditory cortex plasticity but remains effective at reducing oxygen saturation when delivered as a long train to anesthetized rats (Borland et al. 2016). This result indicates that the plasticity reported in this study does not result from a peripheral action and is consistent with our earlier demonstrations that plasticity depends on the release of neuromodulators in the central nervous system (Hulsey et al. 2016, 2019).

Clinical relevance. Individuals with disorders that have an auditory processing component, such as tinnitus, exhibit alterations in the neural response to sounds across the auditory pathway (Eggermont and Roberts 2004; Melcher et al. 2000; Smits et al. 2007). Individuals with tinnitus exhibit alterations in excitability and spontaneous firing in both subcortical as well as nonprimary cortical regions (Eggermont and Kenmochi 1998; Imig and Durham 2005; Robertson et al. 2013). While previous findings suggest that VNS-sound pairing can restore these neural response deficits in A1 (Engineer et al. 2013, 2017), it is not currently known how VNS-sound pairing impacts other dysregulated auditory regions. In rats with tinnitus, both neural A1 and behavioral deficits are restored following VNS-tone pairing (Engineer et al. 2011). Clinical studies suggest that VNS-tone pairing can also improve neural and behavioral deficits in tinnitus patients (De Ridder et al. 2014, 2015; Tyler et al. 2017; Vanneste et al. 2017). The present study raises the prospect that VNS-sound pairing drives these behavioral changes by altering responses throughout the auditory pathway, both subcortically and cortically. Future studies are needed to dissect the functional consequences of the effects of VNS in each auditory region. This defines testable hypotheses for future human and animal studies to characterize plasticity throughout the auditory pathway and functional consequences in the context of disease.

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DISCLAIMERS

Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the Defense Advanced Research Projects Agency Biological Technologies Office.

DISCLOSURES

M. P. Kilgard is a paid consultant for MicroTransponder, Inc., and C. T. Engineer is married to an employee of MicroTransponder, Inc.

AUTHOR CONTRIBUTIONS

M.B., W.A.V., M.P.K., and C.T.E. conceived and designed research; M.B., W.A.V., N.A.M., E.A.F., E.P.B., and S.V. performed experiments; M.B., W.A.V., S.V., M.P.K., and C.T.E. analyzed data; M.B., W.A.V., M.P.K., and C.T.E. interpreted results of experiments; M.B. and C.T.E. prepared figures; M.B. and C.T.E. drafted manuscript; M.B., W.A.V., N.A.M., E.A.F., E.P.B., S.V., M.P.K., and C.T.E. edited and revised manuscript; M.B., W.A.V., N.A.M., E.A.F., E.P.B., S.V., M.P.K., and C.T.E. approved final version of manuscript.

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