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Cortical Map Plasticity as a Function of Vagus Nerve Stimulation Rate

E.P. Buell^{1,2}, K.W. Loerwald^{1,2}, C.T. Engineer^{1,2}, M.S. Borland^{1,2}, J.M. Buell¹, C.A. Kelly², I.I. Khan², S.A. Hays^{1,3}, and M.P. Kilgard^{1,2}

¹Texas Biomedical Device Center, Richardson, TX 75080

²The University of Texas at Dallas, School of Behavioral Brain Sciences, 800 West Campbell Road, GR 41, Richardson, TX 75080-3021

³The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, BSB 11; Richardson, TX 75080

Abstract

Background—Repeatedly pairing a brief train of vagus nerve stimulation (VNS) with an external event can reorganize the sensory or motor cortex. A 30 Hz train of sixteen VNS pulses paired with a tone significantly increases the number of neurons in primary auditory cortex (A1) that respond to tones near the paired tone frequency. The effective range of VNS pulse rates for driving cortical map plasticity has not been defined.

Objective/Hypothesis—This project investigated the effects of VNS rate on cortical plasticity. We expected that VNS pulse rate would affect the degree of plasticity caused by VNS-tone pairing.

Methods—Rats received sixteen pulses of VNS delivered at a low (7.5 Hz), moderate (30 Hz), or high (120 Hz) rate paired with 9 kHz tones 300 times per day over a 20 day period.

Results—More A1 neurons responded to the paired tone frequency in rats from the moderate rate VNS group compared to naïve controls. The response strength was also increased in these rats. In contrast, rats that received high or low rate VNS failed to exhibit a significant increase in the number of neurons tuned to sounds near 9 kHz.

Conclusion—Our results demonstrate that the degree of cortical plasticity caused by VNS-tone pairing is an inverted-U function of VNS pulse rate. The high temporal precision of VNS-tone pairing protocols helps to explain the cellular mechanisms responsible for the beneficial effects of precisely timed VNS during restoration of sensory or motor function.

Keywords

plasticity; auditory cortex; vagal nerve stimulation; inverted-u function; frequency

*Corresponding author information: The University of Texas at Dallas, School of Behavioral and Brain Sciences, 800 West Campbell Road, BSB11, Richardson, TX 75080-3021, ebuell@utdallas.edu, Tel: 1-469-939-0422.

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Introduction

Repeatedly pairing VNS with a sensory or motor event drives robust experience-dependent plasticity in the auditory and motor cortices [1–4]. Pairing brief bursts of VNS with a movement expands the area associated with that movement in the motor cortex and increases the synaptic connectivity from the associated muscles to motor cortex [5–7]. VNS paired with a pure tone increases the number of cortical neurons that respond to the paired tone [1,4]. VNS-directed neural plasticity is being developed to treat a variety of disorders, including tinnitus and stroke [3,4,8–15]. Optimization of VNS parameters is a critical next step in the development of these therapies. Parameter optimization may also help to elucidate the neural mechanisms that allow brief bursts of VNS to direct plasticity that is long lasting and specific to the experience paired with VNS.

Previous studies indicate that VNS parameters, such as the interval between stimulation events, stimulation intensity and stimulation rate, can influence the effectiveness of VNS in producing cortical plasticity [1,16,17]. The stimulation intensity paired with a tone drives cortical plasticity as an inverted-U function [1]. Moderate intensity (0.4–0.8 mA) VNS drives plasticity, while high intensity (1.2–1.6 mA) VNS does not. VNS-tone pairing produces maximal plasticity when the interval between events is greater than thirty seconds [16]. Increasing VNS pulse width enhances plasticity when low currents are used [17]. These studies support the notion that the timing and intensity of stimulation influence the degree of VNS-dependent plasticity, but the effect of VNS rate on cortical plasticity remain unknown.

Changing VNS rate has significant effects on seizure suppression and activity patterns in brainstem nuclei [18]. VNS drives robust activity in the locus coeruleus (LC) across a wide range of stimulation rates [19]. In contrast, activity in the dorsal raphe increases as an inverted-U function of VNS rate [20]. Some studies report that moderate VNS rates are most effective in suppressing seizures [21,22], while others report that higher stimulation rates are most effective [23]. Together, these studies indicate that VNS pulse frequency affects engagement of neuromodulatory networks and may impact the clinical efficacy of VNS, pointing to the relevance of optimizing this parameter.

The purpose of this study was to evaluate whether delivering VNS-tone therapy using different VNS rates changes the degree of plasticity in the auditory cortex. We tested the hypothesis that stimulation rate would affect the degree of VNS-dependent plasticity in primary auditory cortex by evaluating cortical changes after pairing low (7.5 Hz), moderate (30 Hz), or high (120 Hz) rate VNS with a 9 kHz tone. Understanding how brain plasticity is affected by the rate of VNS paired with an event is critical to identify the optimal stimulation settings for clinical applications.

Methods

Data from forty-one adult female Sprague–Dawley rats (250–400 g) was analyzed in this experiment. Seventy-five rats were implanted with vagus nerve cuff stimulators, as in our previous studies [1,4,5,24]. Animals were removed from this study due to surgical deaths

(n=16), head cap failure (n=7), or a broken VNS lead (n=10, confirmed by an inability to reduce blood oxygen saturation in anesthetized rats via continuous VNS) [25]. All animals included in the analysis demonstrated a drop in blood oxygen saturation during the terminal surgery procedure which indicates a functional cuff. Twelve rats served as naïve controls and were not implanted. Rats were interleaved and randomly assigned to receive low rate (7.5 Hz) VNS (n=9), moderate rate (30 Hz) VNS group (n=10), or high rate (120 Hz) VNS group (n=10). All rats were housed in a 12:12 hour reversed light–dark cycle. All handling, housing, stimulation, and surgical procedures were approved by The University of Texas at Dallas Institutional Animal Care and Use Committee and by the Animal Care and Use Review Office of the United States Army Medical Research and Materiel Command Office of Research Protections.

Vagus nerve surgery

Animals were anesthetized using ketamine hydrochloride (80 mg/kg, intraperitoneal (IP) injection) and xylazine (10 mg/kg IP). Animals were given supplemental doses as needed. A Ringer's lactate and dextrose solution was given to the rats to prevent dehydration. Cefotaxime sodium (2 × 10 mg, subcutaneous (SC) injection) solution was administered to animals after the surgery to prevent infection. All animals were implanted with a skull mounted connector. Marcaine (1 mL, SC) was injected into the scalp at the incision site. Bregma and lambda landmarks on the skull were exposed by an incision of the scalp running anterior to posterior. Bone screws were placed near the bregma suture, near the sagittal suture, near the lambda suture and over the cerebellum. The connector headcap was secured to the screws with acrylic. A custom made cuff electrode was placed around the left vagus nerve as in previous studies [1,4,5,24]. Because the right vagus nerve innervates the sino-atrial node, only the left vagus nerve was implanted to avoid cardiac complications [26]. Brain activation following left VNS is bilateral [27].

After lidocaine (2%, 0.5 mL SC) was injected in the neck at the incision site, the vagus nerve was exposed through an incision and blunt dissection of the neck. The cuff electrode was placed around the nerve. Two Teflon coated multi stranded platinum iridium wire leads from the electrode were run subcutaneously between the eye and the ear, and connected to the headcap on top of the skull. The headcap and cuff pins were secured with acrylic. After connection, confirmation of cuff function was obtained by observation of a blood oxygen drop while stimulating the nerve (A-M Systems isolated pulse stimulator model 2100). The animal's neck was sutured closed, and a topical antibiotic cream was applied to the neck and head. Animals were given amoxicillin (5 mg) and carprofen (1 mg) for 2 days after surgery to prevent infection and facilitate recovery.

Vagus nerve stimulation

After 5–7 days of recovery from surgery, the rats received VNS paired with a 9 kHz tone 300 times per day for 20 days, as in previous studies [1,4,17]. Animals were placed in a 25 cm × 25 cm × 25 cm wire cage within a 50 cm × 60 cm × 70 cm chamber lined with acoustic insulating foam. A speaker hanging above the cage presented a 9 kHz tone at 50 dB SPL paired 150 ms after the onset of VNS. VNS was delivered using 100 µs charge balanced biphasic pulses. Experimental groups received 16 pulses of VNS delivered at rates of 7.5 Hz,

30 Hz, or 120 Hz, such that the pulses are distributed over 2000, 500, or 125 ms (Figure 1). VNS intensity was 0.8 mA for all experimental groups. Stimulation was delivered 50% of the time every 15 seconds, such that the average interval between stimulations was 30 seconds. The impedance of the cuff electrode was checked daily and only animals with measured cuff impedances between 1 and 10 k Ω remained in the study.

Auditory cortex recordings

Using standard procedures, multi-unit responses were collected from the auditory cortex twenty-four hours after the final VNS-tone pairing session [1,4,16,28]. Rats were anesthetized with sodium pentobarbital (50 mg/kg) and anesthesia was maintained using supplemental doses of diluted pentobarbital by evaluating anesthesia levels between cortex recordings every 30–60 minutes (0.2–0.4 ml, 8 mg/ml). One mL of a one to one ratio of dextrose (5%) and standard Ringer's lactate solution was administered between every recording to prevent dehydration. To minimize respiratory problems under pentobarbital, a tracheotomy was performed. In addition, cerebral edema was minimized by opening a cisternal drain. A craniotomy exposed the right primary auditory cortex. A durotomy over this exposed section of cortex further exposed the cortex and allowed electrodes to be placed easily. A thin film of silicone oil was placed over the exposed cortex to maintain hydration. Four parylene coated tungsten microelectrodes (1.5–2.5 M Ω , FHC) were lowered to layer IV/V of the primary auditory cortex. Pure tones were delivered from a speaker positioned 10 cm from the animal's left ear in a foam-shielded double-walled sound-attenuated chamber. Neuronal responses were recorded using Brainware software (TDT). Recording sites were tracked using a photo of the cortex in Canvas 16 software. Various tones were presented at 81 logarithmically spaced frequencies spanning 1–32 kHz in 0.0625 octave steps at 16 intensities from 0 to 75 dB SPL in 5 dB steps. These tones were used to determine auditory tuning curves at each of the evaluated sites (Figure 2). Tones were presented every 500 ms and randomly interleaved. For each auditory map, experimenters were blind to the animal's stimulation parameters. At the conclusion of the map, cuff function was confirmed by testing an oxygen saturation drop in response to 10 seconds of continuous vagus nerve stimulation. If an O₂ saturation drop could not be confirmed, the animal was removed from the study.

Data analysis

All groups were analyzed using an automated MATLAB program. This program determines receptive fields based on the characteristics of responses at each site as determined by previous work [28]. The characteristic frequency (CF) was defined at the frequency at which the lowest intensity evokes a response at a particular site. Response threshold was defined as the lowest intensity capable of evoking a response (Figure 2). Spontaneous firing rate was the rate of firing evoked across all tone frequencies when presented at an amplitude of 0 dB. The time it takes for maximum neural responses to occur was defined as the peak latency. For percentage of cortex responding analysis, experimental and control groups were compared using a one-way ANOVA with post-hoc Bonferroni correction for multiple comparisons. For response strength analysis, a mixed-effects model using SPSS software was used to account for the different number of sites recorded for each animal. The fixed factor was the experimental group and the random factor was the individual animals. Simple

contrast analysis was used to determine whether there were statistically significant differences in response strength after VNS-tone pairing.

Results

VNS-tone pairing shifts A1 receptive fields if moderate VNS is used

Plasticity in the auditory cortex was evaluated by quantifying the percentage of A1 neurons that responded to individual tones across a wide range of frequency and intensity. In experimentally naïve control rats, approximately 40% of A1 neurons responded to 50 dB SPL 8–16 kHz tones. VNS-tone pairing significantly altered the percentage of A1 neurons responding to the paired tone frequency ($F(3, 37) = 11$, $p = 0.00003$). After pairing 9 kHz tones with moderate rate VNS, significantly more A1 neurons responded to these tones (Figure 3, $p = 0.001$). This observation represents the fifth independent replication of the finding that pairing VNS with tones alters the organization of auditory cortex [1,4,16,17]. Pairing the same tones with an identical amount of VNS (i.e. sixteen 100 μ s pulses at 0.8 mA) delivered at a lower or higher rate did not significantly alter frequency tuning compared to control rats ($p > 0.05$). These findings demonstrate that cortical plasticity is an inverted-U function of the VNS rate used during VNS-tone pairing.

Figure 4 shows the proportion of A1 neurons that responded to each tone frequency and intensity combination. Across all groups, more A1 neurons were activated as sound intensity was increased (Figure 4, A–D). The only group with a consistently expanded response to tones was the moderate VNS group, which exhibited a significant increase in the number of neurons tuned to tones with a frequency between 8 and 16 kHz and intensities above 20 dB SPL. This difference is best seen by subtracting the proportion of A1 neurons that responded in experimentally naïve control rats from the proportion of A1 that responded in the moderate VNS group (Figure 4F). Rats from the low rate VNS group and the high rate VNS group did not exhibit a significant increase in the number of A1 neurons that responded to any tone when compared to control rats ($p > 0.05$, Figures 4E and 4G).

VNS-tone pairing alters the A1 response strength if moderate VNS is used

VNS-tone pairing significantly altered the number of action potentials evoked in A1 in response to frequencies near the paired tone ($F(3, 34.95) = 6.2$, $p = 0.002$). Tones between 8–16 kHz evoked significantly more action potentials in the moderate rate VNS group compared to the control group (0.93 ± 0.08 spikes vs. 0.62 ± 0.07 spikes, $p = 0.01$, Figure 5). In contrast, the low rate VNS group and the high rate VNS group response strength to these tones were not significantly different than naïve control rats ($p > 0.05$, Figure 5). There was a significant interaction between experimental group and tone intensity ($F(45, 33930.85) = 2.83$, $p < 0.0001$, Figure 6). VNS-tone pairing produced a 50% increase in the A1 response to the paired tone frequency across a wide range of tone intensities (Figure 6) when moderate rate VNS ($p = 0.03$) was used and failed to alter the cortical response when higher or lower VNS rates were used ($p > 0.05$). This observation is consistent with the inverted-U function for receptive field plasticity.

No significant changes in response threshold ($F(3, 40.43) = 1.24, p = 0.31$), spontaneous firing rate ($F(3, 34.12) = 0.21, p = 0.89$), bandwidth ($F(3, 37.66) = 2.47, p = 0.08$), or response peak latency ($F(3, 44.82) = 0.22, p = 0.88$) were observed in any experimental group compared to the control group.

Discussion

More than a dozen studies have confirmed that repeatedly pairing VNS with an auditory or motor event can drive highly specific and long-lasting plasticity in primary auditory or motor cortex, respectively [1,2,4–6,11,16,17,24,29–34]. Since VNS-directed neural plasticity appears to enhance recovery of auditory and motor function following injury [3,4,6,8,12,14,15,34], it is critical to understand the optimal conditions to drive therapeutic plasticity. Previous papers have systematically evaluated how the stimulation intensity, spacing between pairings, pulse width, and number of VNS-tone pairings influence the degree of neural plasticity [1,16,17]. This paper systematically explored the influence of VNS rate and provides the first demonstration that only moderate rate VNS-tone pairing produces plasticity. When VNS is delivered at a fast or slow rate, VNS-tone pairing fails to produce significant cortical map plasticity. This finding helps to clarify potential mechanisms through which VNS-tone pairing drives neural plasticity and suggests that future studies of other VNS rates near 30 Hz are needed to determine whether 30 Hz is the most effective VNS rate for directing neural plasticity.

Multiple modulatory neurotransmitters influence plasticity, and phasic and tonic levels of these neurotransmitters can have very different effects [35–37]. Numerous studies have documented that VNS alters cortical function through activation of the cholinergic nucleus basalis (NB) and noradrenergic locus coeruleus (LC) [19,29,38–42]. VNS produces robust activation of LC neurons [19]. LC activity releases norepinephrine, which is known to modulate synaptic plasticity [35]. Sixteen pulses of VNS produce the same number of LC action potentials regardless of whether the rate is 7.5, 30, or 120 Hz. However, the temporal pattern evoked is quite different, because the same number of action potentials is spread out over 2000, 500, or 125 ms. It is possible that the inverted-U function of VNS rate on cortical plasticity results from inadequate norepinephrine concentration to activate the high affinity alpha receptors at low rates and overly high norepinephrine concentration that activates low affinity beta receptors at high rates [43]. Pharmacology studies will be needed to test this hypothesis.

A second possible explanation for the inverted-U function of VNS rate on plasticity is based on the observation that neurons in the dorsal raphe nucleus (DRN) are selectively activated by moderate VNS rates. VNS increases DRN firing as an inverted-U function of VNS rate [20]. Since serotonin is known to modulate synaptic plasticity, such changes in DRN activity could contribute to our results [35].

Previous studies revealed that VNS effects on memory, cortical plasticity, hippocampal LTP, and DRN activity are an inverted-U function of current intensity [1,20,44–46]. These effects of current likely arise in the periphery because moderate currents recruit large diameter vagus nerve fibers without recruiting small diameter nerve fibers, which limit plasticity via

an unknown mechanism [47]. In contrast, the inverted-U relationship between plasticity and VNS rate must result from a central action because vagus nerve fibers can fire at rates up to 150 Hz. Thus, VNS current and rate both appear to influence plasticity as inverted-U functions, but appear to result from distinctly different mechanisms. Given the richness of synaptic plasticity rules, future studies are needed to empirically determine how VNS rate, VNS duration, and relative VNS-tone timing influence neural plasticity. It remains to be seen whether other VNS paradigms are more effective at directing neural plasticity.

Understanding how the VNS parameter space influences plasticity may help to elucidate the cellular mechanisms that influence cortical plasticity by providing a clinically viable method to deliver brief bursts of neuromodulator release [11,48]. These findings may facilitate determination of optimal VNS parameters for clinical application. Recent clinical studies of targeted VNS pairing plasticity therapies for tinnitus and stroke suggest non-responder rates between 40 and 50%. These therapies would be more effective if parameter optimization could be used to reduce the number of non-responders [14,49].

In summary, the results in this experiment demonstrate that the rate of tone-paired VNS influences the extent of cortical change. Repeatedly pairing sixteen 0.8 mA pulses of VNS delivered at 30 Hz with a tone effectively drives map expansion in the auditory cortex. The same amount of VNS fails to drive plasticity when delivered at a faster (120 Hz) or slower (7.5 Hz) rate. This inverted U-function of rate likely arises from a central mechanism regulating synaptic plasticity. A better understanding of how VNS regulates these mechanisms could potentially lead to the development of novel adjuvant therapies to improve recovery from sensory, emotional, or motor disorders [3,4,7,11,13–15,48,50].

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Highlights

- VNS paired with a sound strengthens the auditory cortex (A1) response to the sound.
- VNS-tone pairing directs A1 plasticity as an inverted-U function of VNS pulse rate.
- High temporal precision is necessary in VNS-tone pairing protocols.
- Insights derived from this work could lead to development of adjuvant therapies.

Figure 1.

Schematic diagram of the VNS-tone train pairing procedure. A train of biphasic pulses were delivered to the left vagus nerve via a cuff electrode. Each group received VNS at one of three different rates: 7.5 Hz (2 s), 30 Hz (0.5 s), or 120 Hz (0.125 s). Rats received VNS paired with a 9 kHz tone every 30 s, 300 times during each 2.5 hour session for 20 days. Cortical recordings were made 24 hours after the last pairing session.

Figure 2.

Example tuning curve obtained from electrophysiological recordings in the primary auditory cortex (A1). The characteristic frequency (CF) is defined as the frequency with neuronal responses at the lowest intensity. Responses at higher intensity (dB) levels are represented by bandwidth (BW). BW10, BW20, BW30, and BW 40 are responses at 10, 20, 30, and 40 dB above threshold intensity, respectively.

Figure 3.

VNS-tone pairing reorganizes the auditory cortex frequency map as a non-monotonic function of VNS rate. Moderate (30 Hz) rate VNS group exhibits a significant expansion of the percentage of A1 responding to 8–16 kHz frequency tones at 50 dB SPL after 20 days of VNS tone-pairing ($p < 0.005$). Low (7.5 Hz) and high (120 Hz) rate VNS groups do not yield a significant increase in neurons responding to 8–16 kHz ($p > 0.05$).

Figure 4.

Percent of A1 responding to each tone frequency intensity combination for naive control rats (A), low VNS rate (7.5 Hz) rats (B), moderate rate (30 Hz) VNS rats (C), and high rate (120 Hz) VNS rats (D). The difference between the percent of A1 responding for low VNS rats and control rats reveals the range of tones that evoked a response in more neurons (red) or fewer neurons (blue) (E). The difference between the percent of A1 responding in moderate rate VNS rats and control rats (F) and high rate VNS rats and control rats (G) is also shown. Note that moderate intensity VNS-tone pairing alters the cortical response more than low and high rate VNS-tone pairing. White lines delineate the frequency intensity combinations which activate significantly more neurons after VNS pairing with a 9 kHz tone ($p < 0.05$).

Figure 5.

A1 action potentials evoked by 8–16 kHz 50 dB SPL tones significantly increases after animals receive moderate (30 Hz) VNS. This change is not observed with low (7.5 Hz) or high (120 Hz) rate VNS.

Figure 6.

Number of spikes evoked with increasing intensity in naive control, low rate VNS (7.5 Hz), moderate rate VNS (30 Hz), and high rate VNS (120 Hz) animals. Moderate rate VNS yields significantly stronger responses than control for intensities greater than 25 dB SPL ($p < 0.05$).