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Cortical Map Plasticity as a Function of Vagus Nerve Stimulation Intensity

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ABSTRACT

Background: Pairing sensory or motor events with vagus nerve stimulation (VNS) can reorganize sensory or motor cortex. Repeatedly pairing a tone with a brief period of VNS increases the proportion of primary auditory cortex (A1) responding to the frequency of the paired tone. However, the relationship between VNS intensity and cortical map plasticity is not known.
Objective/hypothesis: The primary goal of this study was to determine the range of VNS intensities that can be used to direct cortical map plasticity.
Methods: The rats were exposed to a 9 kHz tone paired with VNS at intensities of 0.4, 0.8, 1.2, or 1.6 mA.
Results: In rats that received moderate (0.4–0.8 mA) intensity VNS, 75% more cortical neurons were tuned to frequencies near the paired tone frequency. A two-fold effective range is broader than expected based on previous VNS studies. Rats that received high (1.2–1.6 mA) intensity VNS had significantly fewer neurons tuned to the same frequency range compared to the moderate intensity group.
Conclusion: This result is consistent with previous results documenting that VNS is memory enhancing as a non-monotonic relationship of VNS intensity.

Introduction

Repeated pairing of a motor or sensory event with a brief burst of vagus nerve stimulation causes a long-lasting increase in the number of cortical neurons that are active during the paired event. For example, pairing a pure tone with VNS expands the region of primary auditory cortex that responds to the paired tone frequency [1]. Cortical map plasticity is associated with skill learning in humans and animals [2,3]. For example, the cortical map of the left hand is increased in professional string musicians [4]. Pairing a rapid train of tones with VNS increases the number of A1 neurons that can respond to rapidly presented sounds [5]. Pairing a movement with VNS expands the region of primary motor cortex that produces the paired movement [6]. These studies demonstrate that VNS-event pairing can drive neural plasticity that is experience-dependent and long lasting.

Cortical plasticity is also associated with rehabilitation and a growing body of evidence suggests that VNS-directed plasticity could be used to treat a variety of clinical conditions [7–9]. Animal studies in models of chronic tinnitus, stroke, and traumatic brain injury suggest that pairing VNS with auditory or motor rehabilitation can improve behavioral outcomes [1,10–13]. A pilot study in tinnitus patients confirmed that pairing VNS with tones that exclude the tinnitus frequency can decrease the loudness and distress of their tinnitus [14,15]. Although these benefits were long lasting, they were incomplete and no patient has yet reported being completely tinnitus free. One possible explanation for the incomplete recovery is that the VNS parameters used to drive therapeutic plasticity have not been optimized.

Increasing VNS current can recruit more vagal nerve fibers which might improve efficacy [16]. Greater VNS intensity triggers greater release of plasticity enhancing neuromodulators [17]. Increasing VNS intensity improves outcomes in epilepsy and depression patients [18,19]. Though VNS changes cortical maps, it is not yet known whether such changes vary with stimulus parameters such as intensity.

Several studies have shown that more VNS is not more effective. Many effects of VNS are an inverted U function of stimulation intensity. Moderate VNS intensity is effective at enhancing memory and plasticity in the hippocampus, while high intensity VNS is ineffective. This inverted U function of VNS intensity on memory was observed in both rats [20,21] and humans [22]. Long term

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potentiation in the rat dentate gyrus is increased after moderate intensity VNS [23]. High intensity VNS has no effect. Similarly, the survival of progenitor cells in the rat dentate gyrus is increased after moderate intensity VNS, but not high intensity VNS [24]. It is not yet known whether VNS-directed cortical map plasticity also exhibits an inverted U function of VNS intensity.

The first goal of this study was to determine whether high intensity VNS paired with tones would result in greater or less map plasticity compared to moderate intensity VNS paired with tones. The second goal was to determine whether it is possible to drive cortical map plasticity using less intense VNS than current protocols. High density microelectrode mapping was conducted in thirty-nine rats that heard the same tones paired with VNS at 0.4 mA, 0.8 mA, 1.2 mA, and 1.6 mA.

Methods

Forty-nine female Sprague–Dawley rats (250–370 g) were analyzed in this experiment. Sixty-eight rats were implanted with vagus nerve stimulators, as in our previous studies [1,5,6]. Twenty-nine rats were removed from this study because of surgical deaths, head cap failures, leads breaking and a lack of VNS induced drop in blood oxygen (O_2). A lack of VNS induced drop in blood O_2 indicates that the VNS implant was nonfunctional [25]. Experimental rats were randomly assigned to one of four groups and they were interleaved in time. Nine rats received VNS at an intensity of 0.4 mA. Ten rats received VNS at an intensity of 0.8 mA. Ten rats received VNS at an intensity of 1.2 mA. Ten rats received VNS at an intensity of 1.6 mA. Ten additional rats were used as naive controls. 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.

Vagus nerve surgery

Rats were anesthetized using ketamine hydrochloride (80 mg/kg, intraperitoneal (IP) injection) and xylazine (10 mg/kg IP) and given supplemental doses as needed. A standard Ringer's lactate solution was given to the rats to prevent dehydration throughout the surgery and recovery. Doses of cefotaxime sodium (2 × 10 mg, subcutaneous (SC) injection) solution were given to the rats before and after the surgery to prevent infection. Sixty-eight of the rats were implanted with a skull mounted connector. Rats were placed in a stereotaxic frame, and marcaine (1 mL, SC) was injected into the scalp at the incision site. An initial incision and blunt dissection of the scalp exposed the bregma and lambda landmarks on the skull. Four bone screws were manually drilled into the skull, one near the bregma suture, one near the sagittal suture, one near the lambda suture and one over the cerebellum. The connector was attached to the cranial screws with acrylic. The experimental groups of rats were implanted with a custom made cuff electrode around the left vagus nerve as used in previous studies [1,5,6]. As in humans, only the left vagus nerve was stimulated because the right vagus nerve contains efferents that stimulate the sino-atrial node and can cause cardiac complications [26]. Additionally, unilateral VNS causes bilateral effects.

The cuff electrode consisted of two Teflon coated multi stranded platinum iridium wires connected to a 4 mm section of Micro Renethane tubing. The wires were spaced 1.5 mm apart along the length of the tubing. An 8 mm region of the wires lining the inside circumference of the tube was stripped of the insulation. A cut was made lengthwise along the tubing to allow the cuff to be wrapped around the nerve and then closed with silk threads. Lidocaine (0.5 mL SC) was injected in the neck at the incision site. An incision and blunt dissection of the muscles in the neck exposed the left vagus nerve. The vagus nerve was placed into the cuff electrode, and leads from the electrode were tunneled subcutaneously to the top of the head. Once the leads were connected to the skull mounted connector, the connector was encapsulated in acrylic. Immediately after surgery, the vagus nerve was stimulated and an oxygen saturation drop was observed to make sure the cuff was working properly. A topical antibiotic cream was applied to both incision sites and the rats were given amoxicillin (5 mg) and carprofen (1 mg) for 2 days after surgery to prevent infection and facilitate recovery.

Vagus nerve stimulation

After 2–7 days of recovery from surgery, the rats were placed in a 25 cm × 25 cm × 25 cm wire cage, located inside of a 50 cm × 60 cm × 70 cm chamber lined with acoustic insulating foam. Sounds were presented from a speaker hanging above the wire cage. The rats were exposed to a 9 kHz 50 dB SPL tone paired with VNS 300 times per day for 20 days (Fig. 1). Depending on the experimental group, each 100 μs charge balanced biphasic pulse was delivered at an intensity of 0.4 mA, 0.8 mA, 1.2 mA or 1.6 mA. The stimulation was delivered as a train of 15 pulses presented at 30 Hz (500 ms train duration). The average interval between stimulations was 30 s. To prevent rats from anticipating stimulation timing, there was a 50% chance that vagus nerve stimulation was delivered every 15 s. Each pairing session lasted two and a half hours. The impedance of the cuff electrodes was between 1 and 10 kΩ. The impedance was monitored every day and was stable across the entire training duration for all of the rats. Sixteen rats were detected with broken cuffs (high electrode impedances) and were removed from the study.

Auditory cortex recordings

Twenty-four hours after the last pairing, rats were anesthetized with sodium pentobarbital (50 mg/kg). Anesthesia depth was maintained throughout the procedure with a supplemental dose of diluted pentobarbital as needed or every 30–60 minutes (0.2–0.4 ml, 8 mg/ml). Dehydration was prevented by using a one to one ratio of dextrose (5%) and standard Ringer's lactate solution. A tracheotomy was performed to minimize breathing problems and breathing sounds. A cisternal drain was made to minimize cerebral edema. The section of the skull over the temporal ridge was removed to expose the right primary auditory cortex. The dura was removed and the cortex was maintained under a thin film of silicone oil to prevent desiccation. Four parylene coated tungsten microelectrodes (1.5–2.5 MΩ, FHC) were lowered simultaneously to depths of approximately 600 μm to ensure that they were in layer IV/V of the primary auditory cortex. During the acute electrophysiology recordings, sounds were delivered in a foam-shielded double-walled sound-attenuated chamber via a speaker positioned directly opposite the left ear at a distance of 10 cm. Frequency and intensity calibrations were performed with an ACO Pacific microphone (PS9200-7016) and TDT SigCal software. Multiunit neural activity was captured using a software program (Brainware, TDT) and each recording site location was logged on a detailed digitized photo of the exposed auditory cortex. Auditory frequency tuning curves were determined at each site by presenting tones at 81 logarithmically spaced frequencies spanning 1–32 kHz in 0.125 octave steps at 16 intensities from 0 to 75 dB SPL in 5 dB steps. The tones were randomly interleaved and presented every 500 ms. Experimenters were blind to the experimental conditions of each rat during electrophysiology recordings. The vagus nerve was stimulated and an oxygen saturation drop was observed to make sure the cuff was working properly, at the end of the electrophysiology recording. Two