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VAGUS NERVE STIMULATION MODULATES CORTICAL SYNCHRONY AND EXCITABILITY THROUGH THE ACTIVATION OF MUSCARINIC RECEPTORS

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Abstract—Vagus nerve stimulation (VNS) is an FDA approved treatment for drug-resistant epilepsy and depression. Recently, we demonstrated the capacity for repeatedly pairing sensory input with brief pulses of VNS to induce input specific reorganization in rat auditory cortex. This was subsequently used to reverse the pathological neural and perceptual correlates of hearing loss induced tinnitus. Despite its therapeutic potential, VNS mechanisms of action remain speculative. In this study, we report the acute effects of VNS on intra-cortical synchrony, excitability, and sensory processing in anesthetized rat auditory cortex. VNS significantly increased and decorrelated spontaneous multi-unit activity, and suppressed entrainment to repetitive noise burst stimulation at 6–8 Hz but not after application of the muscarinic antagonist scopolamine. Collectively, these experiments demonstrate the capacity for VNS to acutely influence cortical synchrony and excitability and strengthen the hypothesis that acetylcholine and muscarinic receptors are involved in VNS mechanisms of action. These results are discussed with respect to their possible implications for sensory processing, neural plasticity, and epilepsy.

Key words: vagus nerve stimulation, epilepsy, acetylcholine, auditory cortex, scopolamine, synchrony.

Electrical stimulation of the vagus nerve (VNS) has been used to treat more than 60,000 patients with drug-resistant epilepsy and is under investigation as a treatment for several other neurological disorders and conditions. Among these, VNS increases alertness (Malow et al., 2001; Rizzo et al., 2003), and enhances recovery of motor and cognitive function in animal models of traumatic brain injury (Smith et al., 2005). Recently, we developed a novel and potentially therapeutic method for inducing targeted neural plasticity by repeatedly pairing brief pulses of VNS with acoustic sensory input. This technique was subsequently used to successfully reverse the neurological as well as the perceptual correlates of hearing loss induced tinnitus in adult animals. VNS-tone pairing closely replicated prior studies which induced robust cortical reorganization by pairing tones with electrical stimulation of the cholinergic nucleus basalis (NB) (Kilgard and Merzenich, 1998a).

Since VNS-tone pairing induced cortical plasticity to a degree strikingly similar to NB-tone pairing, similar mechanisms may underlie both effects. NB stimulation has been shown to acutely enhance sensory processing (Goard and Dan, 2009), as well as learning and memory (Miasnikov et al., 2008) in several animal models and is currently under investigation for the treatment of cognitive deficits in human subjects with mild to moderate Alzheimer's disease. The possibility of replicating these effects with VNS (a less invasive technique, as opposed to the highly invasive nature of NB stimulation) substantiates further investigation into VNS mechanisms of action which are not yet established. Consequently, the purpose of the present study was first to test whether or not, and to what extent, brief activation of VNS acutely modulates cortical synchrony, excitability, and sensory processing in control conditions as well as in the presence of the muscarinic antagonist scopolamine.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Sprague–Dawley rats weighing 350–400 g were used in this study. All experimental procedures comply with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University committee on Animal Research at the University of Texas at Dallas. The number of animals was kept to the minimum necessary to ensure statistical validity. All animals were maintained on a normal 12 h/12 h light/dark schedule, with food and water available *ad libitum*.

Surgical protocol

Animals were anesthetized with sodium pentobarbital (50 mg/kg; Sigma-Aldrich Corp., St. Louis, MO, USA). Supplemental pentobarbital (8 mg/ml) was periodically administered i.p. to maintain a state of areflexia (evaluated by a lack of hind leg withdrawal upon toe-pinch) throughout the surgical procedures and during the recording session. Body temperature was maintained at 37 °C with a heating pad (ATC-1000 WPI). After a surgical level of anesthesia was obtained, the skull was fixed in a palato-orbital restraint and exposed through a rostrocaudal incision. The temporalis muscle was resected and the dura over the right auditory cortex was exposed through a craniotomy of approximately 6 mm by 4 mm. The dura was resected and the cortex was maintained in a bath of sterile physiological saline to prevent desiccation.

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Abbreviations: LC, locus coeruleus; PSTH, peri-stimulus time histogram; RRTFs, repetition rate transfer functions; VNS, vagus nerve stimulation.

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Primary auditory cortex was initially localized based on auditory evoked latencies (10–20 ms) and frequency tuning characteristics using bipolar tungsten electrodes (FHC) (Kilgard and Merzenich, 1998a). Next, 16-channel tungsten/polyimide Omnetics based micro-wire arrays (2 by 8, Tucker-Davis-Technologies, 500 μm electrode separation) were inserted to a depth of 600 μm, corresponding approximately to layer IV/V and the entire exposed cortex was covered with Kwik-Cast silicone elastomer (WPI).

For vagal nerve exposure, a rostro–caudal incision was made in the ventral aspect of the neck on the left side. Using glass probes, muscles were separated and the left cervical-vagus nerve was separated from the carotid artery (see Fig. 1A, B). The vagus nerve was gently guided into a cuff constructed from Micro-Renathane® (0.080" O.D.×0.040" I.D.) tubing and braided platinum iridium (.006" diameter) wire with Teflon insulation. The platinum-iridium wires lined the inside of the cuff, with the insulation removed to provide conductivity, allowing bipolar stimulation only around the nerve. The platinum-iridium wires from the cuff to the head attachment were threaded subcutaneously along the neck to the top of the skull as described previously (Engineer et al., 2011).

Vagus nerve stimulation parameters

Each 500 μs charge-balanced biphasic pulse was delivered at an intensity of 0.8 mA. The stimulation was delivered as a train of 15 pulses presented at 30 Hz (500 ms train duration). Preliminary studies confirmed these stimulation parameters sufficient to attenuate EEG amplitude without otherwise influencing behavior (Engineer et al., 2011).

The effect of VNS on spontaneous multi-unit activity was determined by creating a spontaneous firing rate modulation index (post-VNS−pre-VNS)/(post-VNS+pre-VNS) (Otazu et al., 2009). This index compared firing rate during a 100 ms window before each VNS train to activity in a 100 ms window beginning at the end of the stimulation train (repeated every 10 s for 100 repetitions).

Acoustic stimulation and recording

Tucker Davis Technologies (Alachua, FL, USA) neurophysiology hardware (RX6, RX5, RA16PA) and software (OpenEx Suite) were used for signal filtering (0.3–8 kHz), amplification (10,000×)

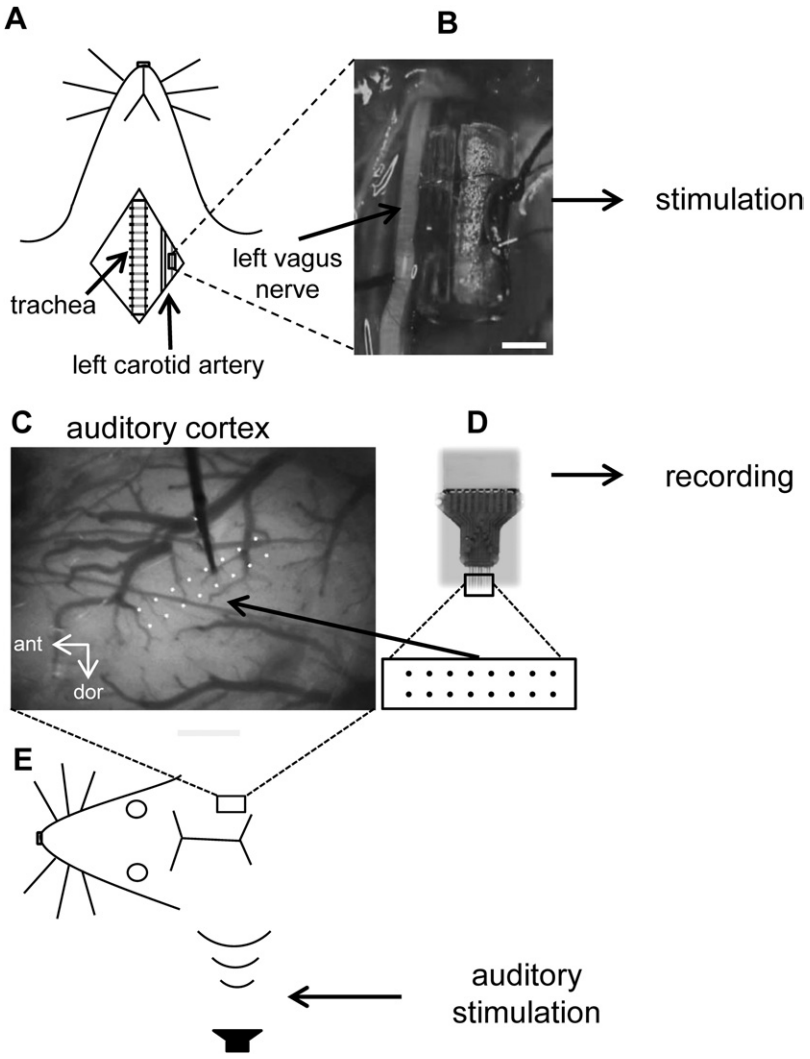

Fig. 1. Experimental setup. (A) Animals were anesthetized and the left vagus nerve was separated from the carotid artery and guided into a bipolar stimulation cuff shown in (B). The auditory cortex was then exposed and initially mapped using bipolar tungsten electrodes (dark region shown in the upper central portion in (C) before 16 channel arrays were inserted to a depth of 600 μm. Typical location of insertion and electrode arrangement are shown in (C, D) respectively. (E) Animals were then placed in a custom holder leaving the ears unobstructed and auditory stimuli were presented in free field. The scale bar shown in (B) = 1 mm.