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Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex

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Although sensory and motor systems support different functions, both systems exhibit experience-dependent cortical plasticity under similar conditions. If mechanisms regulating cortical plasticity are common to sensory and motor cortices, then methods generating plasticity in sensory cortex should be effective in motor cortex. Repeatedly pairing a tone with a brief period of vagus nerve stimulation (VNS) increases the proportion of primary auditory cortex responding to the paired tone (Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake J, Sudanagunta SP, Borland MS, Kilgard MP. 2011. Reversing pathological neural activity using targeted plasticity. Nature. 470:101–104). In this study, we predicted that repeatedly pairing VNS with a specific movement would result in an increased representation of that movement in primary motor cortex. To test this hypothesis, we paired VNS with movements of the distal or proximal forelimb in 2 groups of rats. After 5 days of VNS movement pairing, intracranial microstimulation was used to quantify the organization of primary motor cortex. Larger cortical areas were associated with movements paired with VNS. Rats receiving identical motor training without VNS pairing did not exhibit motor cortex map plasticity. These results suggest that pairing VNS with specific events may act as a general method for increasing cortical representations of those events. VNS movement pairing could provide a new approach for treating disorders associated with abnormal movement representations.

Keywords: cortical plasticity, cortical reorganization, motor cortex, motor training, vagus nerve stimulation

Introduction

Although sensory and motor systems support different functions, both systems can exhibit topographic reorganization of the cortex following training or injury. Tone training increases the representation of the tone in auditory cortex (Bakin and Weinberger 1990; Recanzone et al. 1993). Operant training on a tactile discrimination task significantly increased somatosensory cortical representation of the digit used in training (Recanzone et al. 1992; Wang et al. 1995). Similar changes occur in the motor cortex following training with precise digit movements (Nudo et al. 1996; Kleim et al. 1998). Motivation and frequency of training influence the degree of cortical map plasticity (Recanzone et al. 1992; Kleim et al. 2004; Kleim and Jones 2008). Deprivation caused by peripheral injury changes the organization of sensory and motor cortices (Hubel et al. 1977; Merzenich et al. 1983, 1984; Pons et al. 1991; Yu et al. 2010). For example, digit amputation or nerve transection causes receptive fields in the inactivated somatosensory cortex to shift to neighboring digits (Merzenich et al. 1983, 1984). Likewise, transecting the facial nerve reduces the number of motor cortex neurons that elicit vibrissae movements while increasing the number eliciting forelimb movements (Sanes et al. 1988). Targeted lesions to the sensory or motor cortex cause the surrounding healthy cortical areas to take on some of the damaged area's lost functionality (Eysel et al. 1999; Conner et al. 2005). Drugs that block reorganization of cortical representations in the sensory cortex also block reorganization in the motor cortex (Sawaki et al. 2002; Thiel et al. 2002; Meintzschel and Ziemann 2006; Thiel 2007). Collectively, these results suggest that the mechanisms regulating cortical plasticity are common to both sensory and motor cortices.

Vagus nerve sends afferents to a number of nuclei known to release neuromodulators associated with cortical plasticity, including the locus coeruleus, raphe nuclei, and the basal forebrain (Pettigrew and Kasamatsu 1978; Détári et al. 1983; Gu and Singer 1995; Henry 2002; Hassert et al. 2004; Dorr and Debonnel 2006). The vagus nerve has several efferents to major organs in the body, including the heart; however, a large portion of the vagus nerve consists of afferent connections to several targets in the midbrain (Henry 2002). Low-current stimulation of the left vagus nerve is a commonly used treatment for drug-resistant epilepsy that is associated with minimal risks (Binnie 2000; Ben-Menachem 2001; Groves and Brown 2005; Albert et al. 2009). Complications associated with stimulation to the heart are avoided due to the limited contributions of the left vagus nerve to cardiac activity and the minimal levels of current. Unilateral stimulation of the vagus nerve results in bilateral activation of the nucleus of the solitary tract and its projections to the locus coeruleus and raphe nucleus (Henry 2002). Activation of the locus coeruleus leads to activation of the nucleus basalis through α1 adrenoreceptors (Berntson et al. 1998, 2003). Although the exact mechanisms of action are not entirely yet understood, vagus nerve stimulation (VNS) has demonstrated several beneficial effects for major depression (Rush et al. 2000), mood enhancement (Elger et al. 2000), improved memory (Clark et al. 1999; Ghacibeh et al. 2006), decision making (Martin et al. 2004), and improved cognitive abilities in Alzheimer's patients (Sjögren et al. 2002), and it reduces edema following brain trauma (Clough et al. 2007). Due to the known release of multiple neuromodulators, VNS has recently become an object of study in regulating cortical plasticity.

Our previous study demonstrated that repeatedly pairing VNS with a tone causes a greater representation of that tone in primary auditory cortex (Engineer et al. 2011). This map expansion is specific to tones presented within a few hundred milliseconds of VNS. No previous study has reported the effects

of pairing VNS with a specific movement on cortical plasticity. If the mechanisms regulating map plasticity in the auditory cortex are the same in the motor cortex, then VNS paired with a movement should generate map plasticity specific to the paired movement. In this study, we paired VNS with a specific movement to test if this method could be used to direct specific and long-lasting plasticity in the motor cortex. Movement of the proximal or distal forelimb was paired with VNS with the prediction that only the corresponding area of motor cortex would expand.

Materials and Methods

Overview

Thirty-three rats were randomly assigned to receive a vagus nerve cuff electrode or a nonfunctional sham vagus nerve cuff electrode. After recovery from the surgery implanting the nerve cuff, 31 rats were trained to perform 1 of 2 operant motor tasks using either their proximal or distal forelimb. After the rats learned to reliably generate the required movement, VNS was paired with the movement several hundred times each day for 5 days. For 25 of these rats, intracranial microstimulation (ICMS) was used to quantify the reorganization in the primary motor cortex 24 h after the last training session. Instead of ICMS, 6 of the nonstimulated rats received ischemic motor cortex damage and were retested to confirm that accurate performance on the task requires motor cortex. Motor cortex ICMS was performed on 2 rats that had functional VNS electrodes and received the same amount of VNS but received no motor training. An additional group of 8 experimentally naïve rats that had not received motor training or VNS also underwent motor cortex ICMS.

Comparison of the motor maps from the naïve rats with those of the rats with sham cuffs allowed us to determine if training on the motor tasks without VNS generates cortical plasticity. Comparison of the motor maps from the rats with sham cuffs with the rats with functional cuffs allowed us to determine if pairing VNS with the movements enhances cortical plasticity. Comparison of the motor maps from rats that were performing a task during VNS with rats that were not performing a task during VNS allowed us to determine if the motor task was required to generate motor cortex plasticity.

Subjects

Forty-one adult female Sprague–Dawley rats (264 ± 44 g, mean ± standard deviation [SD]) were used in this experiment. The rats were housed in a 12:12 h reversed light cycle environment to increase their daytime activity levels. During training, the rats weights were maintained at or above 85% of their normal body weight by restricting food access to that which they could obtain during training sessions and supplementing with rat chow afterward when necessary. All handling, housing, surgical procedures, and behavioral training of the rats were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

Implantation Surgery

Rats were implanted with a custom-built cuff electrode prior to training. Stimulating cuff electrodes were constructed as previously described (Dorr and Debonnel 2006; Engineer et al. 2011; Nichols et al. 2011). In brief, 2 Teflon-coated multistranded platinum iridium (0.006″) wires were connected to a 4 mm section of Micro-Renethane tubing (1.8 mm inner diameter). The wires were spaced 2 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. This configuration resulted in the exposed wires being wrapped around the vagus nerve at points separated by 2 mm, while the leads exiting the cuff remained insulated. These insulated wires were tunneled subcutaneously (s.c.) to the top of the skull and attached to an external connector. A second group of randomly chosen rats received similar cuffs but with silk threads in place of the platinum iridium wires.

All the steps of the surgeries were the same regardless of the type of cuff implanted. Rats were anesthetized using ketamine hydrochloride (80 mg/kg, intraperitoneally [i.p.]) and xylazine (10 mg/kg, i.p.) with supplemental doses provided as needed. After rats were no longer responsive to toe pinch, incision sites atop the head and along the left side of the neck were shaved and cleaned with betadine and 70% isopropyl alcohol. The application of opthomalic ointment to the eyes prevented corneal drying during the procedure and a heating pad maintained the rats' body temperature at 37 °C. Doses of cefotaxime sodium (2 × 10 mg, s.c.) and a dextrose/Ringer's (10 × 1 mL total, s.c.) solution were given to the rats before and during the surgery to prevent infection and provide nourishment throughout the surgery and recovery. Bupivicaine (2 × 0.5 mL, s.c.) injected into the scalp and neck further ensured that the rats felt no discomfort during surgical procedures. An initial incision and blunt dissection of the scalp exposed the lambda landmark on the skull. Four to 5 bone screws were manually drilled into the skull at points close to the lambdoid suture and over the cerebellum. After an acrylic mount holding a 2-channel connector was attached to the anchor screws, an incision and blunt dissection of the muscles in the neck exposed the left cervical branch of the vagus nerve. As in humans, only the left vagus nerve was stimulated because the right vagus nerve contains efferents that stimulate the sinoatrial node and can cause cardiac complications (Ben-Menachem 2001).

Eighteen rats received the platinum iridium bipolar cuff electrodes (5–6 kΩ impedance), while another 13 received the sham cuffs in which silk thread replaced the platinum iridium wires. Leads (or silk threads) were tunneled s.c. and attached to the 2-channel connector atop the skull. All incisions were sutured, and the exposed 2-channel connector encapsulated in acrylic. A topical antibiotic cream was applied to both incision sites. After surgery, the rats with silken threads looked identical to the rats with wired cuffs after the surgeries. Rats were provided with amoxicillin (5 mg) and carprofen (1 mg) in tablet form for 3 days following the surgeries and were given 1 week of recovery before training began. During the week of recovery, rats were habituated to having the stimulator cable connected to the 2-channel connector on their heads. This method of cuff electrode construction, implantation, and stimulation delivery has repeatedly been shown to consistently result in VNS that persists over the full-term of the experiment (Engineer et al. 2011; Nichols et al. 2011).

Motor Tasks

Rats were trained on either the wheel spin task (n = 10 rats) or the lever press task (n = 21 rats). Training occurred in 2 daily sessions for 5 days each week. Both tasks involved quick movement of the forelimb in order to receive a sugar pellet reward. Rats initiated each trial, but a delay of at least 2 s was required between trials to allow the rats to eat the sugar pellet. The wheel spin task required the use of muscles located primarily in the distal forelimb, especially the wrist, while the lever press task required the use of the shoulder and the proximal forelimb.

The initial shaping procedures were similar for both motor tasks. Rats were placed in a cage and allowed to freely explore the area. A tether was connected to the rats' heads to familiarize the animals with the feeling of the connection. Each time the rats approached the response device (i.e., the lever or wheel), they received a 45 mg sugar pellet dispensed into a pellet dish located within the cage. Restrictions were gradually placed on rewarding the rats' proximity to the response device until the rats had to be next to, then touching, and finally using the device to receive the reward. An experimenter conducted shaping procedures manually. Rats typically took four 30 min sessions to become familiarized to the response device. After shaping, all training sessions were automated using custom-written MATLAB (MathWorks, Natick, MA) programs.

Wheel Spin Task

Rats that trained on the wheel spin task were required to spin a textured wheel below the floor of the training cage to receive a sugar pellet reward (Fig. 1*A*). Training occurred in two 30 min sessions daily. The acrylic training cage was 28 × 28 × 32 cm with a wheel placed 1 cm

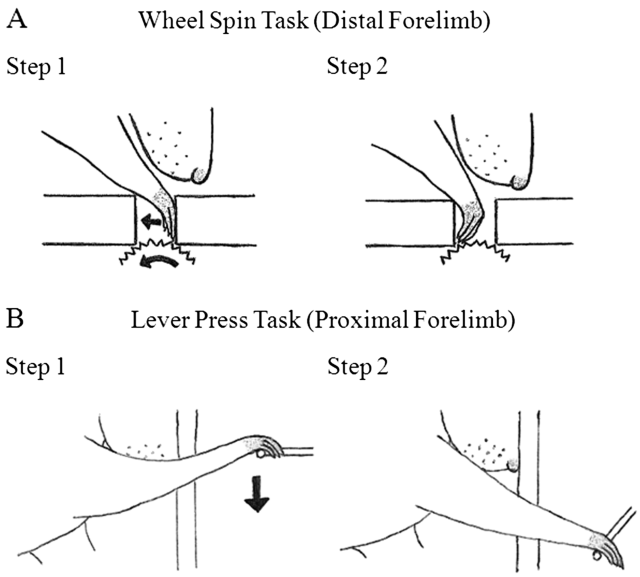

Figure 1. Sketches demonstrate the range of movements necessary to complete the motor tasks. (*A*) The wheel spin task required the rat to spin a textured wheel toward themselves. Rats used movements of the wrist and digits to complete this task. Stimulation and reward occurred after the rat spun the wheel 145° within a 1-s period. (*B*) The lever press task required the rat to depress a spring-loaded lever twice within 0.5 s. The range of motion required to complete this task pivoted primarily around the shoulder joint. Stimulation and reward occurred after the second lever press.

below an opening in the cage floor. The textured wheel was 32 mm in diameter and 9 mm thick. The 32 ridges providing the surface texture were 1.5 mm deep. Interchangeable acrylic pieces with rectangular holes 1 cm wide and ranging from 1.2 to 2.4 cm lengthwise were placed over the wheel to restrict access to the wheel. Reducing the size of the hole prevented broad movements of the forelimb and resulted in use of a flicking motion of the wrist and digits to spin the wheel. Rats learned the wheel spin task in 5 stages. Access to the wheel was progressively reduced at each stage. The size of the opening for each stage was as follows: 1) open access to the wheel, 2) 2.4 × 1 cm, 3) 1.9 × 1 cm, 4) 1.4 × 1 cm, and 5) 1.2 × 1 cm. Trials were initiated by the rats, but rewards were spaced at least 2 s apart by the computer program. Rats were initially rewarded for spinning the wheel 3° within a 1-s period when each new stage began. After 35 successful spins of the wheel, the degree of rotation required for a reward increased to 30°, then 75°, and finally 145°. After 35 rewards at the highest rotational requirement, the rats advanced to the next stage of training (i.e., more restricted access to the wheel) where they repeated all of the levels of increasing rotation again as previously described. Rats demonstrated a paw preference early in training and continued to use that paw for the remainder of the sessions (total left paw $n = 7$, VNS-trained left paw $n = 4$). Paw assignment was not expected to affect the results due to the bilateral effects of unilateral VNS on the nucleus of the solitary tract and its projections to the locus coeruleus and raphe nucleus (Henry 2002).

Lever Press Task

Rats depressed a lever initially located inside the training cage to receive a sugar pellet reward. The training cage was a 20 × 20 × 20 cm wire cage with a Plexiglas wall opposite the door. All training sessions other than the shaping sessions were 15 min long and occurred twice daily. Trials were initiated by the rats, but rewards were only given to trials occurring at least 5 s apart. After receiving 60 pellets in 2 shaping sessions by pressing the lever, the rats learned to press the lever twice in a 3-s period for the same reward. The interval between lever presses that elicited a reward was reduced from 3 to 2 s, then 1 s, and finally 500 ms, with 15 successful trials as the criterion for advancing. After successfully pressing the lever twice within 500 ms forty-five times, the lever was gradually withdrawn out of the cage. The lever was initially located 4 cm inside the cage, then moved to 2 cm inside the cage, and then to 0.5, 1.5, and 2.0 cm outside of the cage. The criterion for retracting the lever was 15 successful double-lever presses for each position, except for 0.5 cm outside the cage, which required 30 successful trials. Rats reached through a 1 × 8 cm window in the Plexiglas wall to reach the lever outside the cage (Fig. 1*B*). The edge of the window was located 2 cm from the cage wall, while the lever was offset so that the middle of the lever lined up with the edge of the window furthest from the wall. This arrangement restricted the rats so that they could only comfortably press the lever with their right paw. This aspect of the task design was important for confirming the importance of the motor cortex for the lever press task with motor cortex lesions.

Effects of Motor Cortex Lesions on Lever Press Task Performance

To confirm that accurate performance on the lever press task requires motor cortex, 6 rats implanted with the nerve cuffs and trained on the lever press task without stimulation received motor cortex lesions and were retested for 2 days following 1 week of recovery. Based on procedures by Fang et al. (2010), the vasoconstrictor endothelin-1 was used to selectively lesion the caudal forelimb area of the motor cortex. Basic surgical procedures for cleaning, anesthesia, and postsurgical care were the same as the cuff implantation surgery. After cleaning the top of the head, an incision was made longitudinally and a craniotomy was performed over the primary motor cortex caudal forelimb area contralateral to the trained forelimb (2.75 to –0.75 mm anteroposterior and 2.25–3.75 mm mediolateral, relative to bregma). Endothelin-1 (0.33 μL of 0.3 μg mixed in 0.1 μL saline) was injected at a depth of 1.8 mm using a tapered Hamilton syringe along a grid within the craniotomy at 2.5, 1.5, 0.5, and –0.5 mm anteroposteriorally and 2.5 and 3.5 mm mediolaterally relative to bregma for a total of 8 sites. KwikCast silicone gel was used to replace the removed skull cap, and the skin was sutured. The lever press task was the only task tested with motor cortex lesions due to the ease with which the forelimb used in the task could be restricted. The lever press task could not be completed with the left forelimb because of the cage design. Lesions were made in the left motor cortex forcing the rat to try to use its impaired right forelimb to complete the task.

VNS Movement Pairing

During the final stage of the motor tasks, reaching through a 1.2 cm wide window and spinning the wheel 145° within a 1-s period or pressing the lever located 2 cm outside the cage twice within 500 ms triggered a food reward and VNS. Stimulations were delivered approximately 75 ms after the wheel reached 145° or the lever triggered the second press. Rats typically continued to spin the wheel or press the lever beyond the required criterion, such that the movements were still occurring during VNS. VNS was always delivered as a train of 15 pulses at 30 Hz. Each 0.8 mA biphasic pulse was 100 μs in duration. The train of pulses was 500 ms in duration. These were the same parameters used by Engineer et al. (2011). Previous studies have demonstrated that the amplitude of electroencephalographic measures are reduced and neuronal desynchrony increases during VNS using the described electrode implantation indicating the successful stimulation of the vagus nerve (Engineer et al. 2011; Nichols et al. 2011). VNS movement pairing during the final stage of training continued for 1 week (i.e., 10 × 30 min sessions for the wheel spin task and 10 × 15 min sessions for the lever press task), delivering around 1200 total stimulations. Previous research has shown that this form of VNS does not alter heart rate, blood oxygenation level, or ongoing behavior, suggesting that the stimulation is neither aversive nor rewarding to the animals (Engineer et al. 2011).

Connections and stimulations from the external stimulator to the rats were identical between rats implanted with functional or sham VNS electrode cuffs. The sham cuffs with silk threads in place of platinum iridium leads did not carry an electrical charge when stimulated. This difference in the cuffs allowed experimenters to remain blind during training to stimulated and sham rats.

Intracranial Microstimulation

The day after the last training session of VNS movement pairing, the organization of primary motor cortex contralateral to the trained paw was defined using standard ICMS mapping procedures (Nudo et al.

1990; Conner et al. 2010). An additional 8 rats (283 and 19 g SD) that did not train or receive VNS also underwent ICMS procedures to the left cortex to compare the effects of training on motor cortex organization. Rats were anesthetized initially with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). Supplemental doses of anesthesia were provided as needed to suppress whisking or response to toe pinch. After placing the rat in a stereotaxic frame with a digital readout, a craniotomy was performed to expose the motor cortex. Parylene-coated tungsten electrodes (~0.7 MΩ impedance) were inserted to a depth of ~1800 μm (corresponding to cortical layers V; Kleim et al. 1998). Stimulation occurred following a grid with 500 μm spacing. Sequential electrode placements were made at least 1 mm apart where possible. ICMS was delivered once per second. Each stimulation consisted of a 40 ms pulse train of 10, 200 μs monophasic cathodal pulses delivered at 286 Hz. Stimulation intensity was gradually increased (20–200 μA) until a movement was observed. If no movement was observed at the maximal stimulation, then the site was deemed nonresponsive. The borders of primary motor cortex were defined based on unresponsive sites and stopped at the posterior-lateral vibrissae area, which is known to overlap the somatosensory cortex (Gioanni and Lamarche 1985).

Motor mapping procedures were conducted with 2 experimenters, both blind to the experimental condition of the rat. The first experimenter placed the electrode and recorded the data for each site. Because the motor cortex is organized with similar movements often occurring in the general vicinity of each other, the second experimenter was kept blind to the electrode placement to avoid potential biasing. The second experimenter delivered stimulations while observing which parts of the body moved in response. Movements were classified based on the part of the body that moved using the threshold stimulation current. Larger movements were obtained using higher current stimulations and were used when necessary to disambiguate movements too small to confidently classify at threshold levels. The first stimulation site was placed in an area often resulting in movement of the distal forelimb. Subsequent stimulation sites were randomly chosen and did not extend beyond established border (i.e., unresponsive) sites. Movements of the vibrissae, face, eye, and neck were classified as "head." Movements of the shoulder, elbow, and proximal forelimb were classified as "proximal forelimb." Movements of the wrist and digits were called "distal forelimb." "Hindlimb" included any movement in the hindlimb of the rat. Cortical area was calculated by multiplying the number of sites eliciting a response by 0.25 mm^2 (0.5 × 0.5 mm). Four sites equal 1 mm^2.

VNS Alone Group

To confirm that VNS alone does not produce motor cortex map reorganization, 2 rats that were never trained to perform a motor task were placed into a training cage and received randomly delivered VNS (i.e., not paired to a specific movement). Except for the movement pairing, VNS in this group was identical to the groups above. Each animal was passively stimulated for two 30 min sessions/day with a 2 h break between sessions and repeated for 5 days. Within each session, stimulation occurred between a range of 8–16 s, giving an average stimulation time of 11.25 s. At the end of each session, 160 stimulations were given, which amounted a total of 1600 stimulations. Animals were ICMS mapped 24 h following the final passive VNS session.

Results

Motor Cortex Map Plasticity

Rats were shaped to the wheel spin task in 4 ± 0.3 sessions and the lever press task in 4 ± 0.3 sessions. Rats reached the last stage of the wheel spin task in 27 ± 5 sessions and the lever press task in 8 ± 1 sessions (mean ± standard error of the mean [SEM]). The percent of successfully completed trials on the wheel spin task on the first day of VNS-paired training was 77 ± 4%. The same measure for the lever press task on the first day of VNS-paired training was 78 ± 4%. Microelectrode mapping techniques were used to determine the organization of the motor cortex after 5 days of VNS-paired training on the last stage. Maps of the motor cortex were derived from 3595 electrode penetrations (average 103 sites per animal).

The general organization of the motor maps obtained in this study is consistent with previous studies (Hall and Lindholm 1974; Gioanni and Lamarche 1985; Neafsey et al. 1986). In all rats tested, the anterior portion of the motor map generated movements of the rat's head, including the jaw, vibrissa, and neck (Fig. 2*A*). The middle region of the map generated

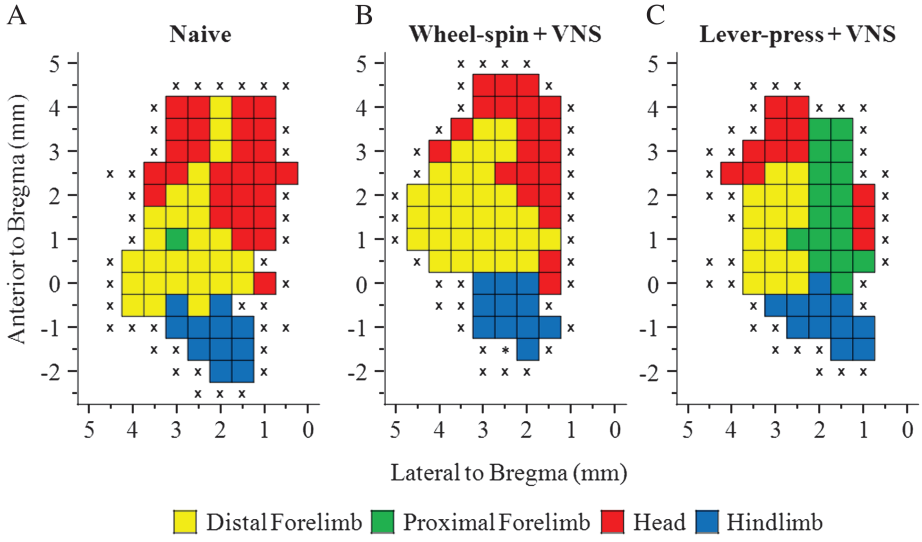

Figure 2. Representative motor maps of a naïve rat (2A), a rat trained with wheel spin paired with VNS (2B), and a rat trained with lever press paired with VNS (2C). Each square represents a 0.25 mm^2 (0.5 × 0.5 mm) area. Electrode penetrations occurred in the middle of each square (±0.05 mm). Movements of the neck, face, vibrissae, and eye were classified as head (represented in red). Movements of the forelimb originating below the elbow were considered distal forelimb (represented in yellow). Movements originating between the shoulder and the elbow, including the elbow, were considered proximal forelimb (represented in green). Any movements in the hindlimb of the rat were classified as hindlimb and are represented in blue. Sites eliciting no response with 200 μA of current are marked with an "x." Sites in the posterior vibrissae area are marked with an asterisk and were considered border sites to the overall somatotopic map in this study.

movements of the forelimb and the posterior region generated movements of the hindlimb. As in earlier reports, it was possible to divide the forelimb area into a small rostral region that is mostly surrounded by head responses and a larger caudal forelimb area that borders the hindlimb area (Neafsey et al. 1986).

The organization of primary motor cortex was not significantly altered by training without VNS. One-way analyses of variance (ANOVAs) comparing the group means within an individual body area were calculated with Tukey post hoc analysis (Table 1). The areas representing the distal forelimb, proximal forelimb, head, and hindlimb were not significantly different across the naïve-, wheel spin-, or lever press-trained rats that had sham VNS cuffs electrodes and received no VNS (Tukey post hoc analysis, $P > 0.05$). Further comparisons of just the groups not receiving VNS confirmed the lack of an effect of training without VNS (Partially repeated measures ANOVA Main Effect of group, $F_{2,18} = 2.8$, $P = 0.09$; Group × area interaction, $F_{6,54} = 0.26$, $P = 0.95$). As a result, these 3 control groups are averaged for group analyses and referred to as the non-VNS group.

Naïve rats had an average rostral forelimb area of 6.4 mm^2, which is comparable to the ~6 mm^2 previously reported in rats just beginning training (Kleim et al. 2004). Unlike naïve rats, the division between the rostral and the caudal forelimb area was indistinguishable in most of the rats that received VNS/movement pairing. For this reason, responses from the rostral and caudal forelimb areas were combined into single groupings of the distal forelimb, proximal forelimb, or shoulder area for further analysis in all groups of rats.

Rats that received VNS paired with the wheel spin task exhibited a significant reorganization of the motor cortex. In the non-VNS rats, the head and distal forelimb occupy approximately the same amount of cortical area (7.00 and 6.75 mm^2, respectively, in the example naïve rat shown in Fig. 2*A*). Hindlimb and proximal forelimb comprise a smaller region of the motor map (2.75 and 0.25 mm^2, respectively, in the example naïve rat shown). Figure 2*B* shows a representative example of motor cortex following wheel spin/VNS pairing. Wheel spin/VNS pairing resulted in a 15% larger distal forelimb area (1.0 mm^2), a 25% smaller head area (–1.75 mm^2), and no proximal forelimb area in this particular animal compared with the naive. These changes in cortical area for the wheel spin/VNS-paired group were significant when compared with the non-VNS group ($P < 0.05$, Fig. 3). On average, pairing VNS with the wheel spin task caused a 32% increase in the cortical area representing the distal forelimb compared with the non-VNS group ($t_{24} = 3.0$, $P = 0.003$). This increase was accompanied by a 38% smaller head area and a 63% smaller proximal forelimb area ($t_{24} = 2.4$, $P = 0.01$; $t_{24} = 1.8$, $P = 0.04$, respectively) but no change in the area devoted to hindlimb. These results suggest that repeatedly pairing VNS with a particular movement can generate a specific increase in the motor cortex representation of that movement.

To confirm that the observed cortical plasticity was specific to the movement paired with VNS, we also documented the reorganization of motor cortex in rats that received VNS paired with a lever press task. Since this task primarily involves movement of the proximal forelimb, we expected to see an increased proximal forelimb representation after lever press/VNS pairing. A cortical map from a representative rat shown in Figure 2*C* exhibited a dramatically larger proximal forelimb area compared with the naïve rat (Fig. 2*A*). The lever press/VNS rat had 1600% (4 mm^2) more area devoted to the proximal forelimb area compared with the naïve rat. Pairing VNS with the lever press movement reduced the head area by 39% (–2.75 mm^2) and distal forelimb area by 59% (–4 mm^2) in this rat compared with the naïve rat. Like the wheel spin/VNS-trained rat, the lever press/VNS rat had the same sized hindlimb representation as the naïve rat. These examples suggest that the motor cortex plasticity observed following VNS movement pairing is specific to the paired movement and not a general effect of VNS.

On average, rats that received VNS during the lever task exhibited a 159% increase in the proximal forelimb area compared with the non-VNS group (Fig. 3; $t_{26} = 4.1$, $P = 0.0002$). The lever press/VNS group had a 23% smaller distal forelimb area and a 29% smaller head area than the non-VNS group ($t_{26} = 2.5$, $P = 0.009$; $t_{26} = 2.3$, $P = 0.01$; respectively). The most striking differences were observed between the wheel spin/VNS rats and the lever press/VNS rats. Although both groups received identical VNS, wheel spin-trained rats had a 72% larger distal forelimb area than the lever press rats and the lever press rats had a 598% larger proximal forelimb area compared with the wheel spin–trained rats ($t_{10} = 9.6$, $P = 1 \times 10^{-6}$; $t_{10} = 3.6$, $P = 0.002$; respectively). These results demonstrate that VNS movement pairing can generate large-scale reorganization of motor cortex and confirm that the reorganization is specific to the movement repeatedly paired with VNS.

We delivered VNS at random times in 2 rats before documenting the organization of motor cortex using ICMS techniques. Motor cortex in these rats was similar to naïve rats and there was no evidence of the reorganizations that were observed after either the lever press or the wheel spin movements were paired with VNS (Main Effect of group, $F_{1,8} = 0.9$, $P = 0.4$; Group × area interaction, $F_{3,24} = 1.4$, $P = 0.3$).

Table 1

Presents the group average sizes of each area (mm^2) in the motor cortex followed by the SEM

Group	*n*	Body area responding to ICMS			
		Distal forelimb	Proximal forelimb	Head	Hindlimb (mm^2)
Naïve	8	6.3 ± 0.5	1.1 ± 0.4	5.7 ± 0.7	3.4 ± 0.3
Wheel spin sham	5	5.5 ± 0.6	0.9 ± 0.4	5.6 ± 0.6	3.1 ± 0.3
Lever press sham	8	5.8 ± 0.6	1.5 ± 0.3	6.5 ± 0.8	3.4 ± 0.4
Wheel spin + VNS	5	7.8 ± 0.2	0.5 ± 0.3	3.7 ± 1.0	3.4 ± 0.4
Lever press + VNS	7	4.5 ± 0.3	3.1 ± 0.6	4.2 ± 0.5	3.0 ± 0.4

Note: One-way ANOVA's comparing the groups means within an individual body area were calculated with Tukey post hoc analysis. ANOVA's revealed significant differences only in the distal and proximal forelimb areas amongst the groups ($F_{4, 28} = 6.1$, $P = 0.001$; $F_{4, 28} = 6.1$, $P = 0.001$, respectively). Brackets demonstrate significant differences between groups as demonstrated in post hoc analysis ($P \leq 0.05$). Naive and sham groups were not significantly different from each other (Partially repeated measures; $F_{2, 18} = 2.8.$, $P = 0.09$), therefore they were grouped together for further analysis.

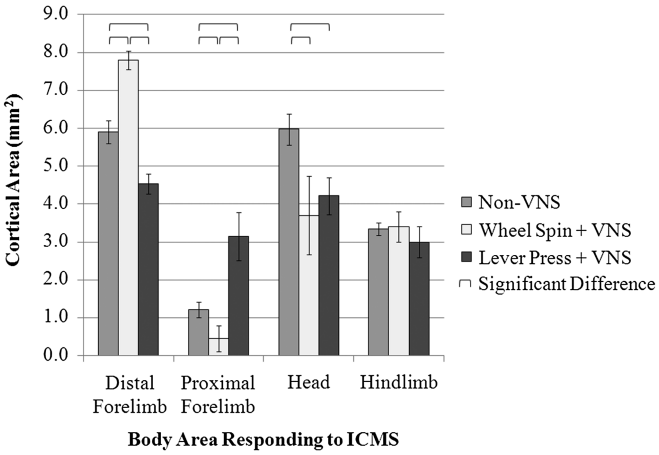

Figure 3. The average amount of cortical area that elicits movement of a specific body area using intracortical microstimulation for rats in the non-VNS group ($n = 21$), rats trained on the wheel spin task paired with VNS ($n = 5$), and the rats trained on the lever press task paired with VNS ($n = 7$). The non-VNS group consists of the naïve rats and the rats trained on the tasks without VNS. Cortical area was calculated by multiplying the number of sites eliciting a response by 0.25 mm^2 (0.5 × 0.5 mm). Four sites are equal to 1 mm^2. Error bars represent the SEM. Brackets represent significant differences as follows between groups within a specific body area ($P < 0.05$).

This observation combined with task specificity of the motor cortex plasticity observed in the trained rats that received VNS suggests that VNS movement pairing is sufficient to generate motor cortex reorganization.

There was no difference in the average stimulation thresholds for the groups receiving movement-paired VNS and the non-VNS group (118 ± 5 μA, mean ± SEM; 116 ± 3 μA; respectively; $t_{31} = 0.53$, $P = 0.3$). These average thresholds are comparable to previously reported levels of stimulation (Ramanathan et al. 2006) and within the maximum amount of current used in other studies using ICMS in the rat motor cortex (Conner et al. 2003, 2005, 2010). The rats trained with VNS paired on the wheel spin task had an average distal forelimb stimulation threshold not significantly different from the wheel spin–trained group with sham VNS cuff electrodes (100 ± 10 μA; 90 ± 6 μA; respectively; $t_8 = 0.94$, $P = 0.2$). The VNS paired with lever press group's proximal forelimb stimulation thresholds was not significantly different from the lever press group trained with sham VNS cuff electrodes (117 ± 9 μA; 105 ± 9 μA; respectively; $t_{13} = 0.97$, $P = 0.17$). Similar stimulation thresholds between paired VNS-trained and non-VNS–trained rats demonstrate that the observed movement representation reorganizations are not due to altered levels of excitability in the cortex. This result is consistent with several papers that have found cortical representation changes in the motor cortex from training occurs without ICMS threshold changes (Kleim et al. 1998; Remple et al. 2001; Ramanathan et al. 2006; Molina-Luna et al. 2008). Morphological changes, such as synaptogenesis, have been observed with past motor cortical reorganization accompanying training and may account for a mechanism of change in movement-paired VNS (Kleim et al. 2004).

Behavioral Analysis

We compared performance on the lever press task before and after ischemic motor cortex damage in 6 rats. Performance was significantly impaired in every rat ($P < 0.05$). Average performance fell from 93 ± 1% successful double-tap attempts for the last 2 days before surgery to 75 ± 5% for the 2 days of testing conducted after a week of recovery ($t_5 = 5.0$, $P = 0.002$). This result confirms that this task like other skilled motor tasks depends on motor cortex for accurate performance (Castro-Alamancos and Borrell 1995; Conner et al. 2005).

We compared task performance in each group to confirm that movement-paired VNS does not make the task more difficult. No behavioral differences were observed between VNS and sham groups on the wheel spin task in the total number of successful trials (VNS = 1584 ± 73 trials, Sham = 1694 ± 148 trials, mean ± SEM, $t_8 = 0.75$, $P = 0.2$), the velocity at which the wheel was spun (VNS = 259 ± 32°/s, Sham = 307 ± 29° per s, $t_8 = 1.3$, $P = 0.1$), or the percentage of successfully completed trials per session (VNS = 68 ± 4%, Sham = 74 ± 5%, $t_8 = 1.1$, $P = 0.2$.). VNS rats showed no impairment on the lever press task and, in fact, exhibited shorter lever press intervals (VNS = 231.6 ± 10.5 ms, Sham = 287.7 ± 14.8 ms, $t_{13} = 3.2$, $P = 0.007$) and triple pressed the lever more often than the sham rats (VNS = 107.3 ± 25.6 trials per session, Sham = 56.3 ± 6.2 trials per session, $t_{13} = 2.2$, $P = 0.04$). Although VNS enhanced some aspects of the lever press task, the percent of successful trials and the total number of successful trials were not different between the VNS and the sham rats (VNS = 1023 ± 46 trials; Sham = 926 ± 48 trials, mean ± SEM; $t_{13} = 1.5$, $P = 0.08$; VNS = 85 ± 3%, Sham = 81 ± 3%, $t_{13} = 1.3$, $P = 0.1$; respectively). These results indicate that VNS is unlikely to have enhanced map reorganization by making the task more difficult.

Discussion

Based on our recent study in the auditory cortex (Engineer et al. 2011), we predicted that repeatedly pairing brief stimulation of the vagus nerve with a specific movement would result in a larger representation of that movement in the motor cortex. To test this prediction, we delivered 0.5 s of VNS each time rats used their distal forelimb to rotate a wheel. After several hundred pairings, the cortical representation of the distal forelimb was significantly larger in these rats compared with naïve rats and rats that performed the same movements without VNS. A second group of rats was trained on a motor task using a different part of their body to confirm that map reorganization was specific to the movement paired with VNS. Pairing VNS with a lever press task that required the use of the proximal forelimb resulted in a significantly larger proximal forelimb representation. Impaired performance in a group of rats following ischemic lesions to the caudal forelimb area confirmed the involvement of the motor cortex in this task. The observations that map expansion was specific to the movement paired with VNS and that neither of the tasks without VNS nor VNS without the task training generated map reorganization indicates that movement-paired VNS is sufficient to direct map plasticity.

Pairing VNS with a motor event generated cortical plasticity comparable to that observed using a similar paradigm in the auditory system. Presenting a tone with a brief period of VNS causes a significant expansion of the paired tone's representation in the auditory cortex (Engineer et al. 2011). Presenting tones or VNS alone did not alter the auditory cortex's tonotopic organization. These 2 studies suggest that the plasticity enhancing mechanisms of event-paired VNS are common to the auditory and motor cortex.

Training without VNS

A number of studies have reported that training on skilled motor tasks increases cortical representations for the movements involved (Nudo et al. 1996; Kleim et al. 1998, 2004; Conner et al. 2003; Molina-Luna et al. 2008). Our results do not contradict these findings, as one of the landmark studies demonstrating training induced cortical plasticity using a skilled reaching task also demonstrated a lack of reorganization for a lever press task (Kleim et al. 1998). The lack of observed cortical change following training on the lever press and wheel spin tasks may be due to a number of reasons. Kleim et al. (1998) have attributed the cortical reorganization observed in a skilled reaching task to the accuracy of the movements necessary to complete the task which may be absent in our lever press and wheel spin tasks. There is also a possibility that the sampling distance of 500 μm is too coarse to see cortical changes associated with tasks in the current study, although this spacing has previously demonstrated training-induced plasticity in the aforementioned skilled reaching task (Conner et al. 2003, 2010; Ramanathan et al. 2009). Another possibility is the cortical changes observed following motor and auditory learning have been shown to be transient, while the acquired skill remains stable over time (Molina-Luna et al. 2008; Ma et al. 2010; Reed et al. 2011). The lever press- and wheel spin-trained rats were mapped approximately 10 and 20 days after their initial training session, respectively, possibly occurring after cortical changes associated with training would have been observed. If this possibility occurred, then the VNS-paired training may have prolonged or reestablished the observed changes in the motor cortex organization.

Potential Mechanisms

The exact mechanisms by which VNS directs plasticity in motor or sensory cortex are unknown. VNS causes the release of several molecules known to enhance cortical plasticity, including acetylcholine, norepinephrine, serotonin, and brain-derived neurotrophic factor (Détári et al. 1983; Hassert et al. 2004; Dorr and Debonnel 2006; Follesa et al. 2007; Albert et al. 2009). Perfusing norepinephrine into an adult cat's visual cortex produces kitten-like plasticity in a test of ocular dominance shifts following monocular deprivation (Pettigrew and Kasamatsu 1978; Kasamatsu et al. 1979). Serotonin-specific neurotoxins and receptor blockers prevent normal ocular dominance shifts in kittens in monocular deprivation, implicating the importance of serotonin for normal plasticity (Gu and Singer 1995). Another important study showed that enhancing serotonin release with fluoxetine can stimulate plasticity in adult cats (Vetencourt et al. 2008). Blocking the release of acetylcholine prevents cortical plasticity and interferes with skill learning and recovery from brain damage (Conner et al. 2003, 2005, 2010). The use of the muscarinic antagonist scopolamine blocks the effect of VNS on spontaneous firing rate in the auditory cortex, further supporting the influence of VNS on the cholinergic system (Nichols et al. 2011). Adding brain-derived neurotrophic factor induces plastic changes in ocular dominance shifts in adult rats following monocular deprivation (Vetencourt et al. 2008). Combining more than one of these elements can lead to greater plasticity than the influence of the elements singularly (Bear and Singer 1986; Seol et al. 2007). The ability of VNS paired with wheel spin or lever press training to produce cortical plasticity supports the importance of the VNS-triggered release of these molecules in enhancing cortical plasticity. VNS is likely to generate cortical map plasticity specific to the associated event through the synergistic action of multiple plasticity enhancing molecules.

Importance of Timing

The simultaneous presentation of VNS with a specific sensory or motor event is sufficient to increase cortical representation of that movement. In this study, we used a sugar pellet to reward the animal's behavior immediately after the completion of a trial. As a result, VNS was delivered during the behavioral task, which finished just a few seconds prior to the animal eating the pellets. It would not have been surprising to see an increased representation of the head and jaw in this study.

In a previous study, our lab demonstrated that changes in auditory cortex were temporally specific to tones paired with VNS (Engineer et al. 2011). Two randomly interleaved tones were presented every 15–45 s for several thousand trials to a rat with only one of the tones paired with VNS (Engineer et al. 2011). The number of sites responding to the VNS-paired tone increased significantly, while the number of sites for the tone presented within tens of seconds of the VNS did not. These observations are consistent with past studies demonstrating that pairing nucleus basalis stimulations with tones only alters the tone's representations when stimulations occurred within seconds of the tone presentation (Kilgard and Merzenich 1998; Weinberger 2003).

The results from the current study demonstrate that the head representations did not increase because of VNS just prior to chewing. This result indicates that the plasticity enhancing actions of VNS are temporally precise, lasting less than 1–2 s. These results demonstrate that brief pulses of VNS can be used to direct highly specific plasticity. Additionally, VNS without paired behavioral training did not result in map reorganization in our study, further supporting our conclusion that the cortical changes triggered by VNS are enhanced by task-specific pairing. Methods for enhancing plasticity that rely on slow-acting mechanisms may not be as effective in generating the same accuracy of plasticity as VNS pairing. Pharmaceuticals often elevate or diminish certain neurotransmitters for several hours. Several movements or sensory events may occur repeatedly during this time, potentially creating unwanted plasticity. The temporal precision of the VNS-pairing method for enhancing cortical plasticity should offer significant advantages in efficiency and efficacy as compared with methods with less precise actions.

Cortical Expansions without Behavioral Consequences

Enhanced task performance (i.e., improved success) did not accompany motor map expansions in rats trained on the VNS-paired wheel spin or lever press tasks. This suggests map changes associated with pairing VNS with a learned motor task are not necessarily reflected by changes in motor function. However, map reorganization has been shown to be important for enhancing behavioral outcomes during the learning process but not after it (Reed et al. 2011). Rats demonstrating increased tonotopic representations for low frequencies following paired nucleus basalis stimulation demonstrated faster learning of a tone discrimination task compared with controls. Rats that had already learned the tone discrimination did not behaviorally benefit from the

induced plasticity. From these results, the authors concluded that "cortical map expansion plays a major role in perceptual learning but is not required to maintain perceptual improvements" (Reed et al. 2011). The rats in the current study had already learned the tasks when they began receiving VNS, otherwise they may have demonstrated an accelerated learning rate compared with the sham groups. The enhanced propensity for cortical reorganization accompanying event-paired VNS may increase success during learning.

Potential Clinical Use of Paired VNS

Stroke and traumatic brain injury often damage movement-controlling areas of the motor cortex resulting in hemiparesis or hemiplegia. Following cortical injury, lost motor representations can partially regenerate in neighboring areas within motor cortex (Eysel et al. 1999; Conner et al. 2005). The size of the regenerated representations is highly correlated with the functional recovery of lost movements, but this recovered area and ability is a fraction of those seen preinjury (Castro-Alamancos and Borrell 1995; Ramanathan et al. 2006). Physical training in healthy animals can greatly increase cortical representation of the muscles used during learning of the task, but rehabilitative physical training in rats after a motor cortical injury is less effective at generating this increased representation (Kleim et al. 1998; Conner et al. 2005; Molina-Luna et al. 2008). Movement-paired VNS in intact rats generates a comparable amount of cortical plasticity in approximately the same amount of time as physical training (Nudo et al. 1996; Kleim et al. 1998; Molina-Luna et al. 2008). Movement-paired VNS is also able to enhance plasticity where plasticity is not observed with training alone. Since increased cortical plasticity is related to increased functional recovery following cortical injury (Castro-Alamancos and Borrell 1995; Ramanathan et al. 2006), it is possible that movement-paired VNS may aid the recovery of specific motor functions following cortical injury (Lozano 2011).

Periodic VNS is FDA approved as a safe and effective treatment of certain types of refractory epilepsy as well as treatment-resistant depression (Binnie 2000; Rush et al. 2000; Ben-Menachem 2001; Groves and Brown 2005; Albert et al. 2009). Protocols for treating epilepsy require 30 s of VNS every 5 min 24 h per day (Groves and Brown 2005; Albert et al. 2009). Periodic VNS using a stimulation protocol similar to that used in treating epilepsy has improved functional recovery in rats with fluid percussion injury to the cortex (Smith et al. 2005, 2006). This protocol requires 145 times the daily current injection compared with what was used in the current study. Our results demonstrate that motor and auditory events must be precisely timed with VNS to significantly alter motor and auditory system organization, respectively. It seems likely that therapies using paired VNS might be a more effective therapy for increasing functional recovery following cortical damage.

Selectively pairing VNS has already shown promise in normalizing abnormal cortical organizations in the treatment of tinnitus in rats (Engineer et al. 2011). The overrepresentation of a tone was reduced by pairing VNS with tones spanning the rats hearing range except for the tones near the tinnitus frequency. This eliminated the behavioral correlate of tinnitus in rats for several months past the cessation of the treatment. A similar strategy of pairing VNS with movements may improve the treatment of disorders related to abnormal representations in the motor system, such as dystonias (Quartarone et al. 2003; Sohn and Hallett 2004; Lin and Hallett 2009; Schabrun et al. 2009). Although the causes are not fully understood, patients with dystonia demonstrate disturbed cortical inhibition that is improved with the application of transcranial magnetic stimulation (Siebner et al. 1999; Sohn and Hallett 2004, Quartarone et al. 2005; Schabrun et al. 2009). Current evidence supports that reducing the overrepresented motor area during these treatments is associated with a reduction in dystonic symptoms (Schabrun et al. 2009). In the current study, the larger representations observed from the VNS-paired movements were accompanied by smaller nearby cortical representations, such as movements of the head. Selectively increasing the size of surrounding muscle representations might decrease the overrepresentation of the dystonic muscles. Movement-paired VNS of nondystonic, surrounding movements may decrease the overrepresentation of the dystonic muscles. The strategic pairing of nondystonic movements with VNS provides a novel potential therapy to treat focal dystonia (Lozano 2011).

Conclusion

Repeatedly pairing VNS with tone presentations enhances the cortical representation of the paired tone (Engineer et al. 2011). We have confirmed that pairing the same VNS with a movement, instead of a tone, causes plasticity in the motor cortex that is specific to the paired movement. Both studies demonstrated the importance of the temporal precision of pairing VNS with the sensory or motor event in order to enhance cortical plasticity. These results suggest that VNS pairing can be used as a general method to generate highly specific cortical plasticity. VNS movement pairing could provide a new approach for treating a range of movement disorders caused by cortical damage or disorders associated with distorted movement representations.

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