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Effects of Tongue Force Training on Orolingual Motor Cortical Representation

David J. Guggenmos¹, Scott Barbay^{1,2}, Crystal Bethel-Brown¹, Randolph J. Nudo^{1,2,3}, and John A. Stanford^{1,2,3,*}

¹Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160

²Landon Center on Aging, University of Kansas Medical Center, Kansas City, KS 66160

³Kansas Intellectual & Developmental Disabilities Research Center, University of Kansas Medical Center, Kansas City, KS 66160

Abstract

Previous research has demonstrated that training rats in a skilled reaching condition will induce task-related changes in the caudal forelimb area of motor cortex. The purpose of the present study was to determine whether task-specific changes can be induced within the orofacial area of the motor cortex in rats. Specifically, we compared changes of the orofacial motor cortical representation in lick-trained rats to age-matched controls. For one month, six water-restricted Sprague-Dawley rats were trained to lick an isometric force-sensing disc at increasing forces for water reinforcement. The rats were trained daily for six minutes starting with forces of 1g, and increasing over the course of the month to 10, 15, 20, 25 and finally 30 g. One to three days following the last training session, the animals were subjected to a neurophysiological motor mapping procedure in which motor representations corresponding to the orofacial and adjacent areas were defined using intracortical microstimulation (ICMS) techniques. We found no statistical difference in the topographical representation of the control (mean = 2.03 mm²) vs. trained (1.87 mm²) rats. This result indicates that force training alone is insufficient to drive changes in the size of the cortical representation. We also recorded the minimum current threshold required to elicit a motor response at each site of microstimulation. We found that the lick-trained rats had a significantly lower average minimum threshold (29.1 ± 1.0 µA) for evoking movements related to the task compared to control rats (34.6 ± 1.1 µA). These results indicate that while tongue force training alone does not produce lasting changes in the size of the orofacial cortical motor representation, tongue force training decreases the current thresholds necessary for eliciting an ICMS-evoked motor response.

Keywords

oromotor; plasticity; movement; licking; tongue; operant; cortical; training

*Correspondence to: John A. Stanford, Ph.D., 2096 KLSIC, MS 3051, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, phone: 913-588-7416, fax: 913-588-5677, email: E-mail: jstanford@kumc.edu.

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Introduction

The mammalian cortex is highly adapted to reorganization in response to learning and experience. Previous research has examined structural plasticity in the cortex related to motor skill learning. Reported changes in neuronal morphology include increased synaptic density [6,9] and dendritic branching [5,23]. Functional changes have also been reported, including expansion of cortical area devoted to the trained movement type [7,15,16], and increased expression of brain derived neurotrophic factor (BDNF) and tyrosine kinase B receptor (TrkB) [10]. These changes are believed to contribute to increased accuracy and speed of learned motor movements (for review, see [2,11]).

One method used to measure functional motor cortical plasticity involves mapping motor cortical representations of muscle movements through the use of intracortical microstimulation (ICMS). Through ICMS it is possible to create a topographical “map” of joint movement representations from different parts of the body [20]. This makes it possible to measure surface area expansion or retraction of individual motor areas following training or insults. An expansion of a motor area during training is correlated with improved motor function [7,14,15,16]. While the cellular/synaptic basis for training-related map plasticity is not completely known, strengthened intracortical connections likely play a role [18].

Previous ICMS studies in the rat have focused on measuring the effects of reaching and grasping tasks on the topography of motor cortical maps [7,15,16]. These studies reported an increase in areal representation for muscle groups involved in performing these tasks. For example, changes in the digit and wrist motor cortical representations were observed in the caudal forelimb area (CFA) following reach and grasp training in rats. The purpose of the current study was to determine whether the results of previous mapping studies involving limb use generalize to a motor task involving movements of the tongue. While many orolingual movements are controlled and mediated through brainstem nuclei, there is substantial orofacial motor cortical representation for tongue and jaw movements in primates and humans, suggesting a cortical role in voluntary movement [12,21,25]. These movements are important in vocalization and mastication, and can become disrupted following brain lesions. Studies using ICMS have characterized orolingual movements evoked by cortical stimulation in mammalian species; for example in primates [12], felines [4] and rodents [13]. In the current study we used a behavioral task involving isometric tongue force training in rats [19,26] to measure changes in orofacial cortical motor representations and current threshold levels necessary to evoke orolingual movements using ICMS.

Methods

Animals

Twelve male Sprague-Dawley rats were obtained from Taconic Farms as retired breeders. At approximately 18 months of age, they were randomly assigned to either a lick-training condition (n=6), or a non lick-training control condition (n=6). Rats were housed individually and were maintained on a 12 hour light/dark cycle. Water access was gradually restricted according to a schedule that allows gradual weight gain. Rat chow was provided *ad libitum*. Protocols for animal use were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Body weights were 538 ± 19 g at the time of surgery.

Behavioral Training

Animals were placed in individual customized Gerbrands rodent operant chambers, each with a front panel containing a 6 cm² hole at floor level. Affixed to the square hole was a 6 cm³ transparent enclosure that, on its lower horizontal surface, contained a 12 mm-diameter hole through which the rat could extend its tongue downward to reach the operandum (see figure 1). The operandum was an 18 mm-diameter aluminum disc rigidly attached to the shaft of a Model 31 load cell (0-250 g range, Sensotec, Columbus, OH). The disc was centered 2 mm beneath the hole in the plastic enclosure. A computer-controlled peristaltic pump, (Series E at 14 rpm; Manostat Corp., New York, NY), fitted with a solid-state relay (Digikey, Thief River Falls, MN) and controlled by a LabMaster computer interface (LabMaster, Solon, OH), delivered water to the center of the lick disc through a 0.5 mm-diameter hole. The force transducer was capable of resolving force measurements to 0.2 g equivalent weights. A PC computer recorded the transducer's force-time output at a rate of 100 samples/s. Software also allowed for programming of a force requirement. For initial training, the force requirement was 1 g to register a response, and 12 licks were required to produce 0.05 ml of water. As animals mastered the task, the force required was gradually increased to 30 g. Trained animals then licked under the 30 g requirement for 4-6 days before cortical mapping sessions.

Electrophysiological Mapping

Within five days of the final training session, standard microelectrode stimulation techniques were used to derive high resolution (250 μ m) maps of the orofacial cortex (i.e., areas of the cortex lateral and rostral to the forelimb areas that, when stimulated, evoke orolingual movements) and bordering areas, and low resolution (>500 μ m) maps of the rostral (RFA) and caudal (CFA) forelimb areas [7]. Animals were anesthetized with ketamine (80 mg/kg ip) and xylazine (5 mg/kg im), and given supplements of ketamine (20 mg/kg im) when needed. The animals were placed in a stereotaxic frame and a craniotomy was performed over the motor area of the right hemisphere. An image of the blood vessel pattern was captured and stored on a computer. A grid with 250 μ m spacings was overlaid onto the image using Canvas (ACDSee). Electrode penetrations were made on the grid intersections using a hydraulic microdrive to a depth of ~1750 μ m (cortical layer V). Stimulation consisted of a 40 ms train of thirteen, 200 μ s monophasic cathodal pulses delivered at 350 Hz at the rate of one train per second. During stimulation, the current delivered was gradually increased from one to 60 μ A. The animal was observed for evoked orolingual movements, such as tongue, jaw and lip movements, by two observers. Movement type and threshold were recorded. If no movement was observed at ≤ 60 μ A the site was deemed nonresponsive. Following stimulation, the coordinates and type of each stimulation point were input into an image analysis program (NIH Image) to determine the total area of the orofacial region of the cortex for each of the animals (Fig. 2).

Results

Representative force-time waveforms recorded during training sessions for one rat are illustrated in figure 3. Repeated-measures analysis of variance comparing peak tongue forces at 1, 20 and 30 grams revealed a significant effect as a function of force requirements, $F = 21.202$, $p = 0.007$ (Fig. 4A). Force requirements did not significantly affect the speed of tongue movements (Fig 4B). Evoked movement maps were derived from ~110 microelectrode penetration locations. Student's t-test (two-tailed, independent; $p < 0.05$) revealed no significant group differences in orofacial area representation [$t(10) = 0.25$; $p = 0.81$] between the control (2.03 ± 0.47 mm²) and trained (1.87 ± 0.46 mm²) animals (Figure 5). There was a statistically significant difference in movement thresholds (minimum current required to evoke movements using ICMS) in the orofacial motor area [$t(10) = 2.85$; $p = 0.017$]. Areas immediately bordering the orofacial region were also mapped. Pooling the thresholds for the control and trained groups, significant differences were found in proximal forelimb movement

thresholds [$t(148) = 3.68$; $p < 0.001$] and neck/trunk movement thresholds [$t(106) = 2.62$; $p = 0.01$] but not in the distal forelimb movement thresholds [$t(94) = 0.66$; $p = 0.51$] (Fig. 6)

Discussion

ICMS was used to characterize functional changes in the orofacial region of the rat motor cortex following a task that required the animals to lick at increasing forces. Those animals that received training exhibited no difference in the areal size of orofacial representation in the motor cortex, when compared to animals in an untrained control group. However, the minimum current needed to evoke motor responses was less in the trained animals.

These results stand in contrast to those obtained using ICMS techniques to derive maps of forelimb representations in motor cortex after training of skilled forelimb use in rats or monkeys [7,14]. In those studies, forelimb representations expanded, but no changes in movement thresholds were found. Further, using transcranial magnetic stimulation (TMS) in human patients trained on a tongue protrusion task, Svensson et al. [21] reported an expansion of cortical tongue motor maps immediately following the training, but a return to baseline levels two weeks post-training.

There are several potential reasons why the present results were inconsistent with previous ICMS findings in rats and monkeys and TMS findings in humans. The lack of expansion in orofacial representation could be task related. Remple et al. [17] found that strength training of the forelimb in rats in a reaching task was insufficient to drive areal representation changes in either the caudal or rostral forelimb areas. Although producing the forces attained by the rats in this study required time and training, licking is a normal behavior for the rat. It could be argued that this task was more consummatory than operant, and that an alternative task requiring tongue force output as a pure operant (i.e., in a context separated from the water reward) would result in a different outcome. It seems unlikely that the differences in rodents and primates would be due to differences in cortical control of these movements. While the rats do appear to have pattern generators in the brainstem that can generate rhythmic licking behaviors [22], studies of decerebrate rats [24] showed these movements were generally uncoordinated. Further, damage to the rats lingual nerve results in alterations of orolingual motor representations [1].

Functional as well as structural cortical plasticity may depend upon the precise behavioral demands of the task. The present study showed a lowering of the current thresholds needed to initiate a movement rather than an expansion of motor movement representations. These results are consistent with the hypothesis that skill and strength training evoke different types of modification in the cortex. Skill training has been associated with map expansions and synaptogenesis [7,8,9,16] while strength or endurance training induces angiogenesis in motor cortex [2,17], but not motor map organization or synapse number [2]. It is possible that the induction of angiogenesis as a result of strength training is related to enhanced synaptic efficacy but not necessarily synaptic number. However, synaptogenesis after repetitive orolingual training cannot be ruled out since it was not examined in the present study. It is also possible that the age of the rats limited the ability of the cortex to undergo plasticity, however, for sensory receptive field plasticity, the decline is use-dependent [3].

We also sampled a portion of the motor areas surrounding the orolingual area and found decreased thresholds for proximal forelimb movements and neck/trunk movements. It is likely that these muscle groups were necessary for proper task positioning and thus also underwent task-related plasticity. Representations for muscle groups not necessary for task positioning (distal forelimb movements) did not display threshold changes.

The results of this study, a decrease in current thresholds required to drive motor movement, but no changes in areal representation of the derived maps, reiterate that there are different avenues through which plasticity can manifest itself following motor learning. Whether the precise mechanisms are due to differences in the portion of the motor representation under study, or differences in behavioral demands of the task, remain to be elucidated.

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Figure 1.
Drawing of a representative rat licking the disc to receive a water reward.

Figure 2.

Representation of orolingual movements in rat motor cortex as defined by intracortical microstimulation. (A) Physiological map of orolingual representation. Each dot represents a stimulation point at an inter-penetration resolution of 250 microns. (B) Point specific map converted to interpolated map for analysis. Key: Yellow – orolingual, Red – distal forelimb, Blue – proximal forelimb, Black – neck/trunk, Orange – vibrissae, Grey – no response

Figure 3.
Raw force-time waveform for 10-s licking bouts as a function of increasing force criteria.
Waveforms are from a representative rat during A) 1g, B) 20g, and C) 30g training sessions.

Figure 4. Effects of tongue force requirements on (A) mean peak force and (B) licking rhythm. Data are means $\pm$ S.E.M. Significant difference from 1g group ($p < 0.05$) denoted by asterisk (*).

Orolingual Motor Area Representation

Figure 5. Total surface area occupied by orolingual movement representations. Orolingual movement representation in lick training group did not differ significantly from control group. Data are means $\pm$ S.E.M.

Figure 6. ICMS thresholds. Mean ($\pm$ S.E.M.) thresholds for evoking orolingual, proximal, and neck/trunk motor movements were significantly ($p < 0.05$) lower for trained rats compared to controls. No significant differences were observed in distal forelimb movements.