Noxious Lingual Stimulation Influences the Excitability of the Face Primary Motor Cerebral Cortex (Face MI) in the Rat

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The mechanisms whereby orofacial pain affects motor function are poorly understood. The aims were to determine whether 1) lingual algesic chemical stimulation affected face primary motor cerebral cortex (face MI) excitability defined by intracortical microstimulation (ICMS); and 2) any such effects were limited to the motor efferent MI zones driving muscles in the vicinity of the noxious stimulus. Ketamine-anesthetized Sprague–Dawley male rats were implanted with electromyographic (EMG) electrodes into anterior digastric, masseter, and genioglossus muscles. In 38 rats, three microelectrodes were located in left face MI at ICMS-defined sites for evoking digastic and/or genioglossus responses. ICMS thresholds for evoking EMG activity from each site were determined every 15 min for 1 h, then the right anterior tongue was infused (20 μl, 120 μl/h) with glutamate (1.0 M, n = 18) or isotonic saline (n = 7). Subsequently, ICMS thresholds were determined every 15 min for 4 h. In intact control rats (n = 13), ICMS thresholds were recorded over 5 h. Only left and right genioglossus ICMS thresholds were significantly increased (≥350%) in the glutamate infusion group compared with intact and isotonic saline groups (P < 0.05). These dramatic effects of glutamate on ICMS-evoked genioglossus activity contrast with its weak effects only on right genioglossus activity evoked from the internal capsule or hypoglossal nucleus. This is the first documentation that introral noxious stimulation results in prolonged neuroplastic changes manifested as a decrease in face MI excitability. These changes appear to occur predominantly in those parts of face MI that provide motor output to the orofacial region receiving the noxious stimulation.

INTRODUCTION

It is well known that motor function is affected by pain. Clinical or experimentally induced pain in the orofacial and spinal motor systems has been shown to result in smaller and slower movements and an inhibition of agonist muscle activity compared with asymptomatic controls (e.g., Dworkin et al. 1990; Graven-Nielsen et al. 1997; High et al. 1988; Lund et al. 1991; Mongini et al. 1989; Schaible and Grubb 1993; Schwartz and Lund 1995; Stohler 1999; Svensson and Graven-Nielsen 2001; Svensson et al. 1995, 1998, 2004; van Dieen et al. 2003). Although it has been well documented that muscle pain has modulatory effects on general motor function at the spinal cord and brain stem levels (Lund et al. 1991; Matre et al. 1998; Rossi and Decchi 1995; Sohn et al. 2000; Svensson et al. 2001; Wang et al. 2000), pain-imaging studies (Casey 1999; Svensson et al. 1997) in the spinal sensorimotor system have demonstrated that the cerebral primary motor cortex (MI) may also be involved. These observations, together with the neuroplastic changes that have been shown to occur in MI in association with peripheral manipulations (Miles 2005; Sanes and Donoghue 2000; Sessle 2006), raise the question as to whether nociceptive afferent activity results in changes within MI that could contribute to the effects of pain on motor function. A number of recent studies indeed point to inhibitory effects of noxious stimulation of the limb on limb MI excitability (Farina et al. 2001; Le Pera et al. 2001; Valeriani et al. 1999, 2001). Further, some of these data suggest that there is a somatotopic association between the MI test stimulus and the noxious conditioning stimulus since the limb MI effects are maximal for the forelimb muscles adjacent to the painful area (Farina et al. 2001; Le Pera et al. 2001).

In contrast to this evidence for a depression of limb MI excitability by noxious stimulation of the limb, some recent transcranial magnetic stimulation (TMS) studies in humans suggest that the excitability of face MI is not affected by experimentally induced orofacial pain (Halkjaer et al. 2006; Romaniello et al. 2000). However, the masseter muscle was specifically tested in the study of Romaniello et al. (2000) and the precontraction of the masseter muscle required to obtain the motor potentials evoked by TMS may have masked any inhibitory effect of the noxious orofacial stimulation (Halkjaer et al. 2006; Le Pera et al. 2001). Further, there may be differences in the MI effects of deep muscle pain compared with mucosal pain since previous studies (Dubner and Ren 2004; Yu et al. 1993) have shown that noxious algesic chemical stimulation of tongue muscle or temporomandibular joint (TMJ) resulted in greater central sensitization than for algesic chemical stimulation of facial skin, consistent with earlier findings (Wall and Woolf 1984) that deep stimuli applied to spinally innervated tissues were more effective than superficial stimuli in producing central sensitization.

We therefore sought to study further the effects of orofacial noxious stimulation on the excitability of the face MI by using a sensitive measure of motor cortical excitability as revealed by the technique of intracortical microstimulation (ICMS). In addition, we used a potent noxious stimulus, 1.0 M glutamate, that previously has been shown to activate craniofacial nociceptive muscu-
oskeletal afferents and to produce trigeminal central sensitization in rats and moderately severe pain in humans (Cairns et al. 2003; Lam et al. 2005a,b; Svensson et al. 2003). Therefore the aims of this study were to determine 1) whether noxious lingual stimulation affected the excitability of the rat’s face MI as defined by ICMS and 2) whether any such effects were limited to the motor efferent MI zones driving muscles in the vicinity of the noxious stimulus. These data have been briefly reported in abstract form (Murray et al. 2005).

**METHODS**

Male Sprague–Dawley adult rats (weight 290–440 g) were used in this study. All procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada). Because many of the procedures have been previously described in detail (Adachi et al. 2007), the following text will concentrate on those methods that have not been reported before.

**Surgical procedures**

Anesthesia was maintained by continuous infusion of ketamine HCl (25 mg/ml) through a femoral vein cannula at 75 mg·kg⁻¹·h⁻¹ during the implantation of bipolar electromyographic (EMG) electrode wires and while a craniotomy was completed over the left sensorimotor cortex at the anterior–posterior plane (AP) −1 to 5 mm and the medial–lateral plane (ML) 1 to 5 mm from bregma, the region within which face MI is located (Adachi et al. 2007; Neafsey et al. 1986). During the period of the experiment in which ICMS was applied (see following text), the infusion rate was kept at a level between 25 and 50 mg·kg⁻¹·h⁻¹. Body temperature was maintained at 37–38°C and heart rate was maintained at 330–430 beats/min. EMG electrodes were implanted into the left and right masseter, anterior digastric, and genioglossus muscles to record any ICMS-evoked activities. EMG electrode placement was confirmed after surgery by visual observation of muscle twitch movements induced by applying stimulus trains (12 × 0.2-ms pulses, 333 Hz, 200–400 μA) to each pair of electrodes. The dura was kept intact and was covered with warm mineral oil (37°C).

**ICMS procedures**

Glass-insulated tungsten microelectrodes were used for ICMS (12 pulses of width 0.2 ms, 333 Hz, total train duration 33.2 ms) as previously described (Adachi et al. 2007; Huang et al. 1989; Murray and Sessle 1992). The face MI (see following text) was grossly mapped by applying ICMS through the microelectrode at ≤60 μA at every 200 μm of depth in each transdural microelectrode penetration track in a systematic series of penetrations (each separated by 0.5 mm, maximum penetration depth: 6,200 μm) made by a micropositioner. The extent of face MI was defined by penetrations that were made from AP 2.5 mm and ML 3 mm and extended laterally until ICMS (60 μA) within that penetration evoked neither EMG activities nor any visible movement. In this initial mapping, an ICMS-positive site was defined when ICMS evoked, in ≥50% of stimulus deliveries at a set intensity, a burst of EMG activity clearly distinguishable from background and at short latency (in the range 8–50 ms), and/or a twitchlike movement in close temporal association with the ICMS stimulus. Penetrations were then applied in the rostral and caudal planes (Adachi et al. 2007; Lee et al. 2006; Neafsey et al. 1986).

**Experimental design**

Of 48 rats used in the study, 38 were divided into three groups to test whether glutamate infusion into the tongue (see following text) affected ICMS-evoked jaw and/or tongue EMG activity. Based on sample size calculations to ensure sufficient numbers for statistical power, the three groups were: intact control group (n = 13), isotonic saline infusion group (n = 7), and glutamate infusion group (n = 18). Of the remaining 10 rats, 4 rats were used solely for an extensive ICMS mapping study to investigate the cortical jaw/tongue muscle representation, as previously described (Adachi et al. 2007; Lee et al. 2006). In the remaining 6 rats, additional microelectrodes were stereotaxically placed in the left internal capsule (n = 6; AP −2.5 to −3.5 mm, ML 3.0 to −3.5 mm, depth 7,200–7,600 μm) or left hypoglossal nucleus (n = 5; AP −13.5 mm, ML 0.5 mm, depth 8,600 μm). Microstimulation with the same parameters as those for ICMS was delivered through these subcortical microelectrodes to assess whether glutamate infusion affected jaw and/or tongue EMG activity evoked from these subcortical sites. After defining the gross topographical map in each of the 38 animals (see earlier text), the coronal plane located near the caudal end of the left face MI (AP 3 mm) was selected to fix three microelectrodes (1: 4.0 mm; 2: 3.5 mm; 3: 3.0 mm lateral from the midline) in a linear array at ICMS-positive sites from which jaw and/or tongue muscle EMG activities could be evoked at the lowest ICMS intensities for that penetration.

In the two infusion groups, the thresholds for ICMS-evoked jaw and/or tongue muscle EMG activities were obtained every 15 min for 1 h before and 4 h after infusion. For the intact control group, ICMS thresholds were recorded every 15 min for 5 h. At each time point and at each microelectrode, four to six trains of ICMS were delivered at 1-s intervals, initially at 60 μA and then at 10–20 μA, to establish the current intensity at which no response was evoked; current was then progressively increased until ICMS threshold was determined on the basis of an evoked response in 50% of stimuli at a set intensity. At each site for evoking EMG activity, the mean threshold value of the first 1-h epoch prior to the infusion (thresholds at 15-min intervals corresponding to IT1, IT2, IT3, and IT4) was defined as the initial threshold (IT). To minimize cortical damage (Asanuma and Arnold 1975), ICMS currents usually never exceeded 60 μA to determine initial thresholds (IT1–IT4) (Table 1), although in two glutamate infusion and two saline infusion animals, initial thresholds approached 60 μA and it was necessary for the ICMS thresholds to be increased >60 μA after infusion. In these four animals, maximum currents were limited to 80 μA for threshold determination in the postinfusion period.

In the two infusion groups, a 27G needle was inserted 10 mm into the right anterior tongue muscle and the needle remained in the tongue without fixation until termination of the experiment. At 10 min after needle insertion, the right anterior rat tongue was then infused for 10 min (20 μl, 120 μl/h) with the algesic chemical glutamate [L-glutamic acid monosodium salt (Sigma, St. Louis, MO), 1.0 M, n = 18] or was infused for 10 min with nonalgesic isotonic saline (0.9%, pH = 7.14, n = 7). At the end of the infusion, ICMS thresholds at each EMG electrode site were determined every 15 min for 4 h and the mean of each 1-h epoch was obtained (1st h, T1; 2nd h, T2; 3rd h, T3; and 4th h, T4). The EMG thresholds evoked from the subcortical microelectrodes were also obtained after glutamate infusion. No swelling of the tongue was noted following any of the infusions. At the end of each experiment, electrolytic lesions (10 μA, 10 s, cathodal DC) were placed at each ICMS site through each of the three microelectrodes for subsequent histological verification of ICMS sites.

**Data acquisition and analysis**

EMG activity was processed off-line through a custom program. For each muscle, the EMG activities for the 10-ms period preceding and for the 100-ms period following the onset of ICMS were rectified and digitally smoothed (moving average, 4-ms window). The mean ± 2SDs of the background activity was obtained from the initial 10-ms period. A site was defined as a positive ICMS site for evoking activity in a muscle when this amplitude level (i.e., mean ± 2SD) was
ANOVA) comparing IT1 thresholds at IT2, IT3, and IT4. LAD, RAD: left, right anterior digastric muscle; LGG, RGG: left, right genioglossus muscle. The lowest ICMS intensity that induced such an EMG response in the jaw and/or tongue muscles in 50% of ICMS deliveries was determined as the ICMS threshold for that site. At some time points, no muscle responses could be evoked at ICMS currents of 60 μA (see earlier text) and even ICMS currents of 80 μA failed to induce jaw or tongue muscle responses. For the purposes of data entry in the statistical analyses, thresholds were assigned these maximum values (i.e., 80 μA) at these time points for these animals. The latency of EMG activities induced at threshold ICMS intensity was also obtained. The averaged ICMS thresholds for each 1-h epoch in the 4 h after infusion (i.e., times T1–T4) were normalized with respect to initial threshold for data expression and analysis. Within-group data were compared by one-way ANOVA (across initial thresholds IT1–IT4) followed by Dunnett’s test for post hoc analysis. The comparison across groups was analyzed by one-way ANOVA (for mean value of initial threshold for data expression and analysis). The comparison across groups was analyzed by one-way ANOVA (across initial thresholds IT1–IT4) followed by the Tukey–Kramer test for post hoc analysis. A probability level of $P < 0.05$ was considered statistically significant. All values are expressed as mean ± SE.

Histology

At the termination of the experiment, each rat was killed by an overdose of anesthetic and fixed by a transcardial perfusion of isotonic saline followed by 10% buffered formalin (Fisher Scientific, Morris Plains, NJ). The brain was removed and stored in 10% buffered formalin. Cross sections of the brain, which covered all of the penetrations, were cut at 50-μm thickness by a vibratome and were stained with cresyl violet or hematoxylin and eosin for reconstruction of microelectrode penetrations. The structures of the brain were described according to the rat brain atlas (Paxinos and Watson 2005).

RESULTS

Jaw and tongue representations in MI

In the initial mapping study ($n = 4$), ICMS ($\pm 60$ μA) at each MI site in the rat sensorimotor cortex evoked positive EMG responses in one or more of the left and right anterior digastric, genioglossus, or masseter muscles, or visible twitch movements of vibrissa(e), neck, forelimb, hindlimb, or trunk regions. These ICMS-positive sites were widely distributed from AP 0.5 to AP 4.0 (Fig. 1, A and B) and were verified histologically (Fig. 1C) to be located in the face region of MI, the secondary motor cortex (SII), the primary somatosensory cortex (SI), the forceps minor of the corpus callosum, the caudate putamen, or the claustrum. The ICMS-positive sites for left and right anterior digastric, genioglossus, and masseter in the face MI were located between AP 2.5 and 4.0 and between 3.0 and 4.0 mm lateral from the midline (Fig. 1). The jaw and/or tongue representations within the face MI were surrounded rostrally and laterally by negative penetrations (i.e., penetrations from which neither EMG responses nor twitch movements could be evoked by ICMS) and were surrounded caudally and medially by ICMS-positive sites for vibrissae, neck, limb, and/or trunk (see Fig. 1, A and B). The histologically defined SI also contained a few positive ICMS sites for the jaw and/or tongue muscles, although the location (AP 1.5–2.0 and lateral 3.5–4.5 mm) was different from those of the face MI, and there were only seven ICMS sites effective for evoking masseter activity. Therefore the SI data and the masseter MI data were not included in further analyses.

ICMS thresholds and latencies within face MI and effects of intramuscular glutamate infusion

The ICMS threshold values obtained at initial thresholds IT1, IT2, IT3, and IT4 from microelectrodes 1–3 across all three groups (intact, isotonic saline infusion, and glutamate infusion) were distributed in the range 10–60 μA (Table 1). Across all animals, the mean initial ICMS thresholds (i.e., mean of initial thresholds IT1, IT2, IT3, and IT4) for evoking genioglossus and anterior digastic activity (left and right data combined) were, respectively, 20.5 and 30.9 μA (microelectrode 1), 27.9 and 26.5 μA (microelectrode 2), and 23.8 and 26.2 μA (microelectrode 3). In each experimental group, for each muscle and at each ICMS microelectrode site, threshold values at initial thresholds IT2, IT3, and IT4 were not significantly different from that at IT1 (see Table 1). There were also no significant differences between groups (intact vs. isotonic saline infusion vs. glutamate infusion).

<table>
<thead>
<tr>
<th>Group</th>
<th>Microelectrode 1</th>
<th>Microelectrode 2</th>
<th>Microelectrode 3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Threshold Mean</td>
<td>Threshold Mean</td>
<td>Threshold Mean</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Range, μA</td>
<td>P</td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>8</td>
<td>14–46</td>
<td>0.71</td>
</tr>
<tr>
<td>RAD</td>
<td>10</td>
<td>10–50</td>
<td>0.40</td>
</tr>
<tr>
<td>LGG</td>
<td>7</td>
<td>13–19</td>
<td>0.56</td>
</tr>
<tr>
<td>RGG</td>
<td>5</td>
<td>10–20</td>
<td>0.10</td>
</tr>
<tr>
<td>Isotonic saline infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>3</td>
<td>20–32</td>
<td>0.41</td>
</tr>
<tr>
<td>RAD</td>
<td>5</td>
<td>14–40</td>
<td>0.39</td>
</tr>
<tr>
<td>LGG</td>
<td>3</td>
<td>18–22</td>
<td>0.52</td>
</tr>
<tr>
<td>RGG</td>
<td>3</td>
<td>18–25</td>
<td>0.66</td>
</tr>
<tr>
<td>Glutamate infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>6</td>
<td>18–42</td>
<td>0.60</td>
</tr>
<tr>
<td>RAD</td>
<td>5</td>
<td>20–44</td>
<td>0.85</td>
</tr>
<tr>
<td>LGG</td>
<td>5</td>
<td>10–48</td>
<td>0.49</td>
</tr>
<tr>
<td>RGG</td>
<td>5</td>
<td>15–48</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The $n$ values are less than the total numbers of rats in each of the three groups because ICMS at each microelectrode site did not necessarily activate a particular muscle in all animals of the group. Columns labeled P show, for each muscle in each group and at each microelectrode site, the statistical analysis (one-way ANOVA) comparing IT1 thresholds at IT2, IT3, and IT4. LAD, RAD: left, right anterior digastric muscle; LGG, RGG: left, right genioglossus muscle.
The effect of repeated application of ICMS over 5 h on ICMS thresholds for the intact control group is plotted in Fig. 2 (open squares). Some threshold values were significantly increased compared with those at initial threshold (IT) (one-way ANOVA followed by Dunnett’s test) for the anterior digastic muscles at microelectrode 1 [right anterior digastic: T3, P < 0.01 (++); right anterior digastic: T4, P < 0.05 (+)] and microelectrode 2 [left anterior digastic: T4, P < 0.01 (++); right anterior digastic: T4, P < 0.01 (++)], although the ICMS thresholds for left and right genioglossus at each microelectrode site were not significantly different between IT and 1st- to 4th-h thresholds (T1–T4).

The ICMS thresholds for evoking orofacial muscle activities from each ICMS microelectrode site were compared among the glutamate infusion, intact control, and isotonic saline infusion groups to determine whether glutamate infusion into the tongue affected the ICMS thresholds (Fig. 2). For the ICMS thresholds for the genioglossus and anterior digastic muscles, there were significant time (P < 0.01; each muscle from each microelectrode site) and time × treatment effects (i.e., intact, isotonic infusion, glutamate infusion) effects [left genioglossus: microelectrode 1, F(8,60) = 3.15, P < 0.01; right genioglossus: microelectrode 1, F(8,45) = 3.47, P < 0.01; two-way ANOVA], as well as significant treatment effects [left anterior digastic: microelectrode 1, F(2,70) = 4.14, P < 0.05; right anterior digastic: microelectrode 1, F(2,85) = 4.29, P < 0.05; microelectrode 2, F(2,120) = 4.02, P < 0.05; microelectrode 3, F(2,95) = 3.12, P < 0.05; left genioglossus: microelectrode 1, F(2,60) = 31.79, P < 0.0001; right genioglossus: microelectrode 1, F(2,45) = 34.19, P < 0.0001; microelectrode 2, F(2,75) = 5.10, P < 0.01; microelectrode 3, F(2,40) = 10.39, P < 0.01]. Further post hoc analyses revealed that glutamate infusion significantly increased ICMS thresholds for the left genioglossus from the most lateral microelectrode site (i.e., microelectrode 1) at 1st-h threshold T1 (vs. isotonic saline group), 2nd-h threshold T2 (vs. isotonic saline group), 3rd-h threshold T3 (vs. isotonic saline group), and 4th-h threshold T4 (vs. intact and isotonic saline groups) (Tukey–Kramer test, Fig. 2). There were similar effects of glutamate infusion on the ICMS thresholds for evoking right genioglossus activities from the most lateral microelectrode site (i.e., microelectrode 1) for 1st-h threshold T1 (vs. intact group), and 2nd-, 3rd-, and 4th-h thresholds (T2, T3, T4 vs. intact and isotonic saline groups), at microelectrode 2 (T1: vs. isotonic saline group and microelectrode 3 (T1, T4: vs. intact group) (Tukey–Kramer test) (Fig. 2). There were no significant differences between the isotonic saline infusion and intact groups for ICMS thresholds for each muscle and at each ICMS microelectrode site. Figure 3 (left panels) shows representative raw data from one rat from the glutamate infusion group (microelectrodes 1, 2, and 3). Note the dramatic increases in thresholds for evoking activity in the right genioglossus at all three microelectrode sites in this rat.

The latency values associated with the threshold EMG responses evoked by ICMS in face MI during the first 1-h period from all animals were in the range 8.2–49.9 ms and these latencies are generally consistent with earlier findings (Adachi et al. 2007). Table 2 shows the range of latency values in the first 1-h period in all groups. With respect to
threshold, latency was stable in the first 1-h period with no significant differences between initial threshold values (IT1–IT4, Table 2), except the latency value of the left anterior digastric at microelectrode 2 of the intact group [F(3,36) = 3.07, P < 0.05]. When comparing latencies across initial threshold to 4th-h thresholds (IT–T4), there was a significant difference [F(2,14) = 5.59, P < 0.05: one-way ANOVA] for the left genioglossus response evoked from microelectrode 1 between the three groups (intact group: 27.9 ± 2.0 ms; isotonic saline infusion group: 33.4 ± 1.8 ms; glutamate infusion group: 25.3 ± 2.7 ms) with the saline infusion group latency being significantly (P < 0.05, Tukey–Kramer test) longer than that in the glutamate group (see Table 2). For the latencies of the ICMS-evoked responses, there were some significant treatment effects [right anterior digastric: microelectrode 1, F(2,82) = 16.19, P < 0.0001; microelectrode 2, F(2,118) = 5.13, P < 0.01; left genioglossus: microelectrode 2, F(2,92) = 5.52, P < 0.01; right genioglossus: microelectrode 1, F(2,42) = 4.05, P < 0.05; microelectrode 2, F(2,67) = 3.53, P < 0.05; two-way ANOVA] and treatment × time effects [right anterior digastric: microelectrode 1, F(8,82) = 2.20, P < 0.05; two-way ANOVA], but there were no significant effects on response latencies of glutamate infusion as assessed by post hoc analysis. Figure 4 shows latency data for left genioglossus and right genioglossus muscles.

**Thresholds within internal capsule and hypoglossal nucleus and effects of intramuscular glutamate infusion**

From the internal capsule site, activity was evoked in the anterior digastric (mean IT: 22.5 μA) and/or genioglossus muscles (19.0 μA). From the hypoglossal nucleus site, activity was evoked in the anterior digastric (mean IT: 9.9 μA) and/or genioglossus muscles (9.5 μA). Initial thresholds at IT2, IT3, and IT4 were not significantly different from those at IT1 when evoked from the internal capsule or hypoglossal nucleus for left and right anterior digastric and genioglossus muscles (P > 0.05, one-way ANOVA). After glutamate infusion, there were no significant differences (P > 0.05, one-way ANOVA) between IT and the 1st-, 2nd-, 3rd-, and 4th-h thresholds (T1, T2, T3, and T4) for left and right anterior digastric activity evoked from the internal capsule (n = 6) or hypoglossal nucleus (n = 5), and for left genioglossus activity evoked from internal capsule (n = 6) or hypoglossal nucleus (n = 6). There was a small but significant increase (P < 0.05, one-way ANOVA) in threshold at the 2nd h (T2) and the 3rd h (T3) (maximum: 155%) for right genioglossus activity evoked from the internal capsule, and at the 4th h (T4) only (maximum: 149%) for right genioglossus activity evoked from the hypoglossal nucleus.
However, these small increases were significantly less ($P < 0.05$, two-way ANOVA) than the large increases ($\leq 350\%$) documented in ICMS thresholds of both left and right genioglossus after glutamate infusion (Fig. 5). Furthermore, the thresholds for genioglossus or anterior digastric activity evoked from internal capsule and hypoglossal nucleus after glutamate infusion were not significantly different from the ICMS thresholds at each microelectrode cortical site in the intact and isotonic saline groups ($P > 0.05$; one-way ANOVA). Figure 3 shows representative data from one rat from the glutamate infusion group with microelectrodes in the internal capsule and hypoglossal nucleus (right panels). Histological analysis in all six rats verified that microelectrode sites were located in the internal capsule and in the hypoglossal nucleus or immediately adjacent solitary tract nucleus.

### Table 2. Ranges of latencies for each muscle at first 1-h period of experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle</th>
<th>Latency, ms (IT1–IT4)</th>
<th>$P$ (IT1–IT4) (vs. Int/Sal)</th>
<th>Latency, ms (IT1–IT4)</th>
<th>$P$ (IT1–IT4) (vs. Int/Sal)</th>
<th>Latency, ms (IT1–IT4)</th>
<th>$P$ (IT1–IT4) (vs. Int/Sal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>LAD</td>
<td>9.1–43.0</td>
<td>0.99</td>
<td>10.4–43.3</td>
<td>0.04*</td>
<td>9.8–43.9</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>RAD</td>
<td>9.5–42.0</td>
<td>0.43</td>
<td>8.8–49.4</td>
<td>0.86</td>
<td>10.8–35.3</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>LGG</td>
<td>12.0–42.2</td>
<td>0.17</td>
<td>10.1–44.7</td>
<td>0.19</td>
<td>10.7–48.5</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>RGG</td>
<td>9.1–45.4</td>
<td>0.90</td>
<td>8.8–44.3</td>
<td>0.77</td>
<td>18.1–41.3</td>
<td>0.92</td>
</tr>
<tr>
<td>Isotonic saline infusion</td>
<td>LAD</td>
<td>10.1–41.1</td>
<td>0.95</td>
<td>8.2–42.4</td>
<td>0.89</td>
<td>10.8–38.4</td>
<td>0.32</td>
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<tr>
<td></td>
<td>RAD</td>
<td>9.6–37.9</td>
<td>0.27</td>
<td>9.1–38.1</td>
<td>0.61</td>
<td>9.3–35.0</td>
<td>0.26</td>
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<tr>
<td></td>
<td>LGG</td>
<td>27.4–42.1</td>
<td>0.78</td>
<td>9.4–43.6</td>
<td>0.63</td>
<td>16.2–41.8</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>RGG</td>
<td>12.0–39.1</td>
<td>0.86</td>
<td>12.5–40.8</td>
<td>0.91</td>
<td>11.7–41.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Glutamate</td>
<td>LAD</td>
<td>12.0–49.1</td>
<td>0.44</td>
<td>9.8–40.3</td>
<td>0.18</td>
<td>9.0–40.1</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>RAD</td>
<td>9.8–45.3</td>
<td>0.73</td>
<td>9.8–40.1</td>
<td>0.78</td>
<td>8.8–39.3</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>LGG</td>
<td>11.0–39.5</td>
<td>0.20</td>
<td>9.1–41.4</td>
<td>0.95</td>
<td>14.0–42.2</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>RGG</td>
<td>19.0–47.8</td>
<td>0.43</td>
<td>21.5–41.6</td>
<td>0.95</td>
<td>20.0–45.1</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Columns labeled $P$ (IT1–IT4) show, for each muscle in each group and at each microelectrode site, the results of the statistical analysis (one-way ANOVA) comparing initial threshold IT1 latency with latencies at initial thresholds IT2, IT3, and IT4. Columns labeled $P$ (vs. Int/Sal) show, for each muscle at each microelectrode site, the results of the statistical analysis (two-way ANOVA) comparing latency across IT1–IT4 between glutamate infusion, isotonic saline infusion, and intact groups. LAD, RAD: left, right anterior digastic muscle; LGG, RGG: left, right genioglossus muscle.
DISCUSSION

The present study has provided the first documentation that infusion of the algesic chemical glutamate into orofacial tissues affects the excitability of the contralateral face MI. The data demonstrate that glutamate infusion, but not isotonic saline (vehicle) infusion, into the rat’s tongue resulted in a significant increase in ICMS thresholds in the left and right genioglossus muscles at the most lateral ICMS microelectrode site within face MI. Glutamate infusion did not result in any significant change in ICMS thresholds within the right or left anterior digastric muscles nor were there any clear effects on response latencies to EMG responses in anterior digastic and genioglossus muscles. These dramatic effects of glutamate on ICMS-evoked genioglossus activity contrast with its weak effects only on right genioglossus activity evoked from the internal capsule or hypoglossal nucleus. Together with the findings that the thresholds for genioglossus or anterior digastic activity evoked from the internal capsule and hypoglossal nucleus after glutamate infusion were not significantly different from intact or saline ICMS thresholds at each microelectrode site, this study indicates that the effects of noxious stimulation on MI excitability may be attributed principally to intracortical mechanisms.

Comparison with previous findings

Comparable volumes and concentrations of glutamate as used in the present study activate TMJ and muscle nociceptive primary afferents and produce central sensitization in trigeminal brain stem nociceptive neurons (Cairns et al. 1998; Lam et al. 2005b) and also result in pain when injected into human jaw or neck muscles or skin (Cairns et al. 2001; Svensson et al. 2003, 2005). The present findings of the effects of glutamate on the excitability of MI are generally consistent with previous findings in the spinal motor system that noxious stimulation of cutaneous or deep tissues results in a reduction in the excitability of the contralateral or ipsilateral MI (Farina et al. 2001; Le Pera et al. 2001; Urban et al. 2004; Valeriani et al. 1999, 2001). Furthermore, it has recently been shown that capsaicin-induced intraoral pain could interfere with the increased excitability (i.e., decreased thresholds) of tongue MI associated with human learning of a tongue-protrusion task (Boudreau et al. 2007). However, no significant effects on human masseter (Romaniello et al. 2000) or human tongue (Halkjaer et al. 2006) motor-evoked potentials were observed in response to hypertonic saline-evoked masseter muscle pain or capsaicin-evoked cheek skin pain, or in response to capsaicin applied to the mucosa of the tongue. These differences between human spinal and trigeminal studies may reflect differences in the corticomotor integration of trigeminal and spinal sensory afferent inputs (Halkjaer et al. 2006), methodological issues; e.g., the preconstriction of the masseter muscle required in the study of Romaniello et al. (2000) may have masked any inhibitory effect with pain (Halkjaer et al. 2006; Le Pera et al. 2001). It is also possible that the differences in effects could be explained by differences in MI effects of deep muscle pain (as in the present study) and mucosal pain (used in Halkjaer et al. 2006).

Selective effects in face MI

Our findings that genioglossus or anterior digastic EMG activity could be evoked not only from face MI but also from the internal capsule or hypoglossal nucleus and immediately adjacent solitary tract nucleus are consistent with earlier findings (De Laat et al. 1998; Dellow and Lund 1971; Dubner et al. 1978; Lowe 1981; Lund et al. 2008). However, our data further suggest that the decreased excitability after glutamate infusion occurred predominantly in face MI and in those parts of face MI providing the motor drive to the orofacial region subjected to the noxious stimulation. First, noxious stimulation of tongue muscle by glutamate only modestly influenced the threshold for evoking EMG activity from the internal capsule or brain stem sites and, for the right genioglossus, only at limited time periods. Second, glutamate decreased the excitability of the representation in the face MI of left or right genioglossus (the main protrusive muscle of the tongue) at all time periods tested but did not affect the excitability of the representation of the anterior digastic (a major jaw-opening muscle). It is possible therefore that the representation affected within face MI provides the output to those motor units in the vicinity of the noxious stimulation (i.e., in the tongue) and also that this tongue MI representation receives somatosensory feedback especially from the site of noxious stimulation within the
Mechanisms of increased thresholds

There is clear evidence for local brain stem mechanisms involved in the effects of noxious stimulation on orofacial motor activity (Bratzlavský 1978; Cadden 2007; Crusçu et al. 1986; De Laat et al. 1998; Komiyama et al. 2005; Schwart and Lund 1995; Van der Glas et al. 2000; Westberg et al. 1997; Yu et al. 1993; for review see Dufner et al. 1978; Lund et al. 2008). However, the raised face MI ICMS thresholds following glutamate infusion and the evidence for an intracortical mechanism for the effects of noxious lingual stimulation on MI thresholds, cited earlier, all support the likelihood that face MI also makes an important contribution to these effects. A cortical origin for similar inhibitory EMG effects following noxious forelimb stimulation has also been demonstrated (Le Pera et al. 2000; Valeriani et al. 1999) and there is good imaging evidence that the cortical processing of nociceptive information includes MI (Apkarian et al. 2005; Casey 1999; Casey et al. 2001; Coghill et al. 1994, 1999; Melzack 1995; Moulton et al. 2005; Svensson et al. 1997; Timmermann et al. 2001). The changes in jaw muscle activity observed in previous studies of algesic chemical injections into deep tissues (e.g., Cairns et al. 1998) are unlikely to contribute to the present findings, given that these previously reported changes dissipate within 10 min of cessation of algesic chemical injection (i.e., before the tests of MI excitability used in the present study).

The gradual increase in ICMS thresholds for evoking activity in the tongue over the 4-h period following glutamate infusion may reflect a gradual buildup of general anesthetic, tonic nociceptive inputs from surgical sites, and/or a deterioration in the ICMS microelectrodes and/or the local cortex. Although threshold increases were also noted in the intact and isotonic groups (see following text), the noxious glutamate effects were nonetheless significantly greater for the genioglossus muscles. Previous studies have also reported prolonged effects of noxious stimuli on spinal sensorimotor system excitability that may last several hours after recovery from pain (e.g., Henderson et al. 2006; Hoheisel and Mense 1989; Le Pera et al. 2000, 2001; Matre et al. 1998; Wall and Woolf 1984). In the trigeminal system, evidence for a central inhibitory effect induced by noxious stimulation of deep tissues has been demonstrated (Dufner and Ren 2004; Tambeli et al. 2001; Yu et al. 1994) that might explain in part the decrease in MI excitability.

Strengths and limitations of this experimental paradigm

This study made extensive use of controls for the volume of the injected solution, the noxious stimuli associated with the
needle insertion into the tongue, the surgical procedures, and the 5-h period of repeated ICMS. The microelectrodes within the hypoglossal nucleus and internal capsule provided a control for the effects observed as being at least partly mediated by circuitry involving the face MI (see earlier text), and for possible direct effects of glutamate on tongue muscle fibers. Activation of the anterior digastic muscle (in addition to the genioglossus muscle) with stimulation of the hypoglossal motor nucleus is most probably due to activation of neurons within the nearby solitary tract nucleus that have connections with the trigeminal motor nucleus (Dubner et al. 1978; Lowe 1981). A limitation of the study was that the use of only male rats precluded any assessment of possible sex differences that have been demonstrated in the sensory and motor effects of noxious stimuli in rats and humans (Cairns et al. 2001, 2002, 2003; Komiyama et al. 2005). The depressive effects of anesthetia on MI ICMS thresholds (Huang et al. 1988, 1989; Sessle and Wiesendanger 1982) may have influenced the manifestation of possibly more subtle effects of the glutamate-evoked nociceptive activity on the face MI ICMS thresholds. A future direction could be to test the effects of noxious stimulation in awake rodent sensorimotor models (e.g., Bejat et al. 2008; Ro 2005). It was also unclear whether other parts of tongue MI or the MI representations of other muscles (e.g., facial) were affected by the noxious stimulation.

Conclusions

These novel data suggest that 1) intraoral noxious stimulation can result in prolonged neuroplastic changes within the face MI that are manifested as a decrease in face MI excitability, 2) this decreased excitability occurs predominantly in those parts of face MI that provide motor output to the orofacial region receiving the noxious stimulation, and 3) these effects are at least partly mediated via intracortical mechanisms. The decreases in face MI excitability may be related to reports that individuals with orofacial pain experience motor weakness and difficulty in movements (Dworkin et al. 1990; Helkimo et al. 1975; High et al. 1988; Lund et al. 1991; Molin 1972; Møller et al. 1984; Murray and Peck 2007; Sae-Lee et al. 2008; Schaible and Grubb 1993; Stohler 1999; Svensson and Graven-Nielsen 2001). The decreases in face excitability associated with orofacial pain might provide a possible suprabulbar mechanism contributing to the limitation of movement and protection of the musculoskeletal system from further injury that is proposed by the Pain Adaptation Model (Lund et al. 1991; Murray and Peck 2007).

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