FACE MOTOR CORTEX NEUROPLASTICITY ASSOCIATED WITH ALTERATIONS IN THE ORAL ENVIRONMENT OF THE ADULT RAT

by

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Abstract

Neuroplastic changes in motor representations within the primary motor cortex (M1) have been described after peripheral manipulations and implicated in motor learning and adaptation processes. It is unclear whether dental manipulations, which may result in altered oral sensorimotor functions, are associated with analogous changes within face-M1. This project applied intracortical microstimulation (ICMS) and recordings of evoked muscle electromyographic (EMG) activity to test if changes occur in the ICMS-defined motor representations of tongue-protrusion (genioglossus, GG) and jaw-opening (anterior-digastric, AD) muscles within face-M1 and adjacent face primary somatosensory cortex (face-S1) following trimming or extraction of the rat's right mandibular incisor, or a change in diet consistency.

ICMS mapping was carried out in anaesthetised adult male rats. Consistent with previous findings, AD and GG had extensive motor representations showing considerable overlap in naïve and sham control rats. AD and GG motor representations were also found within face-S1. Left and right AD (LAD, RAD) had significantly larger representations with shorter onset latency of ICMS-evoked EMG responses within contralateral face-M1.

A change in diet consistency for 2-3 weeks was not associated with significant changes in AD and GG motor representations within face-M1. Compared to control rats,

incisor trimming out of occlusion for a period of 1 week resulted, 1 day later, in a significantly longer GG onset latency in ipsilateral than in contralateral face-M1; 1 week later, despite a regain of normal occlusion, GG and GG/AD overlapping representations were significantly larger and the centre of gravity (at AP 4.0) was significantly deeper in contralateral than in ipsilateral face-M1. Incisor extraction was associated, 1 week later, with significantly larger RAD and RAD/GG overlapping representations and a lateral shift of LAD and RAD centre of gravity. Extraction also induced significant changes in AD and GG motor representations within the contralateral face-S1.

These novel findings indicate that face-M1 can undergo neuroplastic changes in association with intraoral manipulations and also suggest similar neuroplastic capabilities for face-S1 motor outputs. These findings contribute to our understanding of the role of face-M1 and face-S1 in sensorimotor adaptations to an altered oral state and provide the basis for several future studies.

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LIST OF ABBREVIATIONS

AD anterior digastric
AP anteroposterior
CMA cortical masticatory area
CPG central pattern generator
EMG electromyographic
fMRI functional magnetic resonance imaging
GABA γ-amino butyric acid
GG genioglossus
ICMS intracortical microstimulation
InterV intertrigeminal region
JuxtaV juxtatrigeminal region
LAD left anterior digastric
LTD long-term depression
LTP long-term potentiation
M1 primary motor cortex
ML mediolateral
PMA premotor cortex
PO thalamic posterior nucleus
RAD right anterior digastric
S1 primary somatosensory cortex
S2 secondary somatosensory cortex
SD standard deviation
SEM standard error of the means
SMA supplementary motor area
SupraV supratrigeminal region
TMJ temporomandibular joint
TMS transcranial magnetic stimulation
VB thalamic ventrobasal nucleus
VBSNC trigeminal brainstem sensory nuclear complex
VL thalamic ventrolateral nucleus
VLc thalamic ventrolateral nucleus par caudalis
VLm medial division of the thalamic ventrolateral nucleus
VLo thalamic ventrolateral nucleus par oralis
VPLc thalamic ventroposterolateral nucleus par caudalis
VPLo thalamic ventroposterolateral nucleus par oralis
VPM ventroposteromedial nucleus of the thalamus
V - trigeminal
Vm trigeminal motor nucleus
VII facial nucleus

CHAPTER 1

LITERATURE REVIEW

It is well known that the primary motor cortex (M1) representing the orofacial region (face-M1) plays a crucial role in the generation and control of orofacial motor functions (*e.g.* jaw opening, tongue protrusion, mastication). It is also known that the somatosensory system, including the face primary somatosensory cortex (face-S1), provides somatosensory feedback to further assist in the control of orofacial motor functions and may also play a role in the generation of these motor functions. Recent studies in monkeys and in humans suggest that face-M1 also plays a role in adaptation and learning processes associated with alterations in the orofacial sensorimotor functions. This role of face-M1 is reflected in its capability to change its neural features and be modelled throughout life.

The main purpose of the present thesis was to study the neuroplastic capabilities of the jaw and tongue motor representations within the face-M1 of adult rats following various manipulations to the oral tissues such as tooth extraction, tooth trimming or a change in diet consistency. Motor representations can be delineated as the movements that can be evoked by electrical stimulation of face-M1 which activates brainstem motoneurons. Nonetheless, brainstem motoneurons can be activated by descending projections from motor centres other than face-M1. In addition, face-M1 receives inputs from cortical and subcortical areas that can further modulate its motor outputs. All these projections are probably part of the neural substrate modulating and coordinating the activities of the orofacial muscles. Therefore, this literature review will first outline the anatomical substrate of the orofacial sensorimotor system, *i.e.*, the somatosensory inputs and motor outputs of the sensorimotor cortex representing the orofacial area including a description of its cytoarchitecture. Obviously, the sensorimotor system is very complex and therefore, this literature review will focus on a description of the sensory inputs and motor outputs of the primary sensorimotor cortex. This will be followed by a discussion of the role of face-M1 and face S1 in the control of orofacial motor functions including face-M1 neuroplastic capabilities. Then I will discuss the possible mechanisms underlying face-M1 neuroplasticity. Since the rat is the animal used in this thesis project and monkeys are also used in our laboratory's ongoing studies, emphasis will be given to the features of the rat and monkey sensorimotor cortex. Finally, the intracortical

microstimulation (ICMS) technique that was extensively used in the present study will be described.

1. Anatomical substrates

1.1. Peripheral receptors

Orofacial tissues are characterized by high tactile sensitivity attributed to the high innervation density of exteroceptors, proprioceptors and nociceptors (for reviews, see – Capra, 1995; Dubner and Sessle, 1978; Hildebrand et al., 1995; Hu, 2004; Jacobs and van Steenberghe, 1994; Macefield, 2005; Paxinos, 2004; Svensson and Sessle, 2004; Trulsson and Essick, 2004). Skin and mucosa have free nerve endings and specialized mechanoreceptors that function as exteroceptors as well as proprioceptors in response to deformations of underlying muscles during orofacial movements (e.g. facial expressions, chewing and speaking). These receptors are also important for the control of facial and jaw-opening muscles that have significantly fewer or no muscle spindles (Lennartsson, 1980; Rokx et al., 1984). The periodontal ligament has specialized mechanoreceptors distributed along the dental roots and function as exteroceptors as well as proprioceptors. The dental pulp has a rich innervation with free nerve endings sensitive to mechanical, thermal or chemical stimuli. In contrast, in the continuously erupting incisor teeth of rats, pulpal innervation is relatively diminutive (Naftel et al., 1999) and most of the axons can be found in the labial odontoblastic layer of the pulp but not in the lingual odontoblastic layer or within the dentinal tubules (Zhang et al., 1998).

The rich orofacial innervation provides the peripheral feedback and feedforward information needed for the control of masticatory muscles (for reviews, see Johansson et al., 2006a; Paxinos, 2004; Trulsson, 2006; Trulsson, 2007; Trulsson and Essick, 2004). Hence, any change in the orofacial environment, from changes in diet consistency to dental manipulation by trimming or extraction, may conceivably affect the exteroceptive, proprioceptive and perhaps nociceptive inputs. Furthermore, altered occlusal contacts resulting from tooth trimming or tooth extraction may alter the patterns of jaw and tongue movements during mastication (Endo et al., 1998b; Klineberg and Jagger, 2004; Miehe et al., 1999a; Ramirez-Yanez et al., 2004; Shi et al., 2005), thereby

affecting proprioceptive as well as exteroceptive inputs from orofacial tissues involved in the altered orofacial movements.

1.2. Primary afferents and their projections to brainstem

In humans, primary afferents project through branches of the trigeminal (V) nerve. The maxillary nerve innervates the maxillae and upper teeth. The mandibular nerve innervates the mandible, lower teeth and anterior 2/3 of the tongue (lingual nerve). All lower teeth are innervated by the inferior alveolar branch of the mandibular nerve. In rats, the inferior alveolar branch innervates only the lower incisor, the 1st molar and the mesial half of the 2nd molar while the lingual branch innervates the distal halves of the 2nd molars and the 3rd molars (Naftel et al., 1999). Primary afferents from posterior tongue, larynx and pharynx project through the glossopharyngeal (IX) and the vagus (X) nerves. Ear and jaw angle afferents project through the facial (VII), the glossopharyngeal (IX) and the vagus (X) nerves. The cell bodies of these afferents are located in sensory ganglia associated with these nerves. Afferents of cranial nerves VII, IX, and X form the solitary tract and terminate in the nucleus of the solitary tract. However, many have a mechanoreceptive function and terminate in the V brainstem sensory nuclear complex (VBSNC) (For reviews, see Capra, 1995; Paxinos, 2004; Sessle, 2000; Trulsson and Essick, 2004).

Most of the exteroceptive and all the proprioceptive primary afferents innervating the orofacial area project in a somatotopic manner along the V nerve to terminate mainly in the ipsilateral VBSNC which consists of the V main sensory nucleus as well as the V spinal tract nucleus that is subdivided into subnuclei oralis, interpolaris and caudalis (For reviews, see – Capra, 1995; Paxinos, 2004; Sessle, 2000).

Exteroceptive primary afferents have their cell bodies located in the V ganglion while the proprioceptive primary afferents have their cell bodies located in the V ganglion or the V mesencephalic nucleus. Primary afferents can diverge and converge to terminate in VBSNC subnuclei (for reviews, see Capra, 1995; Dubner and Sessle, 1978; Hu, 2004; Paxinos, 2004; Sessle, 2000; Trulsson and Essick, 2004). A significant number of the primary afferents also terminate in the adjacent solitary nucleus, brainstem

reticular formation, para V nucleus, supra V nucleus, and some project to the cerebellum, cuneate, vestibulum and even to the dorsal horn of the cervical spinal cord (C1-C7). Some afferents project to the contralateral VBSNC and a significant number of the afferents terminate in the V motor nucleus (Vm) either directly (mainly proprioceptive) or indirectly through mainly inhibitory, but also excitatory, premotoneurons that project from the supra V area, reticular formation as well as from the V main sensory nucleus and subnuclei oralis and interpolaris (Rats: Dessem et al., 1997; Luo and Dessem, 1995; Luo et al., 2001; Luo and Li, 1991; Marfurt and Rajchert, 1991; Matesz, 1981; for reviews, see Capra, 1995; Paxinos, 2004; Sessle, 2000; Trulsson and Essick, 2004).

1.3. Projections to the thalamic relay nuclei

Most second-order axons arising from the V spinal tract nucleus cross the midline and project in a somatotopic manner through the ventral trigeminothalamic tract to the main thalamic sensory relay nucleus, the ventral posterior medial nucleus (VPM) (ventroposterolateral par caudalis and par oralis, VPLc, and VPLo in monkeys). Second-order axons arising from the V main sensory nucleus ascend bilaterally as the dorsal trigeminothalamic tract and also project in a somatotopic manner to VPM. Other second-order axons that project to the posterior nuclei (PO) and the medial thalamus have more divergence and their projections are less somatotopically organized (Rat: Chiaia et al., 1991; Diamond et al., 1992; Henry and Catania, 2006; Pierret et al., 2000; Monkeys: Asanuma et al., 1980; Bushnell and Duncan, 1987; Iyengar et al., 2007).

The main thalamic motor nuclei (VL in rats and VLo and VLc in monkeys) receive inputs mainly from the cerebellum and basal ganglia. In monkeys (and cats), it has been demonstrated that VL, especially at the border area with the sensory thalamus (VP), also receives exteroceptive but mainly proprioceptive sensory inputs from the limbs (Monkeys: Stepniewska et al., 2003; Vitek et al., 1994; for review, see Asanuma, 1989). Only 1 study in monkeys has demonstrated that VLo receives peripheral sensory inputs from the face area (Vitek et al., 1994). VL can receive somatosensory inputs indirectly through the cerebellum, but no data are available of the direct somatosensory

inputs from the face to the rat thalamic VL nuclei (Monkeys: Iyengar et al., 2007; Rausell and Jones, 1991b; Rats: Tabata et al., 2002; Welker, 1971).

1.4. Thalamic neurons and their projection to the sensorimotor cortex

1.4.1. Projections to the primary somatosensory cortex (S1)

Thalamic neurons project in a somatotopic manner from the main thalamic sensory nuclei (VPLc in monkeys and VPM in rats) to S1 (Rats – Chiaia et al., 1991; Diamond et al., 1992; Henry and Catania, 2006; Pierret et al., 2000; Urbain and Deschenes, 2007; Monkeys: Iyengar et al., 2007; Rausell and Jones, 1991a; for reviews, see Asanuma, 1989; Kaas, 1983; Kaas et al., 2006; Mountcastle, 1997). In both rats and monkeys, thalamic neurons also project from the thalamic motor nuclei to S1 but in a less somatotopic manner; in primates, the projections are mainly from VL to area 3a (see below) (Huffman and Krubitzer, 2001b) and in rats, from VL to the granular cortex, and in particular to the limb area (Donoghue et al., 1979; Henry and Catania, 2006).

1.4.2. Projections to the primary motor cortex (M1)

The thalamic motor nuclei, VL in rats and VLo and VLc in monkeys, are the main motor nuclei relaying somatosensory information to M1 (Rats: Aldes, 1988; Cicirata et al., 1986a; Donoghue and Parham, 1983; Herkenham, 1980 1979; Miyashita et al., 1994; Urbain and Deschenes, 2007; Zhang and Sasamoto, 1990; Monkeys: Asanuma et al., 1980; Hatanaka et al., 2005; Jones et al., 1979; Simonyan and Jurgens, 2005; Strick, 1975; Strick, 1976; Vitek et al., 1994). These thalamocortical neurons project bilaterally but in a less somatotopic manner than the projections from the thalamic sensory nuclei to S1.

The thalamic sensory nuclei PO and VPLo in monkeys (Hatanaka et al., 2005; Simonyan and Jurgens, 2005) and PO in rats (Henry and Catania, 2006; Miyashita et al., 1994) also project directly to ipsilateral M1. However, while there is evidence for VPM projections to limb-M1 (Cicirata et al., 1986a; Cicirata et al., 1986b; Donoghue et al., 1979), apparently there is no evidence for the projections of VPM in rats and VPLc in monkeys to face-M1.

1.5. Organization of somatosensory inputs to the somatosensorimotor cortex

1.5.1. Cytoarchitecture of the sensorimotor cortex

1.5.1.1. Cytoarchitecture of the face-S1

In primates, S1 has 4 distinct cytoarchitectonic areas: area 1, 2, 3a and 3b (Mountcastle, 1957) that extend along the postcentral sulcus. Area 3b receives the majority of peripheral somatosensory inputs and is referred to as area S1-proper (Iyengar et al., 2007; Jain et al., 2001; Kaas et al., 2006; Qi et al., 2002). S1 is characterized by a granular layer IV of densely packed cells, and therefore is regarded as the granular cortex (Monkeys: Burish et al., 2008; Huffman and Krubitzer, 2001a; for review, see Kaas, 1983). Rats lack a central sulcus and they also lack the 4 distinct cytoarchitectonic areas 1, 2, 3a and 3b characteristic of primates. In rats, the granular cortex is thought to correspond to area 3b in primates and a dysgranular cortex (see below) corresponds to area 3a (Monkeys: Iyengar et al., 2007; Krubitzer and Kaas, 1990; Rats: Donoghue and Wise, 1982; for review, see Kaas, 1983). Layer IV is further characterised by discrete areas of small densely packed and darkly stained aggregates of neurons known as 'barrels' or ‘isomorphs’ (*e.g.* barrels representing rodents’ whiskers). These cell-dense areas are distinctly separated from each other and are surrounded by less dense 'septa'. Each cell-dense area contains neurons with a specific peripheral receptive field (see below). In monkeys, the less dense areas reflect discontinuities in peripheral receptive fields. In rats, the less dense areas are referred to as dysgranular areas (Monkeys: Huang et al., 1988; Iyengar et al., 2007; Jain et al., 2001; Kaas et al., 2006; Rats: Chapin and Lin, 1984; Donoghue and Wise, 1982; Wallace, 1987; Welker, 1971; Welker, 1976; Welker and Woolsey, 1974; Welker et al., 1984; Woolsey and Van der Loos, 1970; Woolsey et al., 1975).

1.5.1.2. Cytoarchitecture of the face-M1

In primates, the M1 involves area 4 that extends along the precentral gyrus. In rats, M1 is located more medial and rostral to S1. In primates and rats, the M1 is characterized by large pyramidal cells within layer V and a lack of a prominent granular layer IV, and therefore is referred to as the agranular cortex (Monkeys: Burish et al., 2008; Huntley and Jones, 1991a; Sessle and Wiesendanger, 1982; Rats: Donoghue and

Wise, 1982). In rats, the agranular cortex has been further divided into medial and lateral agranular areas. The medial agranular area, also referred to as the secondary motor cortex (M2), has been characterized by a pale-staining layer III and a relatively dense layer II. The lateral agranular area referred to as the M1, lies between M2 and S1 and has been characterized by a more homogenous appearance of layers II and III and a broader layer V. At the border between S1 and M1 there is a transition zone where layer IV gradually thins (Donoghue and Wise, 1982) (see Chapter 2, Fig. 2-5).

1.5.2. Somatosensory inputs to face-S1

1.5.2.1. Projections from thalamic nuclei

Within face-S1, the thalamocortical projections are in the direction of the radial fibres to layers IV (and III) where they branch extensively and spread horizontally (0.4 - 0.5 mm in the cat limb-S1), thereby establishing the morphological basis of the sensory columns (Rats: Chiaia et al., 1991; Diamond et al., 1992; Donoghue et al., 1979; Pierret et al., 2000; Urbain and Deschenes, 2007; Monkeys: Iyengar et al., 2007; Rausell and Jones, 1991a; for reviews, see Asanuma, 1989; Kaas, 1983; Kaas et al., 2006; Mountcastle, 1997).

In primates, exteroceptive inputs from superficial afferents (cutaneous, mucosa, tongue and teeth), project in a somatotopic manner through VPM primarily to isomorphs (see 1.5.1.1.) within layer IV of area 3b but also to area 1 and some to areas 2 and 3a. These discrete isomorphs are organized in a contiguous somatotopic manner where each isomorph represents a different contralateral oral region (*i.e.*, teeth, tongue, lips, etc.). Proprioceptive inputs from muscle spindles project to area 3a (and M1) and proprioceptive inputs from deep receptors located in teeth, joints and muscles project to area 2 (Iyengar et al., 2007; for review, see Kaas et al., 2006).

In rats, exteroceptive inputs from superficial afferents (Chiaia et al., 1991; Henry and Catania, 2006) project in a somatotopic manner through VPM primarily to isomorphs within the granular area (Diamond et al., 1992; Donoghue et al., 1979; Henry and Catania, 2006; Henry et al., 2006; Koralek et al., 1988; Welker, 1971). Somatosensory information is also relayed in a somatotopic manner to the dysgranular areas through the thalamic PO nucleus (Chiaia et al., 1991; Diamond et al., 1992; Fabri

and Burton, 1991; Koralek et al., 1988), however, their neurons have larger receptive fields than VPM neurons and they relay exteroceptive but mainly proprioceptive information (Chiaia et al., 1991; Welker et al., 1984). Furthermore, VPM neurons and PO neurons receive a large amount of efferent inputs from face-S1 that play a role in the modulation of somatosensory inputs to face-S1 (Diamond et al., 1992; Urbain and Deschenes, 2007).

1.5.2.2. Corticocortical projections

In primates, there is a considerable amount of reciprocal connections among areas 1, 2, 3a and 3b within each hemisphere and between homotopical cortical areas. In addition, area 2 projects to area 4 (M1) and area 3a has reciprocal connections with face-M1 (Huffman and Krubitzer, 2001b; Iyengar et al., 2007; Krubitzer and Kaas, 1990; for review, see Kaas et al., 2006).

In rats, face-S1 also has extensive intra- and inter-hemispheric connections. It has been demonstrated that the areas representing the lower incisors have ipsilateral reciprocal connections with neighbouring areas representing the tongue, buccal pad, and chin (Henry and Catania, 2006). In addition, the granular area and especially the dysgranular area have reciprocal connections with homotopic areas in the contralateral hemisphere (Chapin and Lin, 1984; Hayama and Ogawa, 1997; Henry and Catania, 2006) and while both project to M1, the face-M1 receives projections mainly from the granular cortex (Donoghue and Parham, 1983; Miyashita et al., 1994).

1.5.3. Somatosensory inputs to face-M1

1.5.3.1. Projections from thalamic nuclei

Somatosensory inputs project through the thalamic motor nuclei (VL) in a somatotopic manner to cortical layer V of M1. In addition, projections from a wide area of the thalamus terminate in cortical layer III where they branch less densely than the thalamic projections to S1 but spread to a wider area horizontally (1 mm in the cat limb-M1) (for review, see Asanuma, 1989; Monkeys: Rausell and Jones, 1995; Rats: Aldes, 1988; Cicirata et al., 1986a; Cicirata et al., 1986b; Donoghue et al., 1979; Miyashita et al., 1994).

1.5.3.2. Corticocortical projections

In addition to receiving direct somatosensory input through the thalamus (see above), face-M1 neurons receive indirect somatosensory inputs through face-S1. Most of the studies on the existence of such projections have been in monkeys and those in rats have concerned the vibrissa-M1, and very limited information is available of other face-M1 regions. In rats, anatomical studies demonstrate that the face-M1 neurons receive direct somatotopically organized inputs from the vibrissae-S1 (Izraeli and Porter, 1995; Porter, 1996). While limb-M1 neurons receive proprioceptive and exteroceptive inputs from the dysgranular (layers II, III, V, VI) and granular (layers V, VI) areas of limb-S1 (Chakrabarti and Alloway, 2006; Donoghue and Parham, 1983), vibrissa-M1 neurons receive inputs primarily from the granular cortex (Miyashita et al., 1994) and these are primarily exteroceptive inputs (Henry and Catania, 2006; Welker, 1976). In monkeys, area 3a neurons receive direct proprioceptive inputs from muscle spindles and indirect exteroceptive inputs through areas 3b and 1, and project largely to face-M1 (Hatanaka et al., 2005; Huffman and Krubitzer, 2001a; Iyengar et al., 2007; Lin et al., 1993; Lin et al., 1998; Yao et al., 2002b; for reviews, see Kaas, 1983; Kaas et al., 2006). Other studies in cats reveal that area 2 neurons also project somatotopically to limb-M1, primarily to layers II and III (Cats: Caria et al., 1997; Kosar et al., 1985) and these (M1 – area 2) interconnected neurons also receive direct inputs (through thalamic VL nucleus) from the same or similar areas in the periphery (Cats: Limbs: Caria et al., 1997). Therefore, it is possible that the inputs arriving at area 2 neurons projecting to specific neurons within M1 are actually arising from receptors activated by the contraction of the muscle to which those M1 neurons project (Cats: Caria et al., 1997). In addition, there are also other diffuse somatosensory projections from S1 to M1 through long horizontal interneurons and axon collaterals (Keller et al., 1990; Schwark and Jones, 1989).

M1 also receives a large amount of inputs from other cortical areas such as the cingulate cortex, insular cortex, orbital cortex, (Iyengar et al., 2007; Tokuno et al., 1997), and secondary somatosensory cortex (S2) (Jones, 1982; Rats: Donoghue and Parham, 1983; Henry and Catania, 2006).

1.6. Motor outputs from the face sensorimotor cortex

The corticobulbar tract (*i.e.*, pyramidal tract) is the main descending system influencing motor activity in the orofacial region and rest of the body. Most of its axons originate in the M1 but a large amount of axons originate from S1. In monkeys and in rats, neurons within the M1 and S1 consist of pyramidal cells (cortical efferents) and non-pyramidal stellate cells (intracortical interneurons) organized in 6 horizontal layers (I- VI). Anatomical studies show that M1 efferents and some of S1 efferents have their cell bodies located in layers II-VI but most prominently in layers III and V and their axons (*i.e.*, in the corticobulbar tract) project bilaterally but mainly to contralateral brainstem motor nuclei to facilitate cranial nerve motoneurons innervating the orofacial muscles (from M1: Rat: Neafsey and Sievert, 1982; Ohta and Saeki, 1989; Ohta and Sasamoto, 1980; Wise et al., 1979; Zhang and Sasamoto, 1990; Monkeys: Chase et al., 1973; Sirisko and Sessle, 1983; From S1: Rats: Wise and Jones, 1977b; Zhang and Sasamoto, 1990; Monkeys: Jones, 1976). The motoneuron pool includes all the motoneurons innervating a specific muscle. Brainstem motor nuclei include several motoneuron pools. Each corticobulbar tract neuron diverges extensively and many innervate several motoneuron pools within a particular brainstem motor nucleus or within different motor nuclei. However, neighbouring corticobulbar tract neurons converge to innervate most heavily a particular motoneuron pool that innervates a particular muscle (for reviews, see Miles et al., 2004; Schieber, 2001).

Although M1 neurons can project directly to brainstem motor nuclei, to directly activate motoneurons, it has been documented in primates and subprimates (including rats) that most of the projections related to the masticatory jaw movements are multisynaptic and project to brainstem motoneurons through brainstem premotoneurons located at the brainstem reticular formation regions, as well as the V main sensory nucleus and the subnuclei oralis and interpolaris (Monkeys: Hatanaka et al., 2005; Takada et al., 1994, Rats: Bourque and Kolta, 2001; Holstege and Kuypers, 1977; Holstege et al., 1977; Inoue et al., 1992; Mizuno et al., 1983; Shigenaga et al., 2000; Travers and Norgren, 1983; Zhang and Sasamoto, 1990; Rats: Dessem et al., 1997; Luo and Dessem, 1995; Luo et al., 2001; Luo et al., 1995; Luo and Li, 1991; Marfurt and

Rajchert, 1991; Matesz, 1981; for reviews, see Capra, 1995; Paxinos, 2004; Sessle, 2000; Trulsson and Essick, 2004). Furthermore, these M1 projections to premotoneurons may also project either directly or indirectly through the basal ganglia, red nucleus, vestibular nuclei, superior colliculus and cerebellum (Monkeys: Hatanaka et al., 2005; Takada et al., 1999; Rat: Hatanaka et al., 2005; Satoh et al., 2006a; Takada et al., 1994) In addition, Vm receives inputs from CMA and SMA in monkeys: (Hatanaka et al., 2005; Takada et al., 1994) and the agranular insular cortex in rats (Zhang and Sasamoto, 1990). Moreover, a large amount of somatosensory afferents are also relayed to the brainstem motor nuclei from the skin, teeth, muscles and joints either directly or indirectly through the premotoneurons (Rats: Borke et al., 1983; Dessem et al., 1997; Luo and Dessem, 1995; Luo et al., 2001; Luo et al., 1991; Marfurt and Rajchert, 1991; Matesz, 1981; Tolu et al., 1993; Tolu et al., 1994a; Travers and Norgren, 1983) Lastly, there are many brainstem interneurons (for reviews, see Dubner and Sessle, 1978; Lund et al., 1999), brainstem commissural connections (Rabbit: Donga et al., 1990) and connections among various brainstem motor nuclei such as between Vm and the XIIm motor nuclei and Vm and VIIm (Rat: Luo et al., 2006; Manaker et al., 1992; Zhang et al., 2001).

The V motoneurons project from the Vm through the motor branch of the V mandibular nerve to innervate the ipsilateral masticatory muscles (*e.g.* anterior digastric, masseter, temporalis and pterygoids). The hypoglossal (XII) motoneurons project from the brainstem XII motor nucleus through the XII cranial nerve to innervate the ipsilateral tongue muscles (*e.g.* genioglossus, hyoglossus, styloglossus). The palatoglossus muscle of the tongue is innervated by the vagus (X) nerve. The facial (VII) motoneurons project from brainstem facial motor nucleus through the facial cranial nerve to innervate the ipsilateral muscles of facial expression and the vibrissae in rodents. Each muscle fibre is innervated by 1 motoneuron. Each motoneuron innervates many muscle fibres (*i.e.*, motor unit). In comparison to muscles involved in coarse motor movements, muscles involved in fine motor movements (*e.g.* tongue) have more motor units with a smaller innervation ratio (*i.e.*, motor unit size – the average number of muscle fibres innervated

by a single motoneuron) (for reviews, see Miles et al., 2004; Paxinos, 2004; Schieber, 2001).

1.6.1. Corticocortical projections

In monkeys and in rats, while the pyramidal tract neurons project to innervate brainstem motoneurons, their dendrites and axon collaterals project vertically into all cortical layers as well as obliquely and horizontally (for ~1-3 mm) within all layers, but mainly within layers II-III and V to synapse on neighbouring pyramidal cells. These monosynaptic excitatory projections with glutamate as their main neurotransmitter provide strong excitatory inputs to adjacent pyramidal neurons. There are also stellate cells which are polysynaptic intracortical interneurons with GABA as their main neurotransmitter, and they provide inhibitory inputs to adjacent pyramidal neurons. Altogether, these intrinsic connections form an extensive intracortical network of neurons that can contribute to the excitation and inhibition of motor efferents and thereby affect the area of effective motor outputs (Monkeys: DeFelipe et al., 1986; Huntley and Jones, 1991b; Kwan et al., 1987; Rat: Aroniadou and Keller, 1993; Hall and Lindholm, 1974; Huntley, 1997a; Sanderson et al., 1984; for reviews, see Keller, 1993; Mountcastle, 1997; Schieber, 2001).

Of note is that the face-M1 also has reciprocal connections with the cortical masticatory area (CMA), premotor (PMA) and supplementary motor cortex (SMA) (Barbas and Pandya, 1987; Hatanaka et al., 2005; Pandya and Vignolo, 1971). In addition, there is a considerable amount of inter-hemispheric connections (Darian-Smith et al., 1990; Donoghue and Parham, 1983; Gould et al., 1986; Huntley and Jones, 1991b).

2. Roles of face-M1 in the control of elemental and semiautomatic orofacial motor functions

The orofacial area is involved in a large amount of complexly coordinated bilateral movements including elemental movements such as jaw-opening, jaw-closing, tongue-protrusion or tongue-retrusion and semiautomatic movements such as mastication and swallowing as well as whisking movements in rodents. Several methods

have been used for studying the role of the sensorimotor cortex in the generation and control of these functions (for review, see Toga and Mazziotta, 2002). Electrophysiological methods include single neuron recordings and electrical stimulation of the sensorimotor cortex. Single neuron recordings use transdural microelectrodes and application of peripheral stimuli to determine the somatotopic map of S1 and the mechanoreceptive field properties of sensorimotor cortex neurones. Electrophysiological mapping of motor representations within the sensorimotor cortex uses electrical stimulation delivered to specific sites within the sensorimotor cortex to observe the evoked muscle movements or record evoked electromyographic (EMG) muscle responses. Electrical stimulation can be delivered by surface cortical stimulation or by subdural microelectrodes (*i.e.*, intracortical microstimulation, ICMS) in animals or by transcranial magnetic stimulation (TMS) in humans. Studies using these techniques in conjunction with various central and peripheral manipulations to the sensorimotor systems have revealed that the sensorimotor cortex plays an important role not just in the generation and control of orofacial motor functions but also in adaptation and learning processes. Since this thesis used ICMS to delineate face-M1 role in association with manipulation to the oral tissues, the following sections emphasise the roles of face-M1 in orofacial motor functions and the electrophysiological evidence to support them.

2.1. Evidence from ICMS studies of face-M1

The cortical sites for which electrical stimulation can evoke responses in a particular muscle define that muscle's cortical motor representation. Although electrical stimulation applied to several cortical areas including the M1, SMA, CMA, the cingulate motor area and the S1 can evoke orofacial muscle responses, M1 is the most ICMS-excitable area since it has the lowest ICMS threshold for evoking a muscle twitch (Monkeys: Clark and Luschei, 1974; Hatanaka et al., 2005; Huang et al., 1989a; Huang et al., 1989b; Lin et al., 1994a; Lin et al., 1994b; Martin et al., 1999; Martin et al., 1997; Murray et al., 1991; Murray et al., 2001; Murray and Sessle, 1992a; Murray and Sessle, 1992b; Murray and Sessle, 1992c; Narita et al., 1999; Sirisko and Sessle, 1983; Yao et al., 2002a).

2.1.1. Functional organization of elemental motor movements

ICMS studies of M1 reveal extensive somatotopic organization of the skeletal muscles whereby low-threshold, short-duration ICMS (*e.g.* 0.2 ms pulses, 333 Hz, 35 ms, <30μA) can evoke elemental movements of the orofacial muscles from within the so-called face-M1. Within face-M1, functional motor subdivisions represent the orofacial muscles (or movements) (*e.g.* tongue, jaw muscles and vibrissae). These motor representations are organised in a mosaic-like region where each region represents a muscle (or movement) or group of muscles (or movements) (*i.e.*, overlapping motor representations). Although contralateral representations predominate, ipsilateral representations are quite extensive (Anaesthetised Rats: Adachi et al., 2007; Brecht et al., 2004; Gioanni and Lamarche, 1985; Lee et al., 2006; Miyashita et al., 1994; Neafsey et al., 1986; Tandon et al., 2008; Awake rats: Sapienza et al., 1981); Awake monkeys: Burish et al., 2008; Clark and Luschei, 1974; Huang et al., 1989b; Huang et al., 1988; Martin et al., 1999; Martin et al., 1997; McGuinness et al., 1980; Murray et al., 1991; Murray and Sessle, 1992a; Murray and Sessle, 1992b; Murray and Sessle, 1992c; Yao et al., 2002a; Anaesthetised monkeys: Huang et al., 1989b); for reviews, see Sanes and Donoghue, 2000; Sanes and Schieber, 2001; Schieber, 2001; Sessle et al., 2007). Analogous studies in humans using TMS and recording of evoked muscle responses as well as neuroimaging techniques such as fMRI also reveal extensive representation of the orofacial region within face-M1 (Boudreau et al., 2007; Corfield et al., 1999; Gooden et al., 1999; Martin et al., 2004; Meyer et al., 1997; Nordstrom, 2007; Svensson et al., 2003b; Svensson et al., 2006). Such an extensive bilateral representation of the orofacial region within face-M1 may point to its role in the control of bilateral orofacial movements.

Studies in rats and monkeys have revealed that the jaw and tongue muscles have a considerable amount of overlapping motor representations whereby a specific site within face-M1 represents both jaw and tongue muscles/ movements as reflected in similar excitability for ICMS-evoked muscle responses (Anaesthetised rats: Adachi et al., 2007; Lee et al., 2006; Neafsey et al., 1986; Awake monkeys: Huang et al., 1988; Murray and Sessle, 1992a; Murray and Sessle, 1992b; Sessle and Wiesendanger, 1982;

Anaesthetised monkeys: Burish et al., 2008; for reviews, see Murray et al., 2001; Sanes and Donoghue, 2000; Sanes and Schieber, 2001; Schieber, 2001; Tehovnik et al., 2006). Such an overlapping of motor representations is further supported by anatomic and physiologic studies showing a considerable amount of intrinsic connectivity as well as convergence and divergence of motor outputs on brainstem motoneurons (see above). Sanes et al have termed this overlapping of motor representations a "shared neural substrate" for movement of a body part that is considered to be important for the coordination of several muscles (or movements) (Sanes et al., 1995). Furthermore, the rich synaptic network involving excitatory monosynaptic projections of axon collaterals projecting horizontally for several mm, suggests that neurons within different representation areas within M1 are interconnected, and the polysynaptic interneurons of primarily an inhibitory nature are thought to limit the postsynaptic responses beyond the monosynaptic excitatory connections (for reviews, see Keller and Asanuma, 1993; Mountcastle, 1997; Schieber, 2001). Such neuronal interconnections may play a role in the spatiotemporal coordination of muscle activity during orofacial movements (Aroniadou and Keller, 1993). Indeed, based on recent studies in monkeys, Graziano et al (Graziano and Aflalo, 2007; Graziano et al., 2002a; Graziano et al., 2002b) have shown that long-duration ICMS trains (matching the time course of the motor function being studied) delivered to face-M1 can evoke coordinated, complex movements that are also arranged within the M1 in a map of meaningful body postures in space, suggesting a combined and weighted activation of a group of different efferent areas. For example, long-duration ICMS trains in the orofacial representation area can evoke consistent short-latency (<33 msec) movements of the mouth, lips and tongue towards a specific orofacial posture (Awake Monkeys: (Graziano et al., 2002a; Graziano et al., 2002b). These observations point again to the role of face-M1 in the control of coordinated orofacial motor functions.

While some orofacial movements such as tongue-protrusion, jaw-opening movements and vibrissal movements can be readily evoked by ICMS (Anaesthetised Rats: Adachi et al., 2007; Donoghue and Wise, 1982; Lee et al., 2006; Neafsey et al., 1986; Anaesthetised monkeys: Burish et al., 2008; Huang et al., 1989b; Awake

monkeys: Clark and Luschei, 1974; Huang et al., 1989b; Huang et al., 1988; McGuinness et al., 1980; Murray and Sessle, 1992a; Yao et al., 2002a), other movements such as jaw-closing movements are not commonly evoked by similar ICMS parameters (Anaesthetised rats: Neafsey et al., 1986; Awake monkeys: Clark and Luschei, 1974; Huang et al., 1988; McGuinness et al., 1980; Murray et al., 1991; Murray and Sessle, 1992a). These finding may suggest that face-M1 plays an important role in the generation of some but not all orofacial movements. However, since jaw-M1 neurons discharge more rapidly during a stronger bite force (Hoffman and Luschei, 1980; Luschei et al., 1971) and show different patterns of firing rates during different jaw-closing and biting tasks (Murray et al., 2001; Murray and Sessle, 1992b), it is suggested that face-M1 does play some role in the control of jaw-closing movements. Furthermore, it has been reported that cold block of face-M1 results in a significant increased spontaneous EMG activity of the masseter muscles through possible disinhibition of the masseter motoneurons (Yamamura et al., 2002). Therefore, it is also possible that the masseter representation in face-M1 principally involves inhibitory effects of face-M1 neurons on masseter motoneurons (Chase et al., 1973). Alternatively, the masseter representation is masked by an inhibitory effect of intracortical interneurons as it has been shown in the cat limb-M1 that the motor representations of antagonistic limb muscles are inhibited by cortical interneurons and the inhibitory effect can be reversed by GABAA receptor antagonists (e.g. bicuculline, picrotoxin) resulting in simultaneous activation of antagonistic muscles by ICMS (Ethier et al., 2007),

2.1.2. Functional organization of semiautomatic motor movements

Mastication and swallowing in all mammals as well as vibrissal whisking in rodents are complex, semiautomatic movements that require coordinated activity of the orofacial muscles. It has been well documented that these semiautomatic movements are controlled by subcortical brainstem neuronal networks (*i.e.*, central pattern generator (CPG) (Rats: Berg and Kleinfeld, 2003; Gao et al., 2001; for reviews, see Dubner and Sessle, 1978; Jean and Car, 1979; Lund and Kolta, 2006b; Lund et al., 1999; Sawczuk and Mosier, 2001). Nevertheless, electrophysiological studies in rats, monkeys and in humans suggest that face-M1 also plays a role in the control of semiautomatic orofacial

movements (Aziz et al., 1996; Hamdy et al., 1996; Huang et al., 1989a; Martin et al., 2001; Martin et al., 1999; Martin et al., 2004; Yamamura et al., 2002; Yao et al., 2002a). In monkeys and rats, long-duration ICMS trains (*e.g.* 0.2ms pulses, 50Hz, 3sec, <60μA) can evoke rhythmic masticatory and swallowing movements from an extensive area within face-M1 (Awake monkeys: Huang et al., 1989a; Martin et al., 1999; Yamamura et al., 2002; Yao et al., 2002a; Anaesthetised monkeys: Hatanaka et al., 2005; Huang et al., 1989a; Takada et al., 1999; Anaesthetised rats: Sasamoto et al., 1990; Satoh et al., 2006a; Zhang and Sasamoto, 1990). Furthermore, different patterns of ICMS-evoked rhythmic jaw movements are associated with different patterns of EMG activity (Awake monkeys: Huang et al., 1989a; Martin et al., 1997). In addition, ICMS within face-M1 can modulate brainstem and basal ganglia neuronal activity associated with the rhythmical jaw movements (Anaesthetised rats: Inoue et al., 1992; Nishimuta et al., 2002; Satoh et al., 2006a; Zhang and Sasamoto, 1990).

Similar to rhythmic jaw movements, ICMS of vibrissal-M1 in awake rats can evoke rhythmic vibrissal movements that mimic exploratory whisking (Berg and Kleinfeld, 2003; Haiss and Schwarz, 2005) and it appears that vibrissa-M1 regulates whisking through its actions on a subcortical CPG (Cramer and Keller, 2006).

2.2. Evidence from movement-related face-M1 neuronal activity

2.2.1. Elemental orofacial movements

Limited data are available of the movement-related face-M1 neuronal activity in rats. Yet studies of the vibrissal-M1 in awake freely moving rats show task-related neuronal activity whereby the discharge of M1 neurons correlates with the level of muscles' output as measured by vibrissal EMG activity (Carvell et al., 1996). Movement-related fMRI studies in humans (Corfield et al., 1999; Hamdy et al., 1999; Martin et al., 2004) and single neuron recordings of the ICMS-defined face-M1 areas in monkeys (Hoffman and Luschei, 1980; Murray et al., 2001; Murray and Sessle, 1992b; Murray and Sessle, 1992c; Yao et al., 2002a) have revealed that many face-M1 neurons are active in relation to a tongue or jaw movement task. However, this movement-related neuronal activity may also be related to activation of other orofacial muscles

associated with the tongue or jaw movements such as facial muscles (Moustafa et al., 1994). Some of the face-M1 neurons representing the tongue muscles (tongue-M1) and jaw muscles (jaw-M1) can change (increase or decrease) their firing rate before the onset of a tongue or jaw muscle activity (Hoffman and Luschei, 1980; Luschei et al., 1971; Murray and Sessle, 1992b), different trajectories of tongue movement have different patterns of neuronal firing (Hoffman and Luschei, 1980; Luschei et al., 1971; Murray et al., 2001; Murray and Sessle, 1992c; Yao et al., 2002a) and some of the jaw-M1 neurons discharge more rapidly during a stronger biting force (Hoffman and Luschei, 1980; Luschei et al., 1971). Nevertheless, since many of face-M1 neurons can be activated by mechanical stimulation of orofacial mechanoreceptive fields (Huang et al., 1989b; Huang et al., 1988; Murray et al., 2001; Murray and Sessle, 1992a; Sirisko and Sessle, 1983) and since there are extensive intracortical projections from S1 to M1 (see above), it is possible that some of the movement-related face-M1 neuronal activity is a reflection of the sensory inputs generated by the orofacial movement and projected to face-M1 either directly or indirectly through face-S1 (Yao et al., 2002a) (see below). Collectively, these various studies do point to an important role of face-M1 in the integration of sensorimotor information and the control of orofacial movements.

2.2.2. Semiautomatic orofacial movements

In rats, vibrissal-M1 neuronal activity is phase-locked with trained exploratory vibrissal rhythmic movements (Ahrens and Kleinfeld, 2004). Single neuron recordings studies in monkeys have revealed that many face-M1 neurons have differential activity associated with different phases of the chewing and swallowing cycles (Huang et al., 1989a; Martin et al., 1997; Yao et al., 2002a; for reviews, see Murray et al., 2001; Sawczuk and Mosier, 2001). These movement-related neuronal activities suggest that face-M1 plays an important role in the generation and control of orofacial semiautomatic movements.

2.3. Evidence from face-M1 cold block and ablation studies

2.3.1. Elemental orofacial movements

Bilateral lesioning or reversible bilateral cold block-induced inactivation of face-M1 severely impairs the animal's ability to perform a tongue-protrusion task (Monkeys: Murray et al., 1991; Rats: Castro, 1972; Castro, 1975) but has relatively small effects on the animal's ability to maintain a learned biting task (Monkeys: Luschei and Goodwin, 1975; Murray et al., 1991). Therefore, it is possible that while face-M1 plays a role in the generation of tongue-protrusion and jaw-opening movements, it has a more limited role in the control of jaw-closing movements (also see 2.1.1. above).

2.3.2. Semiautomatic orofacial movements

In monkeys, bilateral but not unilateral ablation of face-M1 alters patterns of masticatory jaw movement (Larson et al., 1980) and bilateral cold block disrupts but does not prevent coordinated masticatory movements (Yamamura et al., 2002). Similarly in rats, unilateral lesioning of the vibrissa-M1 disrupts but does not prevent coordinated rhythmic whisking movements (Gao et al., 2003) and ablation of M1 can alter rhythmical jaw movements (Sasamoto et al., 1990). These observations suggest a possible modulatory role of face-M1 in semiautomatic orofacial movements but not a crucial role in the generation of these movements.

3. Role of the somatosensory system in the control of orofacial movements

Electrophysiological studies reveal that the somatosensory system including face-S1 may also play a role in the control of orofacial movements. This is supported by the existence of 2 parallel projections of direct (through the thalamus) and indirect (through face-S1) somatosensory inputs to face-M1 that can provide important feedback from the orofacial tissues (*e.g.* skin, muscles, joints and teeth) that is crucial for the modulation of orofacial motor movements. In addition, anatomic and ICMS studies of face-S1 provide evidence for efferent projections to motoneurons that can evoke orofacial movements and other corticofugal projections that can further modulate these evoked movements. Therefore, the following sections review the evidence to support these roles of face-S1 in the control of orofacial movements.

3.1. Evidence from ICMS studies of face-S1

3.1.1. Functional organization of elemental orofacial movements

Low-threshold ICMS in awake or anaesthetised monkeys can evoke EMG muscle responses only from face-M1 (Huang et al., 1989b; Lin et al., 1998; Martin et al., 1999). However, it has been reported that in anaesthetised Marmosets (New World Monkeys), low-threshold ICMS can evoke observed orofacial movements (but not tongue movements) also from area 3a as well as from several sites within area 3b but nonetheless, face-M1 has lower thresholds of ICMS-evoked responses (Burish et al., 2008). In anaesthetised or awake rats, low-threshold ICMS of the granular cortex (S1) can also evoke orofacial movements (Awake rats: Sapienza et al., 1981; Anaesthetised rats: Donoghue and Wise, 1982; Lee et al., 2006; Neafsey et al., 1986; Neafsey and Sievert, 1982; Welker et al., 1984) and jaw and tongue EMG muscle responses (Adachi et al., 2007; Lee et al., 2006). ICMS can evoke tongue movement also from the insular taste sensory cortex; however, the motor responses evoked from this area seem to be small tongue twitch responses (Neafsey et al., 1986).

3.1.2. Functional organization of semiautomatic orofacial movements

In awake or anaesthetised monkeys, while short-train ICMS of face-S1 cannot evoke any low-threshold muscle activity, long-train ICMS (*e.g.* 0.2ms pulses, 50Hz, 3sec, <60μA) of face-S1 as well as the insular cortex can evoke rhythmical jaw movements (Huang et al., 1989a; Lin et al., 1998). Similarly, it has been reported in analogous studies in subprimates, that ICMS of S1 in anaesthetised rabbits (Lund et al., 1984) and cats (Hiraba et al., 1997) and ICMS of the insular cortex in rats (Zhang and Sasamoto, 1990) can evoke rhythmic jaw movements. These findings suggest that rhythmic jaw movements can also be induced from the face-S1 and point to a role of face-S1 in the control of orofacial semiautomatic movements.

Although there is anatomical evidence for a direct efferent projection from S1 to motoneurons in monkeys and rats (Jones, 1976; Wise and Jones, 1977a; Zhang and Sasamoto, 1990), it has also been shown that face-S1 has extensive connections with face-M1 through axon collaterals and interneurons (Chakrabarti and Alloway, 2006; Donoghue and Parham, 1983; Henry and Catania, 2006; Iyengar et al., 2007; Izraeli and

Porter, 1995; Miyashita et al., 1994; Porter, 1996; Welker, 1976), and therefore, it is possible that the movements evoked by ICMS of S1 are the result of spread of stimulating currents from S1 to adjacent M1 area through these intracortical connections. However, rhythmical jaw movements can be evoked from only some sites and not all of the face-S1 area and not necessarily only from sites adjacent to face-M1.

3.2. Evidence from movement-related face-S1 neuronal activity

3.2.1. Elemental orofacial movements

In monkeys, single unit recordings reveal that the firing rate of different face-S1 neurons is altered depending on the oral motor task, *e.g.* periodontal-S1 neuronal activity is altered during tongue-protrusion and biting tasks and tongue-S1 neuronal activity is altered during tongue-protrusion but not during biting (Lin et al., 1994a). Furthermore, some of the neuronal activity appears before the start of EMG activity and before the oral movement (Lin et al., 1994a; Lin et al., 1994b; Lin and Sessle, 1994; Murray et al., 2001), indicating that it is not necessarily generated by sensory feedback evoked by the movement. Some of the neuronal activity associated with exteroceptive inputs related to a specific movement is suppressed during that specific movement, thereby compensating for the increased exteroceptive inputs produced during that movement (Lin and Sessle, 1994; Murray et al., 2001). However, this latter suppression of sensory inputs during orofacial movements may involve other modulating projections such as M1 projections to thalamic nuclei (Urbain and Deschenes, 2007), or corticofugal projections from S1 to brainstem sensory nuclei (Olsson et al., 1986), or corollary discharge (Sperry, 1950) or efference copy (Von Holst, 1954) that project from M1 to S1. Hence, it appears that face-S1 may play a role in the preparatory phase as well as the modulation of orofacial movements.

3.2.2. Semiautomatic orofacial movements

Although evidence from face-S1 neuronal activity in rats is limited, different neurons within face-S1 in awake, freely moving rats have different response properties associated with different aspects of their ingestive behaviour (*i.e.*, licking and eating) (Yamamoto et al., 1988) and in cats many face-S1 neurons have neuronal activity related

to different aspects of masticatory movements including rhythmic jaw movements (Hiraba; Hiraba et al., 1997).

3.3. Evidence from face-S1 cold block and ablation studies

3.3.1. Elemental orofacial movements

Unilateral cold block of the monkey's face-S1 results in partial elimination of face-M1 neuronal activity that otherwise appears before the start of an oral movement (Monkeys: Murray et al., 2001; Yao et al., 2002b), and bilateral cold block impairs the success rate of the monkey in performing a tongue-protrusion task but not biting task and also reduces the monkey's ability to maintain steady tongue-protrusive and biting forces (Lin et al., 1993; Murray et al., 2001). Therefore, face-S1 may play a role in the generation of tongue-protrusion but not jaw-closing movements, and once jaw-closing and tongue-protrusion movements are initiated, face-S1 may play a role in the fine control of these movements.

Limited data are available from studies in rats. One study in rats has shown that bilateral lesioning of face-S1 severely impairs the animal's ability to perform a tongue-protrusion task (Castro, 1975) and in cats, lesions within face-S1 could alter the licking and other oral motor behaviours associated with the feeding process (Hiraba, 1999; Hiraba, 2004; Hiraba et al., 2007). In addition, it has been demonstrated that electrical stimulation of the vibrissal pad evokes contralateral M1 neuronal activity with latencies that are shorter than the latencies of electrically-evoked thalamic (VL and PO) neuronal activity (Diamond et al., 1992; Farkas et al., 1999), and unilateral inactivation or ablation of the vibrissa-S1 decreases or eliminates the short-latency electrically-evoked M1 neuronal activity, respectively (Farkas et al., 1999). These results suggest that the main short-latency somatosensory inputs to face-M1 are relayed via face-S1 and further support data in monkeys of the role of these somatosensory inputs via face-S1 in modulating face-M1 motor outputs and consequently orofacial movements.

3.3.2. Semiautomatic orofacial movements

While unilateral cold block of the monkey face-S1 has a limited effect on the monkey's ability to perform chewing and tongue-protrusion movements and no effect on

swallowing, bilateral cold block impairs the monkey's ability to perform rhythmical jaw and tongue movements during mastication and swallowing (Lin et al., 1998; Murray et al., 2001). These findings suggest a role for face-S1 in modulating semiautomatic muscle activity, probably through its effects on face-M1 and the brainstem central pattern generator (Lin et al., 1998; Murray et al., 2001). No data seem available on the effect of S1 ablation on semiautomatic orofacial movements in rats; however, it has been reported that unilateral lesioning of the face-S1 in awake cats results in impaired orofacial motor behaviours associated with feeding, such as impaired lip tension and elongation of the mastication period; however the pattern of masticatory movement does not change (Hiraba, 1999; Hiraba et al., 2000).

3.4. Evidence from functional organization of somatosensory inputs to face-S1

In primates, face-S1 neurons receive some bilateral but mainly contralateral exteroceptive inputs from the orofacial tissues that are organized in a discrete, contiguous somatotopic manner that is considered to be crucial for precise spatial localization of peripheral mechanoreceptive inputs (*i.e.*, teeth, tongue, lips, etc.) (Awake Monkeys: Huang et al., 1989b; Lin et al., 1994a; Anaesthetised monkeys: Cusick et al., 1986; Huang et al., 1988; Iyengar et al., 2007; Jain et al., 2001; Sirisko and Sessle, 1983; for review, see Kaas et al., 2006). Most face-S1 neurons have rapidly adapting responses to orofacial mechanical stimuli (Lin et al., 1994a). Neurons within areas 3a and 2 receive mainly proprioceptive inputs, and in comparison with areas 3b and 1, they have larger and more complex receptive fields, involving more than 1 joint or muscle, usually involving several teeth in either jaw or both jaws. Some neurons have receptive fields in other oral structures such as gingiva, lips, tongue and mucosa (Awake Monkeys: Huang et al., 1989b; Lin et al., 1994a; Toda and Taoka, 2001; Toda and Taoka, 2004; Yao et al., 2002b; Anaesthetised monkeys: Cusick et al., 1986; Huang et al., 1989b; Huang et al., 1988; Sirisko and Sessle, 1983).

In rats, neurons within the granular area of the face-S1 receive bilateral exteroceptive inputs that are also organized in a discrete, contiguous somatotopic manner

(Anaesthetised rats: Chapin and Lin, 1984; Henry et al., 2006; Sanderson et al., 1984; Awake rats: Chapin and Lin, 1984); for review, see Kaas et al., 2006). Similar to area 3a in monkeys, neurons within the dysgranular area receive exteroceptive as well as proprioceptive inputs from the orofacial tissues and in comparison with the granular area, they have larger and more complex receptive fields (Chapin and Lin, 1984; Welker, 1976; Welker et al., 1984).

These extensive bilateral representations with extensive intra- and inter-hemispheric reciprocal connections (Rats: Henry and Catania, 2006; Monkeys: Huffman and Krubitzer, 2001a; Iyengar et al., 2007; Krubitzer and Kaas, 1990 that in turn include projections to M1, may further point to a role for face-S1 in the control and coordination of the bilateral sensorimotor functions that occur in the orofacial region. This role of face-S1 is further supported by mapping studies of the sensory representations within face-S1 of different species of rats. For example, in Sprague-Dawley albino rats, 64% of the total S1 area represents the head and neck and 30% of this area is devoted to the representation of the vibrissae, reflecting the importance of the vibrissae in the exploratory behaviour by rats of their environment (no data are available on the representation of the teeth) (Welker, 1971). In contrast, naked mole-rats live in subterranean colonies and have small eyes and very large incisors that are used for daily sensorimotor functions (*e.g.* tunnel excavation). The importance of the incisors is reflected in their unique and extensive representation within face-S1 that occupy ~50% of the total orofacial representation area as compared to ~7% in Long-Evans rats including the more caudal area of the cortex that, in Long-Evans rats, represents the visual sensory area (Catania and Remple, 2002; Henry et al., 2005 2006). The star nose mole-rat uses its 11 fleshy rays surrounding each of the nostrils for exploring its environment; however, the shortest ray with the smallest number of sensory organs has the highest innervation density per sensory organ and the largest representation within face-S1 (Catania and Kaas, 1997; Catania and Remple, 2002). These studies of differential somatosensory representations within face-S1 of different species reflect not just the high diversity of innervation densities of the orofacial tissues (Welker and Van der Loos, 1986; for reviews, see Catania and Remple, 2002; Henry et al., 2006;

Macefield, 2005; Trulsson and Essick, 2004), but also point to the important role of orofacial sensorimotor behaviour in reshaping the face-S1 somatosensory representations (Catania and Kaas, 1997; Catania and Remple, 2002).

3.5. Evidence from functional organization of somatosensory inputs to face-M1

The functional organization of the somatosensory inputs from the orofacial tissues to face-M1 has been studied in detail in monkeys. These studies reveal that face-M1 receives prominent somatosensory inputs from the orofacial tissues that are characterized by multiple representations of somatosensory inputs from the same orofacial area to several, often non-contiguous sites. Most of these inputs derive from contralateral orofacial sites but a considerable amount of M1 neurons receive ipsilateral or bilateral inputs; while most of the inputs are excitatory, some are inhibitory (Anaesthetised monkeys: Gould et al., 1986; Huang et al., 1989b; Huang et al., 1988; Awake monkeys: Hoffman and Luschei, 1980; Huang et al., 1989b; Luschei et al., 1971; Murray and Sessle, 1992a; Yao et al., 2002b).

In awake monkeys, face-M1 neurons receive primarily exteroceptive inputs from the orofacial tissues and especially from the upper lip, lower lip, and tongue and very limited proprioceptive inputs (Huang et al., 1989b). Nevertheless, jaw-M1 neurons receive exteroceptive and proprioceptive inputs from the jaw muscles and periodontal ligaments (Huang et al., 1989b; Luschei et al., 1971; Murray and Sessle, 1992a). In comparison to awake monkeys, in anaesthetised monkeys, face-M1 neurons receive more proprioceptive inputs (Huang et al., 1988; Sirisko and Sessle, 1983).

Limited data are available from a study in awake rats where face-M1 receives somatosensory inputs from the lips and vibrissae that are primarily exteroceptive inputs (Farkas et al., 1999; Sapienza et al., 1981). Anatomical studies in anaesthetised rats show that ICMS-defined vibrissal-M1 and jaw-M1 neurons receive inputs directly through the thalamic sensory nucleus (PO) but primarily indirectly through the vibrissal-S1 barrel area (Farkas et al., 1999; Hoffer et al., 2005; Hoffer et al., 2003; Izraeli and Porter, 1995; Miyashita et al., 1994). Since neurons within S1 barrel areas receive mainly exteroceptive inputs from the orofacial region (Henry et al., 2006; Welker, 1976), it is

possible that face-M1 neurons in rats, as in monkeys, receive primarily exteroceptive inputs (Farkas et al., 1999; Miyashita et al., 1994). However, there appear to be no published reports on the functional organization of somatosensory inputs to face-M1 representing the oral region in rats.

3.6. Evidence from functional overlapping of somatosensory inputs and motor outputs in sensorimotor cortex

While the S1 and M1 are described as anatomically and physiologically 2 distinct areas, it is apparent that M1 receives sensory inputs and S1 has some motor functions and both have functionally related overlapping representations. M1 neurons may project to a specific muscle to evoke a specific movement, and these same M1 neurons receive somatosensory inputs from peripheral receptors activated by contraction of the muscle to which those M1 neurons project. Similarly, neurons within S1 may receive specific somatosensory inputs from a specific peripheral area, and these same S1 neurons project to a specific muscle to evoke a specific movement within the same peripheral region from which the somatosensory afferents to the S1 neurons derive (Rats: Cicirata et al., 1986a; Cicirata et al., 1986b; Izraeli and Porter, 1995; Sanderson et al., 1984; Monkeys: Huang et al., 1989b; Lin et al., 1998; Murray and Sessle, 1992a). Such spatial contiguity of sensory inputs and motor outputs emphasises the importance of sensory inputs in modulating cortical motor functions. In addition, this spatial contiguity may further be involved in the substrate for cortical neuroplasticity (see below). Nonetheless, it has been shown in awake monkeys and rats that although most face-M1 neurons receive exteroceptive inputs from the same orofacial areas within which movement is evoked by ICMS applied to the same neuronal recording site receiving the exteroceptive input, a substantial number of face-M1 neurons receive exteroceptive inputs from distant orofacial areas that have no close spatial relation with the ICMS-evoked movement area (Awake monkeys: Huang et al., 1989b; Huang et al., 1988; Lin and Sessle, 1994; Murray and Sessle, 1992a; Awake rats: Sapienza et al., 1981); for review, see Murray et al., 2001). This functional organization of somatosensory inputs to face-M1 may reflect the role of face-M1 in sensorimotor integration and the need for extensive exteroceptive

somatosensory feedback from a wide peripheral orofacial area for the fine control, coordination and modulation of the orofacial muscle activities during orofacial movements (Monkeys: Huang et al., 1989b; for reviews, see Murray et al., 2001; Murray and Sessle, 1992a).

3.7. Evidence from peripheral block and lesioning of somatosensory inputs

Evidence for the role of peripheral somatosensory inputs (*e.g.* from teeth and the periodontal mechanoreceptors) in the control of oral motor functions is supported by studies of V deafferentation induced by peripheral nerve lesioning or anaesthetic block in humans and rabbits. Bilateral transections of the mandibular and maxillary branches supplying sensory innervation of the teeth and other orofacial tissues result in altered patterns of mastication in rabbits (Inoue et al., 1989; Lavigne et al., 1987; Morimoto et al., 1989; for review, see Trulsson, 2006). From clinical practice it is well known that mandibular or maxillary nerve block or injury are associated not just with sensory deficits but also with motor deficits reflected in drooling, tongue biting and difficulties with speaking (Haas and Lennon, 1995; Tay and Zuniga, 2007). Clinical studies also indicate that patients with reduced periodontal tissue support and thus altered mechanoreceptive innervation of the teeth demonstrate reduced biting forces and impaired masticatory behaviour (Johansson et al., 2006a; Svensson et al., 2003b; Trulsson and Essick, 2004). Dental implant-supported prostheses are considered to be a good substitute for lost natural teeth although dental implants do lack periodontal ligament (Jacobs, 1998; Zarb, 2002; Zarb and Bolender, 2003). Findings from psychophysical studies in dentate as compared with edentate patients treated with implant-supported prostheses further support the notion that somatosensory inputs from periodontal mechanoreceptors play a role not just in tactile perception but also in the control of orofacial motor functions. For example, in comparison with dentate patients, patients with implant-supported prostheses require greater biting forces to hold and split a peanut with the anterior teeth and they exert the same pattern of muscle activity during chewing of hard and soft diets (Trulsson and Essick, 2004). These studies suggest that

mechanoreceptive inputs from the teeth provide important feedback that is crucial for the control of oral motor functions.

4. Neuroplasticity and the role of face-M1 in adaptive and learning processes

Neuroplasticity describes one of the most striking features of the adult nervous system, its remarkable capacity to change its structure (Greenough et al., 1985; Kleim et al., 1996; Withers and Greenough, 1989) and function (Monfils et al., 2004; Rioult-Pedotti et al., 1998; Teskey et al., 2007) throughout life. Neuroplastic changes may occur at the peripheral level, subcortical level or cerebral cortical level through molecular, cellular or synaptic events. Changes may be structural or functional, may have fast-onset or slow-onset and they may be either short-lived or long-lasting. Most significantly, M1 neuroplasticity has been associated with motor function recovery following central injury (*e.g.* stroke) or peripheral injury. It has also been associated with pain, changes in muscle use or disuse, learning of novel motor skills and adaptive processes. M1 has an extensive network of synaptic connections that provide the neural substrate for changes to occur. Such neuroplastic changes can be reflected in M1 as a functional reorganization of motor representations and changes in cortical excitability (for reviews, see: Buonomano and Merzenich, 1998; Donoghue, 1995; Donoghue, 1997; Ebner, 2005; Sanes and Donoghue, 2000; Sessle, 2006; Sessle et al., 2007). The following sections discuss various manifestations of face-M1 neuroplasticity and include a description of some of the mechanisms thought to underlie these changes.

4.1. Neuroplasticity associated with modified somatosensory experience

4.1.1. Peripheral deafferentation

Neuroplasticity of face-M1 induced by peripheral nerve injury has been demonstrated in several studies, most of which have addressed the effects on face-M1 representing the vibrissae (*i.e.*, vibrissal-M1) in rats (for reviews, see Buonomano and Merzenich, 1998; Ebner, 2005; Sanes and Donoghue, 2000). Injury to the facial nerve supplying motor innervation to the vibrissae results in a decreased vibrissal representation and expansion of neighbouring forelimb and eyelid representations into

the deprived vibrissal representation within the contralateral M1 (Donoghue et al., 1990; Huntley, 1997b; Sanes et al., 1990; Sanes et al., 1988; Toldi et al., 1996). In addition, the ICMS threshold for evoking movements in the expanded representations decreases, suggesting an increased excitability (Sanes et al., 1990). Such changes may occur within 1 hour after the nerve transection, may last for at least 4 months (Donoghue et al., 1990; Sanes et al., 1988) and may be preceded by a rapid-onset (within 4 min) and transient (lasting hours to 1 day) change in ipsilateral vibrissal representation (vibrissae usually have a contralateral representation) (Toldi et al., 1996). However, injury to the infraorbital nerve supplying sensory innervation to the vibrissae results in, 2-3 weeks later, no changes in the vibrissal motor representations but does result in significant increased ICMS thresholds for evoking vibrissal movements, suggesting decreased vibrissal-M1 excitability (Franchi, 2001). In a recent study from our laboratory in rats, a unilateral transection of the lingual nerve supplying sensory innervations to the tongue resulted in significant time-dependent changes of the GG representation within face-M1, 1- 4 weeks later (Adachi et al., 2007). In humans, TMS studies have reported that peripheral deafferentation induced by lingual nerve block is associated with decreased excitability (increased threshold) of tongue-M1 (Halkjaer et al., 2006) and local anaesthesia to lower facial skin is associated with increased excitability (increased motor evoked potentials – MEPs) of jaw- M1 (Yildiz et al., 2004). These different neuroplastic changes associated with the different peripheral manipulations provide evidence that face-M1 has the capability to adapt and be modelled in a task-dependent manner; and may further suggest different underlying mechanisms (see below).

4.1.1.1. Dental extraction

Peripheral soft tissue injury as a result of tooth extraction has been associated with irreversible deafferentation of pulp and periodontal ligament and therefore may conceivably alter the exteroceptive and proprioceptive and perhaps nociceptive inputs from the oral cavity to face-S1 and face-M1. Studies have shown that although there is initial degeneration of neurons immediately following tooth extraction, there is a progressive process of axonal regeneration within the extraction socket (Rats: Fried et al., 1991; Hansen, 1980; Ferrets: Mason and Holland, 1993) and re-innervation of the

regenerated bone and adjacent gingival tissue (Rats: Fried et al., 1991). Such re-innervation of neighbouring tissues may be one explanation for reorganizational changes of receptive fields observed in the mesencephalic nucleus (Cats: Linden and Scott, 1989) and face-S1 (Henry et al., 2005) following tooth extraction. In the latter study by Henry et al, incisor extraction has been performed in young rats and has been reported to result, 5 to 8 months later, in reorganization of face-S1 whereby the entire extent of the region normally representing the incisor has become represented by other orofacial tissues including the contralateral upper incisor, ipsilateral lower incisor, tongue and the buccal pad. Tooth pulp deafferentation has also been shown to induce (within 1-2 weeks) reversible reorganizational changes in the mechanoreceptive fields of neurons within the V brainstem nuclei (Rats and Cats: Hu et al., 1986; Hu et al., 1999; Kwan et al., 1993).

There appear to have been no reports of the effects of dental extraction on face-M1. Yet such information is of clinical significance since loss of teeth is still a common occurrence and face-M1 neuroplastic mechanisms may be crucial for a patient's ability to learn to adapt to the altered oral state. Since the M1 receives a large amount of somatosensory inputs either directly through the thalamus or indirectly through S1 (see above) and since neuroplastic changes after dental deafferentation do occur at the peripheral, subcortical (see below) and face-S1 levels, it is possible that neuroplastic changes also occur in face-M1 analogous to the occurrence of neuroplasticity in limb S1 and limb M1 following limb amputation (Dettmers et al., 2001; Lotze et al., 1999; Manger et al., 1996). While any such neuroplastic changes in face-M1 following tooth extraction might be explained by changes in sensory inputs to M1, it needs to be kept in mind that they may also be explained by other changes such as altered orofacial motor function or pain (see below).

4.1.1.2.. Dental trimming

Trimming of teeth can result in mechanical, thermal and perhaps noxious stimulation and incisal trimming of the rat incisor has been reported to result in a significant decrease in the thickness of the enamel and dentin (Michaeli et al., 1982; Risnes et al., 1995; Weinreb et al., 1985). Reduced occlusal contacts induced by incisal trimming have been associated with a reduction in the size and number of periodontal

nerve endings that reverses once the occlusal contacts are restored (Shi et al., 2005). Therefore, trimming of teeth out of occlusal contacts may conceivably alter the somatosensory inputs from the teeth to the sensorimotor cortex. Since somatosensory inputs to the sensorimotor cortex play a role in M1 neuroplasticity (see above), it is possible that incisal trimming will also result in neuroplastic changes within face-M1.

4.1.2. Post-operative pain

Post-operative pain may induce alterations in nociceptive inputs to face-M1 and face-S1. Tooth extraction results in peripheral tissue injury that can activate peripheral nociceptors directly and in addition causes the release of many inflammatory mediators (*e.g.* bradykinin, prostaglandins, substance P and histamine) that can further increase the excitability of peripheral nociceptors. Evidence is accumulating to indicate that dental pain induced by application of the inflammatory irritant mustard oil to the rat's tooth pulp results in central sensitization in functionally identified V brainstem and thalamic nociceptive neurons (Chiang et al., 1998; Chiang et al., 2005; Zhang et al., 2006).

Chronic pain conditions, such as back pain, phantom limb amputation (Dettmers et al., 2001) or complex regional pain syndrome (Krause et al., 2006) have been associated with increased limb-M1 excitability and reorganization of the S1 as well as M1(Lotze et al., 1999; Tsao et al., 2008); and a regular use of a myoelectric prosthesis in these patients is associated with less cortical reorganization and less phantom limb pain (Lotze et al., 1999). Studies dealing with the effects of experimental acute pain on limb-M1 in humans have shown that reversible noxious stimuli can decrease M1 excitability (Farina et al., 2001; Le Pera et al., 2001; Svensson et al., 2003a). While these studies may suggest that reorganization of M1 and S1 plays a role in acute and chronic limb pain, no study has examined the involvement of M1 and S1 in dental amputation or phantom tooth pain (Marbach, 1993a; Marbach, 1993b; Marbach and Raphael, 2000; Tassinari et al., 2002). Furthermore, the exact interaction between acute orofacial pain and face-M1 neuroplasticity is the subject of controversy. Studies applying hypertonic saline or capsaicin to the masseter muscle, facial skin (Romaniello et al., 2000) or tongue (Halkjaer et al., 2006; Romaniello et al., 2000) in humans have failed to demonstrate any association between pain and face-M1 excitability. Yet experimental pain induced by

topical application of capsaicin to the tongue does prevent the increased tongue-M1 excitability associated with training of a tongue-protrusion task (Boudreau et al., 2007); and a fMRI study in humans has shown that electrically-induced tooth pain activates a cortical network which includes also the M1 (Jantsch et al., 2005). In support of these latter human studies, experimental noxious stimulus to the tongue induced by topical application of glutamate in rats does reduce tongue-M1 excitability, but injection of hypertonic saline to the tongue does not affect tongue-M1 excitability (Adachi et al., 2007). Although the inconsistencies in the results of the different studies may be related to different study designs (*e.g.* species, type and intensity of the experimental noxious stimulus and site of its application), they may also be related to the multidimensional nature of pain and its modulatory effect on face-M1 excitability. Nonetheless, these considerations do raise the possibility that acute pain resulting from tooth extraction may contribute to face-M1 neuroplasticity.

4.2. Neuroplasticity associated with modified motor experience

Numerous studies conducted primarily in limb-M1 of humans, monkeys and rats have revealed that motor representations are altered by motor experience such as following training in a novel limb motor skill (Karni et al., 1998; Kleim et al., 1998; Nudo et al., 1996; Pascual-Leone et al., 1995; Remple et al., 2001; for review, see: Ebner, 2005). Typically, these studies reveal that there is an increased representation of the muscle or muscles involved in the trained movement at the expense of the representations of the less trained muscle(s). Consistent with these studies, training monkeys (Sessle et al., 2007; Sessle et al., 2005) in a novel tongue-protrusion task has been associated with significant neuroplastic changes within face-M1 representing the tongue. In comparison to the pre-training data, training in the tongue-protrusion task for a period of 1-2 months has been associated with a significantly increased representation of tongue-protrusion movement and a decreased representation of tongue-retrusion movement (Sessle et al., 2007; Sessle et al., 2005). In analogous TMS studies in humans, 1 week or even 1 day of training in a tongue-protrusion task has resulted in an increased tongue representation within face-M1 that reverses after 2 or 1 weeks of no training,

respectively (Svensson et al., 2003b; Svensson et al., 2006). Furthermore, even just 15 min of training in the same tongue task has been associated with increased excitability of face-M1 (Boudreau et al., 2007). These findings indicate that the primate face-M1 has the neuroplastic capability to adapt to significant changes in oral motor behaviour and may be modelled in a specific use-dependent manner.

Limited data are available on the neuroplastic capabilities of the rat's face-M1 in association with alterations in orofacial motor behaviour. Rats actively sweep their vibrissae to explore their immediate environment and the vibrissae have a prominent representation within face-M1 (see above). Unilateral trimming of the rat vibrissae induces changes in the exploratory behaviour by the rat as it adopts a motor behavioural asymmetry favouring the use of the intact vibrissae (Milani et al., 1989). However, such changes in the rat exploratory behaviour are not associated with decreased vibrissal representation within the contralateral face-M1 of adult rats (Huntley, 1997b). On the other hand, bilateral trimming of the vibrissae for 5 days does result in changes in the vibrissal motor representations that reverses once the vibrissae are allowed to grow back to normal length (Keller et al., 1996).

4.2.1. Occlusal modifications to the rat incisors

There are no studies of the neuroplastic capabilities of face-M1 in association with alterations in the oral motor behaviour of the rat. In humans, changes in oral motor behaviour (*e.g.* altered pattern of jaw movement) may be induced by modifications to the dental occlusion (Johansson et al., 2006; Klineberg and Jagger, 2004; Proschel and Hofmann, 1988; Trulsson and Essick, 2004)

Normally, rats are engaged in gnawing motor behaviour to compensate for the continuous eruption of their incisors (Burn-Murdoch, 1999; Michaeli and Weinreb, 1968; Michaeli et al., 1974). Dental extraction (Endo et al., 1998; Miehe et al., 1999) or unilateral trimming of an incisor out of occlusion (Ramirez-Yanez et al., 2004) in rats are associated, 1-2 weeks later, with morphological changes in the condyles and masticatory muscles suggesting alterations in oral motor behaviour of the rat. In addition, as mentioned above (see above 3.7.) somatosensory inputs from the orofacial tissues to the sensorimotor cortex play an important role in motor control of orofacial motor

functions and that incisor trimming or extraction may alter such somatosensory inputs from the teeth (see above 4.1.2.2). Therefore, it is possible that changes in somatosensory inputs induced by modifications to the dental occlusion can also induce changes in oral motor behaviour. Consistent with the concept of use-dependent neuroplasticity, it is possible that such changes in the rat oral motor behaviour can contribute to face-M1 neuroplasticity; however this has not been addressed yet in detail in rats.

4.2.2. Effect of diet consistency

A soft diet has different functional demands than a hard diet and somatosensory inputs from orofacial mechanoreceptors to the sensorimotor cortex can provide information regarding food consistency, thereby contributing to the cortical control of mastication (Jacobs, 1998; Miles et al., 2004; Trulsson, 2007) . Therefore, changes in diet consistency may possibly be associated with different biting and chewing loads and a different pattern of mastication. Indeed, it has been documented in humans that food consistency can affect the pattern of chewing movements (Proschel and Hofmann, 1988). In rabbits, diet can affect the pattern of tongue muscle activity during mastication (Inoue et al., 2004) and a change to a soft diet is associated with morphological changes in the masticatory muscles of rats (*e.g.* composition of masseter muscle fibres) (Kiliaridis et al., 1988; Miehe et al., 1999). In mice, food consistency influences the pattern of jaw movements, the activity of masticatory muscles (*e.g.* masseter and anterior digastric) and the chewing rhythms (Okayasu et al., 2003). Food consistency can also affect the central mechanisms regulating the digastric muscle reflex and in turn, the reflex can contribute to the regulation of masticatory force during chewing (Yamamura et al., 1998). These findings raise the possibility that changes in diet consistency may also alter oral sensorimotor functions and affect the organizational properties of the face sensorimotor cortex but this has not yet been explored in any other study.

4.2.3. Individual variability

The topographic organization of motor representation is highly variable across individuals and this individual variability may be related, at least in part, to individual variation in motor experiences manifested as a use-dependent cortical neuroplasticity

(Donoghue et al., 1992; Huntley and Jones, 1991b; Nudo et al., 1992; Nudo et al., 1996; Sessle and Wiesendanger, 1982). This can be further supported by the limb-M1 observation that the representation of the limb in the dominant hemisphere contralateral to the dominant hand is larger and more complex than the representation of the non-dominant hand in the non-dominant hemisphere (Nudo et al., 1992).

4.3. Time-dependent neuroplasticity

M1 reorganization following a peripheral deafferentation may be manifested differently at different points of time. For example, within 4 min of facial nerve transection, the ipsilateral vibrissal representation (vibrissae usually have contralateral representation) takes over nearly the entire deprived contralateral vibrissal representation (Toldi et al., 1996). However, during the following hours to days, the ipsilateral vibrissal representation shrinks and the forelimb and eye representation progressively expand to take over the deprived vibrissal representation. Unilateral lingual nerve transection has been associated at one point of time after the transection (1-2 weeks) with a significantly decreased GG representation and at another point of time (3-4 weeks) with a significantly increased GG representation (Adachi et al., 2007). The above findings suggest that M1 motor representations are dynamic and different changes in motor representation may occur at different points of time.

4.4. Changes in other cortical or subcortical areas

There is evidence to suggest that alterations in orofacial somatosensation may induce neuroplastic changes at cortical and subcortical levels of the somatosensory system (*e.g.* S1, thalamus, brainstem and peripheral nerves) (for reviews, see Jones, 2000; Kaas et al., 2008). Sensory perturbation induced by capsaicin injection to the lip (Katz et al., 1999) or sensory deprivation induced by intraoral local anaesthesia (Nicolelis et al., 1993) induces reorganization of the orofacial receptive fields at both thalamic and S1 levels. Dental deafferentation (*i.e.*, tooth extraction, pulp extirpation) is associated with reorganizational changes of the mechanoreceptive fields within the mesencephalic nucleus (Linden and Scott, 1989) and V brainstem nuclei (Hu et al.,

1986; Hu et al., 1999; Kwan et al., 1993). A recent study in young mole-rats has reported that 5-8 months after incisor extraction there is reorganization of face-S1 whereby the entire extent of the deprived incisor representation becomes represented by other orofacial tissues (Henry et al., 2005). Face-M1 receives somatosensory inputs either directly through the thalamus (Hatanaka et al., 2005; Rausell and Jones, 1995; Simonyan and Jurgens, 2005), or indirectly through face-S1 (Chakrabarti and Alloway, 2006; Hoffer et al., 2005; Iyengar et al., 2007). In addition, it has been demonstrated that neuroplastic changes within the vibrissal-M1 are the result of cortical disinhibition and unmasking of latent inputs from S1 to M1 (Farkas et al., 2000). Thus, it needs to be kept in mind that some of the neuroplastic changes within face-M1 may reflect changes at other cortical (*e.g.* S1) and subcortical afferent relay stations, analogous to the occurrence of neuroplastic changes in limb-S1, limb-M1, as well as in subcortical areas following limb amputation (Dettmers et al., 2001; Florence and Kaas, 1995; Lotze et al., 1999; Manger et al., 1996).

ICMS of M1 evokes EMG responses through activation of brainstem motoneurons which are the final common path integrating a large number of sensory and motor inputs before projecting to evoke muscle activity (Capra, 1995; Paxinos, 2004; Sessle, 2000; Trulsson and Essick, 2004). For example, afferent inputs from jaw muscles and incisors can modulate the activity of the XII motoneurons controlling tongue-protrusion and retrusion (*e.g.* Tolu et al., 1993; Tolu et al., 1994a; Tolu et al., 1994b) or V motoneurons controlling jaw muscles (*e.g.,* Goldberg, 1971; Lavigne et al., 1987; Sessle, 1977; Sessle and Schmitt, 1972). It has been reported that transection of the facial (motor) nerve induces motor reorganization not just within face-M1 (Toldi et al., 1996) but also within brainstem VIIm and V nuclei (Kis et al., 2004). Thus, any change within face-M1 may reflect changes within subcortical efferent relay stations.

4.5. Mechanisms underlying cortical neuroplasticity

Several features of the sensorimotor cortex may provide or at least contribute to the substrate for cortical neuroplasticity such as an extensive network of excitatory and inhibitory connections. Excitatory connections are characterized by monosynaptic

connections of pyramidal axon collaterals projecting horizontally for several mm (for reviews, see Keller and Asanuma, 1993; Mountcastle, 1997; Schieber, 2001). The inhibitory interneurons constitute about 30% of the M1 neurons (Jones, 1993). M1 functional organization is characterized by spatial contiguity of motor representations with a considerable amount of overlapping of motor representations, and spatial contiguity of sensory inputs and motor output (for reviews, see above and Sanes and Donoghue, 2000; Sanes and Schieber, 2001; Sessle et al., 2007; Tehovnik et al., 2006).

There is a clear lack of studies related to the mechanisms underlying face-M1 neuroplasticity and manifested as reorganization of motor representations. Most of the reported studies concern limb-M1, although some data are available from studies of the vibrissal-M1. Different mechanisms can be involved in different forms of neuroplastic processes and different mechanisms may be involved at different points of time or may operate simultaneously. Rapid cortical reorganization, such as following peripheral deafferentation, can occur within minutes and may be explained by mechanisms such as potentiation of previously existing connections by unmasking (*e.g.* through disinhibition) of existing intracortical excitatory synaptic connections which are usually ineffective because of inter- and intra-hemispheric lateral (*e.g.* GABAergic) inhibition (Rat: Farkas et al., 2000; Jacobs and Donoghue, 1991). Mechanisms involved in cortical reorganization following limb motor-skill learning include rapid changes as well as slow and long-lasting changes such as enhanced gene expression (Kleim et al., 1996) and increased neuronal excitability (Aou et al., 1992) during early stages of learning; dendritic branching (Greenough et al., 1985; Jones et al., 1996; Monfils et al., 2004) and synaptogenesis (Kleim et al., 2002a; Kleim et al., 2004; Kleim et al., 1996) during later phases of learning and long-term potentiation (LTP) may play a role at early as well as late phases of the learning process (Hess and Donoghue, 1994; Rioult-Pedotti and Donoghue, 2003; Rats: Monfils and Teskey, 2004a; Rioult-Pedotti et al., 1998; Teskey et al., 2007; for reviews, see Boroojerdi et al., 2001; Chen et al., 2002; Kaas, 1991; Navarro et al., 2007). On the other hand, non-skilled limb training has been associated with lack of cortical reorganization of motor representations (Kleim et al., 2002b; Swain et al., 2003).

4.5.1. Unmasking of existing latent excitatory connections

Neighbouring excitatory regions of M1 are connected via inhibitory interneurons. Damage to a neuron projecting to an inhibitory interneuron reduces the synaptic efficacy of this GABA-mediated inhibitory pathway (*i.e.*, disinhibition). ICMS within the deafferented or deefferented M1 area (such as following peripheral nerve injury) could then excite neighbouring neurons that previously were unresponsive, thereby increasing an ICMS-defined motor representation (Farkas et al., 2000; Huntley, 1997a; Jacobs and Donoghue, 1991).

4.5.2. Modulation of synaptic efficacy

Modulation of synaptic efficacy through LTP or long- term depression (LTD) is an important mechanism that has been attributed to long-lasting changes such as learning and memory but also to short-term functional reorganization of M1. LTP was first described by Bliss and Lomo (Bliss and Lomo, 1973) who recognized that high-frequency stimulation (HFS) of hippocampal excitatory afferents results in increased excitatory postsynaptic potentials (EPSPs) in the hippocampus. Similar mechanisms of synaptic enhancement have since been described in other areas of the peripheral and central nervous system including M1 (for review, see Bi and Poo, 2001).

Enhanced synaptic efficacy resulting in enhanced M1 excitability may also occur as a result of increased release of excitatory neurotransmitters, increased density of postsynaptic receptors and changes in membrane conductance (Jones, 1993). Increased cortical excitability may further facilitate the ability to evoke motor responses by ICMS of M1 areas that previously could not evoke any motor response by a similar stimulus, thereby increasing M1 motor representations (Rats: Monfils et al., 2004). On the other hand, LTD has been associated with decreased motor representations (Rats: Teskey et al., 2007). Consequently, LTD and LTP together can contribute to the bidirectional effectiveness of intracortical horizontal synaptic connections forming the substrate for functional reorganization of motor representations (Teskey et al., 2007).

Some of these features may be important in motor learning. M1 possesses 5 important features that are considered to be crucial for motor learning: 1. Associativity – existence of 2 parallel afferent pathways, one does (S1 projections to M1) and the other

(through the thalamus) does not induce LTP by a tetanic stimulus (Bi and Poo, 2001; Caria et al., 1997; Keller et al., 1990; Kimura et al., 1994); 2. Cooperativity – whereby the 2 parallel afferent pathways converge to induce LTP in postsynaptic M1 pyramidal neuron (Bi and Poo, 2001; Hess et al., 1996; Iriki et al., 1991; McNaughton et al., 1978); 3. Temporal specificity – the temporal order of activation of the 2 pathways determines the LTP and reversing the temporal order results in LTD (Bi and Poo, 2001; Levy and Steward, 1983); 4. NMDA receptor dependence – glutamatergic NMDA receptors are distributed preferentially in M1 superficial layers II and III and in-vitro application of NMDA-antagonist blocks the possibility of LTP induction in M1 horizontal connections (Hess et al., 1996); 5. Horizontal inhibitory connections that can allow for a transient disinhibition of horizontal pathways that is required for LTP induction (Hess et al., 1996). Therefore, any change in afferent inputs that can decrease (or increase) the inhibition may affect the cortical motor organization by unmasking (or masking) horizontal excitatory connections and providing the substrate for LTP (or LTD) to occur (Hess et al., 1996; Hess and Donoghue, 1994).

4.5.3. Dendritic branching and synaptogenesis

Dendritic branching and synaptogenesis are other mechanisms that may underlie slower (days) and long-lasting (days-months) cortical changes. These mechanisms result in an increased number of synapses that can strengthen the intracortical connections, thereby potentially increasing the likelihood for evoking muscle movements during ICMS (Greenough et al., 1985; Jones et al., 1996; Kleim et al., 2002a; Monfils and Teskey, 2004a).

5. The Intracortical microstimulation (ICMS) technique

Electrical stimulation of the sensorimotor cortex has been used for over a century to investigate the functional organization of motor-outputs within the sensorimotor cortex (for reviews, see Asanuma, 1989; Taylor and Gross, 2003). Two main electrophysiological techniques are available today for mapping motor representations. The ICMS technique, developed by Asanuma and colleagues, provides good spatial (at the neuron level, μm) and temporal (msec) resolution giving detailed information of the

organizational features of the sensorimotor cortex; but the inherent invasiveness limits its use to animals (for reviews, see Asanuma, 1989; Boulton et al., 1999; Cheney, 2002; Patterson and Kesner, 1981; Yeomans, 1990). Transcranial electrical stimulation and TMS (for review, see Cheney, 2002) provide a non-invasive technique but have a temporal and spatial resolution of seconds and cm respectively. The ICMS and TMS techniques are time-consuming techniques providing detailed motor maps of a relatively small segment of the brain and as such do not reflect the whole cortical motor function. Imaging methods (*e.g.* fMRI, PET, EEG and MEG) can provide a functional picture of the whole brain at one point of time, but they have lower spatial and/or temporal resolutions (for review, see Cheney, 2002). The electrophysiological and imaging techniques complement each other and combining them could have provided valuable information at various levels of the brain. However, at this time, the thesis used the ICMS technique alone and therefore an outline is provided of this technique.

ICMS is an extracellular stimulation technique whereby a cathode microelectrode delivers small electrical currents to a localized area within the cortex. These currents usually excite pyramidal tract neurons within layers V-VI of the cortex to generate action potentials that propagate along the corticobulbar tract to synapse and activate motoneurons within the brainstem motor nuclei that project to the neuromuscular junction to evoke a muscle response that can be either observed or recorded by an electromyograph (Asanuma, 1989; Greenshaw, 1998; Miles et al., 2004). Systematic microelectrode penetrations and recordings of ICMS-evoked EMG activity can help delineate the cortical area devoted to the motor output of each muscle and thereby form the motor representation map of each muscle.

Features of ICMS-evoked EMG responses include its threshold (the lowest ICMS intensity reliably evoking EMG responses), amplitude, duration, area under the curve and onset latency (relative timing of the associated muscle EMG bursts). Under similar stimulation parameters (*e.g.* intensity, frequency and duration), changes in the features of the ICMS-evoked responses may indicate changes in the strength (or excitability) of the corticobulbar (or corticospinal) projections to the muscles (Asanuma, 1989; Greenshaw, 1998; Ranck, 1975). On the other hand, changes in the stimulation parameters as well as

changes in other parameters such as the cortical depth at which the ICMS is applied, state of anaesthesia, previous stimulation, initial posture of the body part/muscle, as well as individual variations, can all have an effect on the features of the ICMS-evoked EMG responses and thereby may influence the overall extent of muscle/s representation (Asanuma, 1989; Graziano et al., 2002b; Greenshaw, 1998; Neafsey et al., 1986; Sessle and Wiesendanger, 1982; Tandon et al., 2008; Tehovnik et al., 2006).

5.1. Effective extent of stimulating current spread

ICMS activates a collection of neurons in the vicinity of the stimulating microelectrode and therefore, the ICMS-defined motor representation reflects the organization of a stimulated cluster of neurons rather than single neurons. The extent of neuronal activation depends on the effective spread of stimulating current that directly activate nearby neurons and the indirect current spread through transynaptic connections. Electrophysiological studies have calculated that the distance that currents can spread effectively to directly activate nearby neurons is proportional to the ICMS intensity and to the excitability of the neuronal tissue. The excitability of the neuronal tissue depends on the neuronal density and axonal diameter and myelination (Asanuma et al., 1976; Stoney et al., 1968a; for reviews, see Asanuma, 1989; Tehovnik, 1996; Tehovnik et al., 2006). It has been estimated in cats that ICMS intensity of 20 μA can effectively spread within a radius of 100-175 μm (Stoney et al., 1968a; Tehovnik et al., 2006) to directly activate 1-24 large pyramidal tract neurons and 360-4336 small pyramidal neurons (Cheney, 2002; Cheney and Fetz, 1985). In awake rats, an ICMS train of 20 μA (0.2ms pulse, 300Hz) can activate pyramidal cells within a radius of 0.25 mm (Sapienza et al., 1981) and a 50 μA ICMS current (0.2 ms pulse, 300HZ) can activate pyramidal tract neurons within a radius of 0.5 mm (Neafsey et al., 1986; Ranck, 1975). The recurrent axon collaterals of the pyramidal tract neurons can project horizontally 1-3 mm (Rats: Aroniadou and Keller, 1993; Cat: Asanuma et al., 1976; Monkeys: Jankowska et al., 1975) and can spread even further through interneurons that connect neighbouring pyramidal tract neurons. However, since the interneurons are mainly inhibitory, beyond the range of monosynaptic connections of pyramidal neurons, the interneurons are

thought to limit the effective spread of ICMS currents (Asanuma et al., 1976). Overall, stimulating currents can spread horizontally to activate distant neurons, thereby indirectly increasing the effective spread of the stimulating currents (Asanuma, 1989; Gustafsson and Jankowska, 1976; Ranck, 1975; Tehovnik, 1996; Tehovnik et al., 2006). In addition, all the diverging and converging motor outputs and the somatosensory inputs on Vm (see above) may further expand the network of excitatory and inhibitory motor connections, thereby influencing the extent of ICMS-evoked responses.

The use of ICMS at threshold intensity aims at activating the most excitable projection neurons located close to the tip of the stimulating microelectrode. It can activate a combination of neuronal elements including cell bodies and axons of passage (Asanuma et al., 1976; Gustafsson and Jankowska, 1976; McIntyre and Grill, 2000; Nowak and Bullier, 1998a; Rattay, 1999; Stoney et al., 1968a; Stoney et al., 1968b; Swadlow, 1992; Tehovnik et al., 2006). Therefore, a threshold intensity stimulus exciting the most excitable neurons (myelinated, larger diameter) can evoke a short-latency muscle response and at the same time also evoke longer-latency responses of other muscles. Similarly, higher ICMS intensity stimuli can evoke activity in the same muscle from a wider cortical area, in combination with activities in various other muscles (for reviews, see Asanuma, 1989; Tehovnik et al., 2006; Cats: Asanuma et al., 1976; Asanuma et al., 1968; Stoney et al., 1968a; Stoney et al., 1968b; Rats: Sapienza et al., 1981). Since cortical layer III has mainly horizontal connections and layer V is the main corticobulbar (corticospinal) output layer, ICMS of cortical layer V is associated with the lowest threshold values (Asanuma et al., 1976; Hall and Lindholm, 1974; Sapienza et al., 1981). ICMS of layer III evokes responses at higher threshold values, probably mainly due to indirect activation of pyramidal tract neurons (Asanuma et al., 1976). In addition, although each corticobulbar tract neuron diverges extensively and may innervate several motoneuron pools within a particular brainstem motor nucleus or within different motor nuclei, neighbouring corticobulbar tract neurons converge to innervate most heavily a particular motoneuron pool that innervates a particular muscle and therefore, low-threshold ICMS can evoke a short-latency muscle activity in this most heavily innervated muscle (*e.g.* jaw or tongue muscles); in contrast, high-threshold

ICMS can excite several motoneuron pools and thereby can affect more than 1 muscle (*e.g.* jaw and tongue muscles) (for reviews, see Asanuma, 1989; Mountcastle, 1997; Schieber, 2001; Anaesthetized monkeys: Sirisko and Sessle, 1983; Rat: Andersen et al., 1975; Neafsey et al., 1986; Wise et al., 1979). For example, in comparing movements evoked by low-intensity short-train ICMS (0.25 ms, 350 Hz, 50 ms, 100 μA) with movements evoked by high-intensity long-train ICMS (0.25 ms, 350 Hz, 300 ms, 500 μA) in anaesthetised rats, changes in the duration of the movements may occur but not the actual movement evoked (Neafsey et al., 1986).

Excessive ICMS currents (*e.g.* in cats 6 pulses at >80 μA; 0.2 msec; 2.0 msec interval) can produce noxious effects and result in tissue damage or increased activation of lateral inhibitory connections that their sum effect exceeds the excitatory effect of the pyramidal neurons, thereby blocking the effective spread of stimulating currents through trans-synaptic connections (Asanuma and Arnold, 1975).

Another important stimulus parameter is the number of repetitive stimulations (*i.e.*, stimulation train) and their duration. Repetitive stimulation results in temporal summation with a subsequent gradual increase of the amplitude and duration of successive ICMS-evoked EMG responses. Therefore, repetitive ICMS stimulation using long-duration (30-40 msec), high frequency (300-400 Hz) and long trains (> 10 pulses) can evoke EMG responses in an individual muscle at a relatively smaller ICMS threshold (Asanuma et al., 1976; Ranck, 1975).

5.2. Individual variability

Lastly, since different cortical areas differ in their architecture and the layout of horizontal interconnectivity (Johansson, 2006; Johansson and Lansner, 2007; Lund et al., 1993) and since the overall extent of current spread may vary as a function of stimulus measures and neural tissue excitability (see above), the extent of the representation map can vary among different cortical areas and can also vary among different animals (Nudo et al., 1992; Nudo et al., 1996), as noted above.

5.3. Effect of general anaesthesia

Two main experimental approaches exist to study effects of ICMS: acute experiments in anaesthetised animals and chronic experiments in awake animals. Since there is some variability among animals in the location of motor representations within M1 (Nudo et al., 1992; Nudo et al., 1996), the most significant advantage of the chronic experiment is that the effect of various manipulations over time can be observed in the same animal free of general anaesthesia. In acute experiments, data are combined from several animals. Extensive ICMS mapping of motor representations is an invasive and time-consuming technique that may last 10-12 hrs. The threshold of ICMS-evoked muscle activity and thereby the extent of muscle representation can be influenced by many factors such as state of anaesthesia or alertness of the animal as well as muscle position or muscle stretching (Asanuma et al., 1968; Graziano et al., 2002b; Wong et al., 1978). In chronic experiments, awake animals can stay alert in a relaxed muscle position for only a few hours at each mapping session and consequently extensive mapping will require weeks to months to be completed; during this time the animal needs to survive despite the invasiveness of the procedure. In contrast, in acute experiments under general anaesthesia, mapping can be completed within 1 session. However, there are concerns regarding the use of anaesthetics including the use of ketamine in M1 mapping. Ketamine is a dissociative general anaesthetic that is commonly used in ICMS mapping studies because it is one of the few general anaesthetics that does not abolish ICMS-evoked muscle responses (Nudo et al., 2003). Ketamine acts as a non-competitive blocker of NMDA receptors (Ebert et al., 1997; Sessle and Wiesendanger, 1982; Yamakura et al., 2001). Dendrites and cell bodies of cortical neurons have NMDA receptors while axonal branches do not and therefore, ketamine's effect is mainly on cell bodies and dendrites. Since ICMS mainly excites axons and not cell bodies (Nowak and Bullier, 1998a; Nowak and Bullier, 1998b), ketamine's actual effect on ICMS-evoked muscle responses seems to be relatively small.

It has been reported that deeper states of general anaesthesia induced by ketamine (and other general anaesthetics) can influence cortical excitability as reflected in increased thresholds and longer latency of ICMS-evoked muscle responses and these in

turn can be manifested as changes in the extent of motor representations within M1 and in particular S1 (Rats: Gioanni and Lamarche, 1985; Sapienza et al., 1981; Tandon et al., 2008). Nevertheless, EMG responses in orofacial muscles can be evoked by low ICMS intensities in anaesthetised as well as in awake animals (Huang et al., 1989b; Sapienza et al., 1981; Tandon et al., 2008) and similar motor maps have been obtained from different studies using different anaesthetic agents (Monkeys: Frost et al., 2000; Nudo et al., 2003) or different states of anaesthesia (Rats: Tandon et al., 2008; Monkeys: Huang et al., 1989b). ICMS can reveal changes in motor representations over small distances of 250 μm in awake or anaesthetised animals. In addition, it has been shown that relatively close matches of EMG activity can be obtained by spike-triggered averaging in awake animals and repetitive ICMS in anaesthetised animals (Cheney, 2002).

Therefore, ICMS under ketamine anaesthesia can be an appropriate technique to reveal the organizational features of face-M1. The results from anaesthetised animals may be comparable to those derived from studies with awake animals. Yet it is crucial to regulate the administration of the anaesthetic and to maintain a stable level of general anaesthesia that allows for EMG activity evoked by relatively low ICMS intensities (Nudo et al., 2003).

6. Statement of the problem and study objectives

Electrophysiological studies in subprimates and primates have employed ICMS or single neuron recordings in conjunction with reversible cold block or lesioning techniques to underscore the crucial role of that part of M1 representing the orofacial region (face-M1) in the generation and control of orofacial motor functions. Analogous studies have revealed that the somatosensory system including the face-S1 may also play a role in the control of orofacial movements. This is supported by the existence of 2 parallel projections of direct (through the thalamus) and indirect (through face-S1) somatosensory inputs to face-M1 that provide peripheral feedback from the orofacial tissues including the teeth that further assist in the control of orofacial motor functions.

One striking finding of these studies is that ICMS can evoke EMG activity in orofacial muscles from an extensive area of face-M1, suggesting extensive motor

representation of the orofacial muscles. Numerous studies conducted primarily in face-M1 representing the rat vibrissae have revealed that motor representations are altered by peripheral manipulations of vibrissal sensory inputs or motor output. However, limited published data are available on the neuroplastic capabilities of face-M1 following intraoral manipulations. Neuroplastic changes can occur in the tongue motor representation within face-M1 following the training of humans and monkeys in a novel tongue-protrusion task; application of the algesic glutamate to the tongue in rats and capsaicin to the tongue in healthy subjects induces decreased face-M1 excitability and transection of the lingual nerve supplying sensory innervation to the tongue results in time-dependent changes in the tongue representation within face-M1. Nevertheless, no study has addressed whether neuroplastic changes occur in the ICMS-defined motor representations within face-M1 following tooth loss, modification to the dental occlusion or a change in diet consistency and no study has addressed the neuroplastic capabilities of face-S1 motor outputs. Yet this information is of clinical significance since modification to the dental occlusion as a result of loss of teeth or dental attrition are common dental occurrences that may be accompanied by impaired oral sensorimotor functions. Consequently, the most vital functions of eating and speaking may be impaired and jeopardize the patient's quality of life. Oral rehabilitation aims at restoring the lost oral sensorimotor functions. Novel therapies of motor dysfunction as a result of spinal cord injuries or stroke have taken advantage of limb-M1 neuroplastic mechanisms to promote recovery of limb motor functions in animal models as well as in humans. For example, a treatment with a myoelectric prosthesis results in reorganization of limb and lip motor representations that is also associated with less phantom limb pain (Lotze et al., 1999). Recent studies in rats (Adkins et al., 2008), monkeys (Plautz et al., 2003) and humans (Brown et al., 2006) have shown that pairing rehabilitative training with cortical electrical stimulation induces more behavioral improvement than training alone. Thus, clarification of the cortical neuroplastic mechanisms underlying orofacial motor function, malfunction and recovery following peripheral injuries may provide better therapeutic strategies to oral rehabilitation to ensure the restoration of oral functions in patients suffering from oral sensorimotor deficit and thereby improve their quality of life.

Therefore, the purpose of the present thesis was to develop an animal model to study whether neuroplastic changes occur in face-M1 following various alterations in the oral environment.

HYPOTHESIS

Alterations in the oral environment resulting from tooth trimming or extraction as well as changes in diet consistency are associated with neuroplastic changes in the ICMS-defined jaw and tongue motor representations within the rat cytoarchitectonic-defined face-M1 and adjacent face-S1.

OBJECTIVES

To use ICMS and recordings of evoked muscle electromyographic (EMG) activity to test if neuroplastic changes occur in the ICMS-defined motor representations of the tongue-protrusion (GG, genioglossus) and jaw-opening (AD, anterior digastric) muscles within the rat face-M1 and adjacent face-S1 following:

1. A change in diet consistency.
2. Unilateral trimming of the mandibular incisor.
3. Unilateral extraction of the mandibular incisor.

CHAPTER 2

GENERAL MATERIALS AND METHODS

All experimental procedures were approved by the University of Toronto Animal Care Committee, in accordance with the Canadian Council on Animal Care Guidelines and the regulations of the Ontario Animals for Research Act (R.S.O. 1990). One investigator carried out all experimental procedures and data analysis to ensure consistency in the experimental procedures and in the blinded data analysis.

1. Animals

Gender is known to influence brain anatomy and chemistry affecting the function of many brain areas including the brainstem, cerebral cortex and hippocampus (for reviews, see Cahill, 2006; McEwen, 2002). Male and female rats differ in their oral functional behaviour (Cairns et al., 2003; Sessle, 1966) and response to pain (Cairns et al., 2001; Dao and LeResche, 2000) and cortical neuroplasticity can be modulated by motor function (Keller et al., 1996; Kleim et al., 1998; Plautz et al., 2000; for review, see Ebner, 2005) and pain (Boudreau et al., 2007). Cortical neuroplasticity can also be modulated by gender effects (Hattemer et al., 2007; Jonasson, 2005) and be manifested differently at different ages of the rat (Franchi et al., 2006; Huntley, 1997b). Therefore, the experiments reported herein were performed only on young adult male Sprague-Dawley rats (Charles River, Montreal, QC, Canada) (150-250g on arrival, 300-400g on day of cortical mapping).

The rats were housed in individual cages (27 × 45 × 20 cm) containing a PVC tube (used as a shelter and gnawing device), under a controlled temperature (21 ± 1 °C) and humidity (50 ± 5 %), with a 12 hrs light/dark cycle (lights on at 07:00 am). Rats are usually kept on hard diet from their weaning day. Since trimming or extraction may impose some discomfort while chewing on hard diet the rats in these 2 experiments (see below) were kept on soft diet (mashed chow) (Rodent diet #2018M, Harlan Teklad) from the day of their arrival at the vivarium. Since diet consistency may affect masticatory functions (Inoue et al., 2004; Okayasu et al., 2003; Proschel and Hofmann, 1988; Thexton et al., 1980), we also tested whether a change in diet consistency has any effect on the jaw and tongue motor representations and therefore, 1 group was kept on hard

(chow) diet (Rodent Diet # 2018, Harlan Teklad) while the other group was given the soft diet.

Animals were monitored on a daily basis to assess body weight and food consumption to ensure continuous and similar growth rate; any change in general behaviour (*e.g.* exploration, freezing, rearing, jaw motion) (Chudler and Byers, 2005) as well as any post-operative complication (*e.g.* bleeding, inflammation). In none of the rats was there any need for post-operative antibiotics. According to the animal protocol, Buprenorphine hydrochloride (0.05 mg/kg) was given S.C. every 8-12 hours during the first post-operative day to reduce any possible post-operative pain.

Before dental manipulation all study groups (see below) had a similar daily gain of body weight. In comparison with pre-treatment, during the 1 week of trimming or sham-trimming and during the 1 week post-extraction (see below), rats continued to gain weight, although at a slower rate but the "trim recovered" group of rats showed a similar rate of weight gain across the experimental periods (*i.e.*, before trimming, during trimming or during recovery) (Fig.2-1 and Table 2-1).

2. Study groups

Morphological changes in the condyles (Ramirez-Yanez et al., 2004) and periodontal ligament (Shi et al., 2005) may occur within 1-2 weeks following dental manipulation (*e.g.* trimming) suggesting alterations in oral sensorimotor functions. Previous studies have shown in rats that reorganization of face-M1 motor representations may occur within 1 week (Adachi et al., 2007; Sanes et al., 1990) or even within hours of peripheral deafferentation (Donoghue et al., 1990; Huntley, 1997b; Sanes et al., 1988; Toldi et al., 1996). To keep similar experimental time intervals, the time interval between dental manipulations and cortical mapping experiments in all groups was set at 1 week (Fig. 2-2).

Animals were divided into the following study groups:

Experimental Groups

1. "Trim" group (n=6)
    - Under general anaesthesia (see below), rats had the incisal edge of the right lower incisor trimmed every 2 days for a period of 1 week (see below and Fig. 2-3A). ICMS mapping was carried out 1 day following last trimming day (see timeline in Fig. 2-2).
2. "Trim recovered" group (n=6)
    - Like rats of the incisor trim group, under general anaesthesia (see below), rats had the incisal edge of the right lower incisor trimmed every 2 days for a period of 1 week (see below and Fig. 2-3B) and ICMS mapping was carried out 1 week following the last trimming day (see timeline in Fig. 2-2).
3. "Extraction" group (n=8)
    - Under general anaesthesia (see below), rats had the right lower incisor extracted (see below and Figs. 2-3F, 2-3G). ICMS mapping was carried out 1 week following tooth extraction (see timeline in Fig. 2-2).
4. "Soft diet" (naïve) group (n=6)
    - ICMS mapping was carried out following 2-3 weeks of soft diet consumption (see timeline in Fig. 2-2).
    - Rats had neither general anaesthesia nor any dental treatment.
5. "Hard diet" group (n=6)
    - ICMS mapping was carried out following 2-3 weeks of hard diet consumption (see timeline in Fig. 2-2).
    - Rats had neither general anaesthesia nor any dental treatment.

    NOTE: the soft diet group served as a naïve control group in the trim (chapter 4) and extraction (chapter 5) experiments.

Sham control Groups

1. "Sham trim" group (n=7)
    - Under general anaesthesia (see below), rats had the right lower incisor slightly trimmed but without affecting the occlusal contacts every 2 days for a period

of 1 week (see below and Figs. 2-3C, 2-3D, 2-3E). ICMS mapping was carried out 1 day following the last incisor trim (see timeline in Fig. 2-2).

2. "Sham extraction" group (n=6)

- Under general anaesthesia (see below), rats underwent part of the extraction procedure (see below) but the tooth was not extracted. ICMS mapping was carried out 1 week following this procedure (see timeline in Fig. 2-2).

3. Dental manipulation techniques

All dental procedures were carried out under general anaesthesia (inhalation of 2% Halothane in oxygen at 1 L/min) while the animal was in a supine position.

3.1. Incisor trimming

Rat incisors normally erupt continuously at a rate of approximately 1-2 mm per day (Burnmurdoch, 1995; Risnes et al., 1995; Sessle, 1966). The rat incisors have a relatively diminutive pulpal innervation (Naftel et al., 1999) that terminate 2 mm away from the incisal edge (for reviews, see Hildebrand et al., 1995; Paxinos, 2004) and can be found neither in the lingual odontoblastic layer of the pulp nor within the dentinal tubules (Zhang et al., 1998; for review, see Paxinos, 2004). Therefore, in order to keep the right mandibular incisor in a state of reduced occlusal contacts and at the same time to avoid pulp exposure, 1 – 2 mm of its incisal edge were carefully trimmed every 2 days (in total 4 trims)(Fig. 2-3 A). The incisor was trimmed with a dental high-speed turbine (Kavo, Germany (model D-7950) and a diamond wheel bur rotating at a high speed (25,000 RPM) under copious saline irrigation. A dental bonding agent (OptiBond® Solo Plus™ Dual Cure, Kerr Manufacturing Co., California, USA) was applied to seal any exposed dentinal tubules.

3.2. Incisor sham-trim

A dental turbine (as mentioned above) with a carbide round bur trimmed the labial surface of the incisor at the gingival level. This procedure created a cavity within the dentin with a radius of ~1 mm (Figs. 2-3C, 2-3D, 2-3E). Thereafter, every other day,

for a period of 1 week (and in total 4 times), the dentin was re-exposed with the same procedure. Dental bonding agent (as above) was applied to seal any exposed dentinal tubules.

3.3. Incisor extraction

All dental extractions were supplemented with local anaesthesia with 0.1ml, 2% Lidocaine hydrochloride (Lignocaine, Lignospan standard®, Septodont, Ontario, Canada) injected to the labial and lingual sides of the right and left lower incisors. The extraction protocol was carried out under aseptic conditions (Elsubeihi and Heersche, 2004). A scalpel and a periosteal elevator were used to deflect a full thickness mucoperiosteal flap around the right lower incisor. The mental foramen was identified. Under copious saline irrigation, the same dental turbine as above with a size 2 round carbide bur rotating at a low speed was used to remove the labial bone around the right incisor. The incisor was luxated using a modified dental wax knife and pulled out of the socket with a mosquito forceps. The socket was then irrigated with saline and the wound closed with absorbable sutures (4-0 Coated Vicryl TF needle; Ethicon J-743D, Ethicon, INC. NJ, USA).

3.4. Incisor sham extraction

Rats received the same surgical treatment as described above for incisor extraction but the tooth was not actually extracted.

4. Intracortical microstimulation (ICMS)

Electrical stimulation of M1 by a fine microelectrode (ICMS) and electromyographic (EMG) recordings of the resulting evoked muscle activity are techniques that have been used for over a century to map the motor representations of the skeletal muscles (*i.e.*, "motor maps") (Adachi et al., 2007; Asanuma, 1989; Neafsey et al., 1986; Sanes and Donoghue, 2000; Sessle, 2006). ICMS was used in this study to map the motor representations of jaw-opening (left and right anterior digastric, LAD, RAD), jaw-closing (left and right masseter), tongue-protrusion (genioglossus, GG), vibrissae and neck muscles within the face-M1 of rats.

4.1. Rat preparation and anaesthesia

Rats were maintained throughout the ICMS experiments under general anaesthesia with ketamine HCL (Ketaset®, Ayerst Veterinary Laboratories, Ontario, Canada), 175 mg/kg i.m. for the initial preparations (right femoral vein cannulation and EMG electrode insertion); femoral i.v. infusion controlled with a pump (PHD 2000, model 11 Plus, Harvard Apparatus, Inc., Holliston, MA, USA) at 75 mg/kg/hr for the craniotomy and at 25-50 mg/kg/hr throughout the ICMS mapping. ICMS experiments typically lasted ~8 - 12 hrs. During this time, the infusion rate was continuously adjusted to keep the rat within a narrow 'window' of as light as possible anaesthetic depth to retain the excitability of the sensorimotor cortex. This anaesthetic state allowed spontaneous jaw and tongue muscle twitches at a rate of 4-5 twitches per minute and a noxious pinch applied to the hind paw could not induce a flexion withdrawal response. In addition, local anaesthesia [0.1 ml, 2% Lidocaine in 1:100,000 epinephrine (Lignocaine, Lignospan standard®, Septodont, Ontario, Canada) and 0.1 ml 4% Articaine in 1:200,000 epinephrine (Bupivacaine, Septanest N, Septodont, Ontario, Canada)] was applied to each surgical site (*i.e.,* femoral, submandibular and scalp areas).

A heating blanket (Model 73A, YSI, Ohio, USA) regulated by feedback from a rectal thermometer maintained a physiological body temperature of 37 - 38 °C. The EKG ranged from 330 – 350 beats/min. The fur covering the operated areas (right hind limb, neck, head, and submandibular region) was shaved with a fur trimmer.

4.2. Insertion of electromyographic (EMG) electrodes

Pairs of EMG electrodes [40-gauge, single-stranded, Teflon-insulated stainless-steel wires with 0.5-1 mm exposed tip, (Cooner wire, Chatsworth, CA, USA)] were inserted into each of the LAD or RAD and into each of the left or right masseter (jaw-closing) muscles (Fig. 2-4A). A single EMG electrode was inserted into each of the left or right GG, vibrissal pads and neck muscles. To confirm the adequate placement of EMG electrodes and to ensure that the muscle preparation had not deteriorated during the experiment, tetanic stimulation (12 × 0.2 ms pulses, 333 Hz, 200 μA) of each muscle was applied at the end of electrode insertion and at the termination of the mapping

experiments to observe evoked muscle twitch responses, *e.g.* jaw-closing in response to stimulating the masseter muscle, jaw-opening when the AD muscle was stimulated, or tongue-protrusion when the GG muscle was stimulated. Any ICMS-evoked jaw, tongue, vibrissal, neck or forelimb movement was also monitored visually and recorded in a log book.

4.3. Craniotomy

The rat was placed in a stereotaxic apparatus (model 1340, David Kopf, Tujunga, CA, USA) with 2 ear rods and an anterior mouthpiece to fix its head at a standardized position. Incision exposed the scalp. Bregma (Fig. 2-4B) was considered as the 0 reference point from which the coordinates of the stimulating sites were determined (Neafsey et al., 1986; Paxinos and Watson, 1998). A dental low-speed hand piece (Kavo, Germany (model D-7950) with intermittent water cooling exposed the left and right hemispheres, at anteroposterior (AP) coordinates 0.0 to 5.0 mm rostral to Bregma, and at the mediolateral (ML) coordinates of 1.0 mm from the sagital suture (to avoid bleeding from sagittal sinus) and as far lateral as possible (~ 5.5 mm) without damaging the orbital and temporal bones and their associated soft tissues and muscles (Fig. 2-4B). The dura was kept intact and covered with warm mineral oil (37°C).

4.4. Systematic cortical mapping

Custom-made glass-insulated tungsten microelectrodes (1- 5 MΩ, 10-20 µm exposed tip, 125 µm shank diameter and 300µm outer diameter) (Alpha-Omega Engineering, Nazareth, Israel) were used for ICMS (Adachi et al., 2007; Cicirata et al., 1986a; Donoghue and Wise, 1982). The reference electrode was a stainless-steel rod placed under the skin of the neck. A micromanipulator - micropositioner (Kavo, Germany (model D-7950) controlled the horizontal (AP, ML) and vertical position of the stimulating microelectrode relative to midline and cortical surface respectively. Based on previous studies of the approximate location of the face-M1 (Adachi et al., 2007; Lee et al., 2006; Neafsey et al., 1986; Sapienza et al., 1981), and the estimated extent of ICMS current spread of less than 0.5 mm at 60 µA ICMS intensity (Cheney, 2002; Neafsey et

al., 1986; Ranck, 1975), mapping extended from 2.5 to 4.0 mm anterior to Bregma (*i.e.*, AP 2.5, 3.0, 3.5 and 4.0) and 1.5 to 5.5 mm lateral to midline with horizontal spatial resolution of 0.5 mm (Fig. 2-4B). At each AP plane, a series of mediolateral microelectrode penetrations was made until no more jaw or tongue ICMS-evoked EMG activity could be detected, or until the most lateral or medial borders of the exposed brain area had been reached. In most cases, mapping started at AP 3.0, followed by mapping at AP 3.5 and AP 4.0 followed by mapping at AP 2.5.

If ICMS at AP 4.0 evoked EMG activity in jaw or tongue muscles, then AP 4.5 was mapped as well. In all rats either AP 4.0 or AP 4.5 showed no evoked-EMG activity in either AD or GG muscles. If ICMS of AP 4.0 did not evoke EMG activity, then mapping included planes AP 2.5 and AP 2.0. The reasons behind this sequence were: 1. to map the anterior border of face-M1 from which ICMS could not evoke EMG activity in jaw or tongue muscles. 2. To make sure we have at least 4 planes with positive ICMS-evoked EMG activity in AD and GG. 2. AP 2.5 ML 3.0 was always a positive penetration site from which ICMS evoked jaw or tongue EMG responses (*i.e.*, "positive ICMS penetration", see below); obtaining a positive EMG response following negative responses ensures that the experimental conditions had not deteriorated. If mapping sites at AP 2.5 were also negative, the whole mapping experiment was excluded. Penetrations that would penetrate major blood vessels on the dural surface required larger or smaller steps to be avoided or alternatively they were skipped from the systematic mapping sequence and mapped at the end of the mapping session.

Many studies mapping the motor representations of the limbs and vibrissae within the sensorimotor cortex have been using microelectrode penetrations that are perpendicular to the cortical surface (Gioanni and Lamarche, 1985; Hall and Lindholm, 1974; Huntley, 1997b; Neafsey et al., 1986; Sapienza et al., 1981) and parallel to the assumed cortical motor columns (Mountcastle, 1997) and ICMS has been applied at one depth within layer V (Franchi, 2001; Gioanni and Lamarche, 1985; Huntley, 1997b; Kleim et al., 2002a; Kleim et al., 1998; Miyashita et al., 1994; Neafsey et al., 1986). However, perpendicular penetrations for mapping the laterally positioned jaw and tongue motor representations within M1 would have required damaging the orbital and temporal

bones and their associated soft tissues and muscles. In addition, it has been shown that ICMS can evoke muscle activity from the entire depth of layers V and VI as well as layer III (Aldes, 1988; Asanuma, 1989; Asanuma et al., 1976; Asanuma and Rosen, 1972; McGuinness et al., 1980; Neafsey et al., 1986; Sapienza et al., 1981). Furthermore, while threshold ICMS intensity can excites the most excitable neurons located close to the tip of the stimulating microelectrode, suprathreshold ICMS intensities may result "anodal surround block" whereby an excitable axon close to the stimulating microelectrode is not stimulated although it could have been stimulated by a smaller ICMS intensity or if it was further away from the stimulating microelectrode. At the same time it is possible that a small diameter axon that is very close to the microelectrode will be stimulates (Jankowska et al., 1998; Ranck, 1975). Therefore, to reveal the entire extent of effective jaw and tongue motor representations, ICMS was applied at multiple depths throughout the mapped area (Asanuma, 1989; Brecht et al., 2004; Neafsey et al., 1986). Such mapping excluded the necessity for perpendicular penetrations (see Fig. 2-5) and therefore, vertical microelectrode penetrations were used and within each penetration, ICMS was applied at 0.2 mm steps of penetration depths until no ICMS-evoked EMG response could be observed.

4.5. Stimulation parameters

Stimulation parameters were similar to those used in previous ICMS studies in rats (Franchi, 2001; Franchi, 2002; Huntley, 1997b; Kleim et al., 1998; Neafsey et al., 1986). Sequencer and script codes in Spike2 and CED-1401 Plus System (Cambridge Scientific Instruments, UK) were set-up to generate monophasic, cathodal, constant-current stimulation trains of 333 Hz [*i.e.*, 33.2 msec trains comprised of 12 pulses of short (0.2 msec) duration, with 2.8 msec inter-pulses intervals] (Appendix 2-1) delivered through a stimulus isolator (Model A365, World Precision Instruments, Stevenage, UK) to the stimulating monopolar microelectrode.

Previous studies have shown that tetanic trains produce a muscle response noticeable in an EMG record (Asanuma, 1989; Neafsey et al., 1986; Sanes and Donoghue, 2000). The use of biphasic pulses consisting of a negative followed by a

positive phase or discharging the microelectrode electronically after each monophasic pulse could have balanced the charge delivered to the tissue and limit the neural damage that might have occurred due to accumulation of electric charges and microelectrode polarization (Asanuma et al., 1976; Asanuma and Arnold, 1975). In the present study, microelectrode polarization and the subsequent neuronal tissue damage were minimized by using short monophasic ICMS trains with pulse duration of 0.2 msec that is shorter than the chronaxie reported for pyramidal neurons (Ranck, 1975; Stoney et al., 1968a) and ICMS intensities of ≤ 60μA. A cathodal stimulation, as compared with anodal stimulation may further minimize damage to the microelectrode tip (as a result of positive ions removal). Moreover, deep cortical cells (*i.e.*, pyramidal cells) can be excited more effectively by cathodal pulses than by anodal pulses (Asanuma and Sakata, 1967; Ranck, 1975; Rattay, 1999; Stoney et al., 1968a).

The effective extent of current spread by diffusion to directly activate nearby pyramidal neurons has been estimated to be less than 0.5 mm at ICMS intensity of ≤ 60 μA (Cheney, 2002; Neafsey et al., 1986; Ranck, 1975; Stoney et al., 1968a) which was our horizontal mapping resolution. Therefore, at each ICMS site, 5 trains were delivered at 1 Hz and at a suprathreshold ICMS intensity of 60 μA to test if ICMS could effectively evoke a jaw and/ or tongue muscle response (Appendix 2-1). If an EMG response was observed, a second series of 5 trains at 60 μA was delivered to confirm the response, and then 5 trains were delivered at 20 μA and 40 μA and again at 60 μA (Adachi et al., 2007; Lee et al., 2006). Threshold ICMS can evoke a short-latency muscle activity in a specific muscle (Asanuma, 1989; Neafsey et al., 1986; Sapienza et al., 1981; Tehovnik et al., 2006); however, determining the threshold at each stimulation site is very time consuming. In order to allow for extensive mapping at stimulation intensity close to threshold, we limited the lower ICMS intensities to 20 and 40 μA that were within ± 10 μA of the AD and GG mean threshold of 30 μA revealed in preliminary experiments (Lee et al., 2006).

For histological verification of the ICMS sites, electrolytic lesions were placed by passing cathodal DC (10 μA for 10 sec) at the bottom of every positive ICMS penetration (Fig. 2-5). This is consistent with previous ICMS studies (Adachi et al., 2007;

Iriki et al., 1991; Toda and Taoka, 2004) although many other studies have placed electrolytic lesions only at a few selected penetrations at the end of the mapping to minimize damaging neurons within the gray matter during the mapping session (Asanuma and Pavlides, 1997; Butovas and Schwarz, 2003; Tandon et al., 2008).

5. Data acquisition and analysis

EMG activity was amplified using a gain of x1000 and filtered (bandpass 100~1 kHz) by an AC amplifier (A-M system, Washington, USA, model 1700). The signals were digitized at 5 kHz by an A/D converter (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK) which was operated by a personal computer. A customized software written in Spike2 script (CED, Cambridge Electronic Design, Cambridge, UK) and LabView (National Instruments, Austin, TX, USA) were used to analyze data files off-line (Adachi et al., 2007). Data for each muscle was analyzed separately. For each muscle (LAD, RAD, GG, masseter, vibrissae, neck), at each ICMS site, the 5 ICMS-evoked EMG waveforms, corresponding to the series of 5 stimulation trains, were rectified, averaged and smoothed by a 4-msec moving-average window (Baker and Lemon, 1995; Myers et al., 2003; for review, see Cheney, 2002) (Figs. 2-4C, 2-4D).

5.1. ICMS-evoked EMG activity and positive ICMS sites

Consistent with previous studies (Adachi et al., 2007; Lee et al., 2006), 2 criteria were set in the computer algorithm for automatic analysis and identification of EMG signals as "positive ICMS-evoked responses" (Fig. 2-4D): **1**. The rectified, averaged and smoothed EMG responses had a peak activity exceeding the mean value of the initial 10 msec signal plus 2 standard deviations (SDs) (95% confidence interval) and with an onset latency of evoked EMG response of ≤ 40ms. **2.** At least 3 of the 5 ICMS-evoked responses met the first criterion (Hodges and Bui, 1996). Since movement artifact or electrocardiograms (EKG) may obscure the automatic computer-identified onset of EMG activity, each of the computer-identified positive EMG responses was checked visually to ensure a correct and meaningful identification of the positive ICMS response and its computed onset latency (Hodges and Hui, 1996).

Any cortical site from which ICMS could evoke positive EMG response/s from LAD, RAD and/ or GG (as well as masseter, vibrissae and neck) muscles was counted as a "positive ICMS site" and the values of the onset latencies of the evoked responses were noted. In all rats, ICMS could evoke positive EMG responses from 1 of these muscles or from a combination of 2 or 3 of these muscles. For each study group, the mean number of positive ICMS sites was calculated for each muscle or combination of muscles (see Table 2-2) within the left or right face-M1 and in the diet and extraction experiments also for face-S1.

5.2. ICMS-evoked EMG activity and positive ICMS penetrations

For each muscle (LAD, RAD, GG, masseter, vibrissae, neck), an ICMS penetration was defined and counted as a positive ICMS penetration if it contained at least 1 positive ICMS site. In addition, within each penetration, the AP, ML and depth positions of LAD, RAD and/ or GG positive ICMS (60 µA) sites with the shortest onset latency of evoked-response were noted and the mean position and mean latency of these sites were calculated.

In the extraction study (Chapter 5), to illustrate the ML frequency distribution of the positive ICMS penetrations, the mean numbers of positive ICMS (60µA) penetrations within each ML penetration were grouped together irrespective of the AP coordinates and plotted as a function of the ML position from the midline.

5.3. Motor maps and centre of gravity

Cortical maps were created by stimulating different sites within face-M1 at an ICMS intensity of 20, 40 or 60 µA and counting the number of sites from which ICMS evoked EMG activity in LAD, RAD and GG. These sites were plotted on histological coronal sections of the rats' brains or templates from Swanson rat brain atlas (Swanson 2004), corresponding to planes AP 2.5 - 4.0 mm anterior to Bregma, to outline the extent of the muscles' motor representations within face-M1. In these maps, at many of the sites, ICMS evoked EMG activity in more than 1 muscle (*i.e.*, "overlapping motor

representations"). Another variation of motor maps was created by including, at each ICMS site, only the muscle with the shortest onset latency of evoked-response.

The centre of gravity, which defines the mean 3-dimentional centre position of the motor representation (Ridding et al., 2000; Wassermann et al., 1992), was calculated for each of the LAD, RAD and GG muscles by taking into account the mean number of positive ICMS sites obtained at each ML, AP and depth coordinates, thereby providing the position of the motor map weighted relative to the extent of the motor representation (modified from Ridding et al., 2000; Wassermann et al., 1992). The following equation was used: $X=\Sigma aiXi/\Sigma ai$, where ai is the number of positive ICMS sites at a cortical ML coordinate Xi. In a similar way the depth coordinate Yi and the AP coordinate Zi were determined. In a similar way, the centre of gravity weighted relative to the shortest onset latency within each penetration was calculated where ai is the mean onset latency at Xi, Yi and Zi coordinates.

6. Histological procedures and verification of ICMS sites

At the termination of each ICMS mapping experiment, the rat was euthanized by a lethal overdose of ketamine HCL and fixed by a trans-cardial perfusion of normal saline followed by 10 % buffered formalin (Fisher Scientific, New Jersey, USA). The brain was removed and kept in 10 % buffered formalin. A vibratome (Model 3000, TPI, Missouri, USA) was used to slice 100 μm thick coronal sections of the relevant parts of the brain. Alternate sections were stained with Nissl stain (Cresyl violet) or Haematoxylin-Eosin stain. Nissl stain is a basic dye that stains nucleic acids of the neurons' nuclei, and therefore was used here to demonstrate the cellular architecture of the mapped cortex. In the Haematoxylin and Eosin method, haematoxylin labels nuclei in blue while eosin labels the cytoplasm in pink.

A flat-bed scanner digitized all histological sections into computer images at a resolution of 1200 dots per inch (dpi). For measurements' calibration a ruler was scanned with each slide. The public domain Image-J software program (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2006) was used for measurements of the vertical and ML

position of the electrolytic lesions from the cortical surface and the midline respectively. This position was compared to the position recorded by the micropositioner-micromanipulator and corrected accordingly. Consequently, the corrected position of all positive ICMS penetrations and sites was reconstructed on the histological sections to verify that they were within the gray matter of the cortex. Positive ICMS sites located outside the gray area were excluded from the data analysis. Paxinos atlas (Paxinos and Watson, 1998) and Swanson atlas (Swanson, 2004) helped to identify anatomical landmarks and the criteria described by Donoghue and Wise (Donoghue and Wise, 1982) were used to determine the boundaries between the granular and agranular cortex, where the granular cortex (*i.e.*, S1) has a granulated layer IV located in the primary somatosensory cortex (S1) and the agranular cortex lack layer IV and is located more medial and rostral to S1. Within the agranular cortex, the primary motor cortex (M1) lies more laterally and the secondary motor cortex (M2) lies more medially (Fig. 2-5). In the incisor trimming experiments, positive ICMS sites within S1 were excluded from the data analysis. In the diet and extraction experiments, the positive ICMS sites located in S1 were analyzed separately from those of M1.

Although most ICMS studies use Bregma as the AP reference point, individual animal variability in brain size and the alignment between the skull and the brain structures (Paxinos and Watson, 1998; Xiao, 2007) may result in individual differences among the rats in the actual anatomical position of the ICMS planes/ sites. Therefore, only results from those rats where the coronal planes fell within ± 0.5 mm of AP 2.5, 3.0, 3.5 or 4.0 were included in the data analysis.

7. Statistical Analyses

Statistical analyses used The SAS System v.9.1.3 (SAS Institute, Cary, NC, USA). The first series of data analysis is concerned with the effects of the dental manipulations (trimming or extraction) on the dependent variables (number of positive ICMS penetrations and ICMS sites, onset latency, ML position of the ICMS penetrations and the centre of gravity of the ICMS sites) (Appendix 2-2). The mean values of the dependent variables were broken out by muscle (or combination of muscles), stimulation

intensity and cortical side, and compared across treatment groups through a series of ANOVAs (univariate analysis) for comparing 3 groups and independent *t-tests* when only 2 groups were being compared (*e.g.* in the hard vs soft diet study or after pooling the naïve and sham groups into 1 control group). The ANOVA results were followed by *post-hoc* Bonferroni-adjusted pairwise comparisons as appropriate. In the second set of data analysis, for each muscle (or combination of muscles), mixed model repeated-measures ANOVA (MMRM) analyses (multivariate analyses) were used followed by *post-hoc* Bonferroni-adjusted pairwise comparisons as appropriate to determine whether any of the independent effects (study group, cortical side, and stimulation intensity) or any combination of these effects significantly affected the above-mentioned dependent variables. When the results of the multivariate analyses were not significant, only the univariate analyses were reported. In addition, the within-group comparison of ipsilateral vs. contralateral onset latency for LAD, RAD and GG positive ICMS sites was carried out by the paired *t-test*. Series of paired *t-tests* and MMRM ANOVA were used to assess within and across groups differences in mean daily gain of body weight. In all analyses a probability level of $p < 0.05$ was considered statistically significant.

Daily gain of body weight (g/day)
(Mean ± SEM)

Group	Baseline	Mid-Study	Follow-up	Statistical Significance
Extraction	9.85 ± 1.62	6.60 ± 1.09	n/a	p<0.0001
Sham extraction	9.60 ± 1.33	4.98 ± 0.56	n/a	p=0.0009
Trim	9.79 ± 0.60	6.00 ± 1.78	n/a	p=0.0289
Sham trim	9.77 ± 1.34	6.34 ± 1.23	n/a	p=0.0009
Trim recover	9.66 ± 1.91	7.44 ± 2.37	7.36 ± 2..12	p=0.1839
Soft diet	7.78 ± 1.48	n/a	n/a	n/a
Hard diet	8.99 ± 1.29	n/a	n/a	n/a

Table 2-1. This table provides a summary of the daily gain of body weight (g/day) (Mean ± SD) for animals within each study group during the periods before dental manipulation, during the week of dental trimming, or following dental extraction and in the trim recovered group, also during the 1 week period following the dental manipulation. There were no significant differences across the groups in the rate of weight gain during the pre-dental manipulation period (MMRM ANOVA: F=1.66, df=6,37, p=0.16). Within each of the trim and extraction groups there was a significant difference between the rate of weight gain before and after the dental manipulation (paired *t-test*: p<0.05). Within the trim recovered group, there were no significant differences across the rates of before trimming, during trimming or during recovery period (MMRM ANOVA: p=0.18). There was also no significant difference across the trim, trim recovered and extraction groups in the rate of weight gain during the period following the dental manipulation (MMRM ANOVA: F=2.27, df=4,28, p=0.087).

Definitions of the muscles and groups of muscles

Symbol	Activated muscle
LAD only	ICMS sites that could evoke EMG response only in LAD
RAD only	ICMS sites that could evoke EMG response only in RAD
LAD and RAD	ICMS sites that could evoke EMG response in both LAD and RAD *(i.e.* LAD and RAD overlapping representation sites)
GG only	ICMS sites that could evoke EMG response in GG
GG and LAD	ICMS sites that could evoke EMG response in both GG and LAD
GG and RAD	ICMS sites that could evoke EMG response in both GG and RAD
GG, LAD and RAD	ICMS sites that could evoke EMG response in GG, LAD and RAD
LAD	ICMS sites that could evoke EMG response in LAD
RAD	ICMS sites that could evoke EMG response in RAD
AD	ICMS sites that could evoke EMG response in LAD and RAD
GG	ICMS sites that could evoke EMG response in GG
AD and GG	ICMS sites that could evoke EMG response in both AD and GG
AD and/ or GG	the total number of all ICMS sites that could evoked EMG response in either LAD, RAD, GG or any combination of these muscles

Table 2-2. Definitions of the muscles and groups of muscles categories used in the data analysis of the positive ICMS sites. (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus

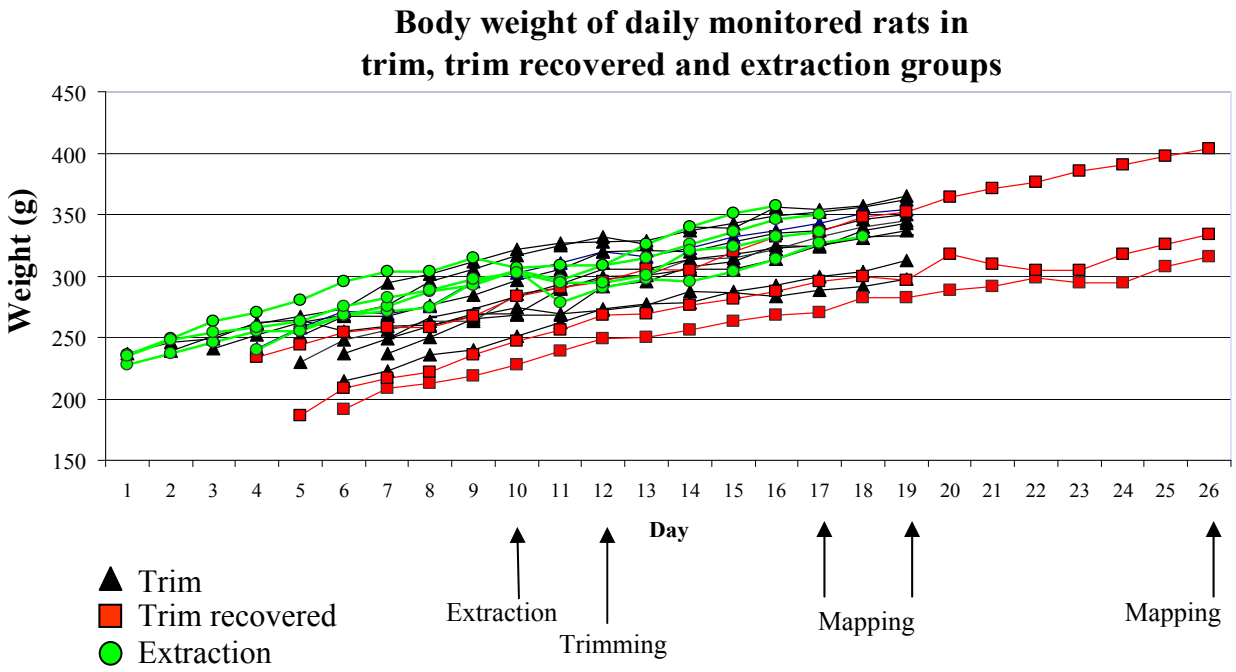


Fig. 2-1. This graph illustrates the daily gain of body weight (g) of individual rats from the day of arrival to the vivarium and up to mapping day, in 10 rats from the trim group (black triangles), 3 rats from the Trim recovered group (red squares) and 4 rats from the extraction group (green circles). Note a steady growth rate with a small decrease in growth rate following dental manipulation (extraction, trimming).

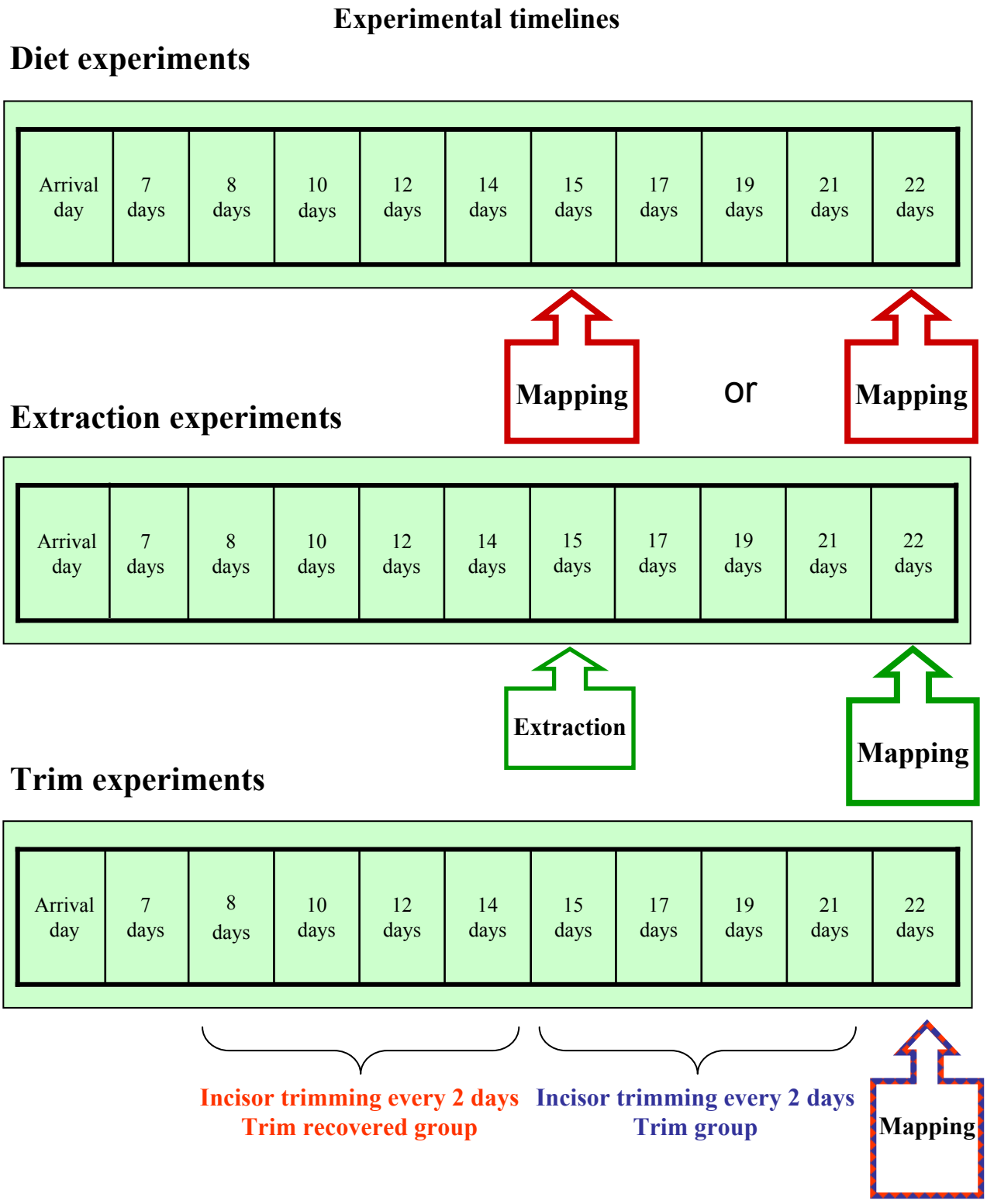


Fig. 2-2. Experiments timelines. To keep similar experimental time intervals, in all study groups, the time interval between dental manipulation and cortical mapping was set at 1 week All mappings were done 3 weeks following rat arrival to the vivarium except for the diet group where in 3 rats mapping was done 2 weeks after arrival

Photographs illustrating clinical procedures

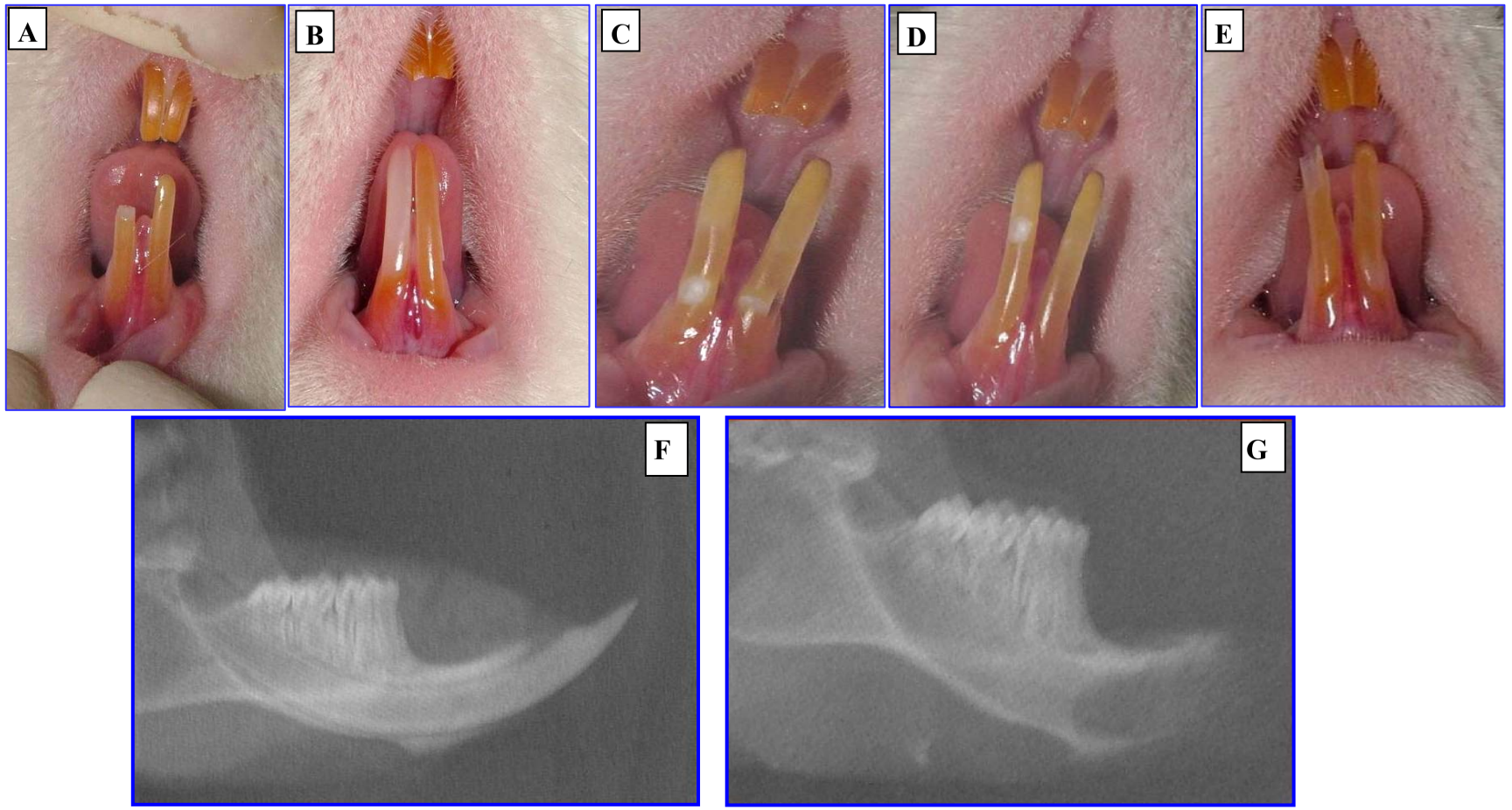


Fig.2-3: Photographs of: A. Trimmed right lower incisor. Note: No overeruption of opposing unimpeded incisor. B. Right lower incisor 1 week following last trim. C-E. Sham trimmed right lower incisor (C. 1st trim, D. 3rd trim, E. mapping day). F. x-ray of mandibular incisor. Note its extension all along the mandibular border. G. x-ray following incisor extraction

The ICMS mapping procedure

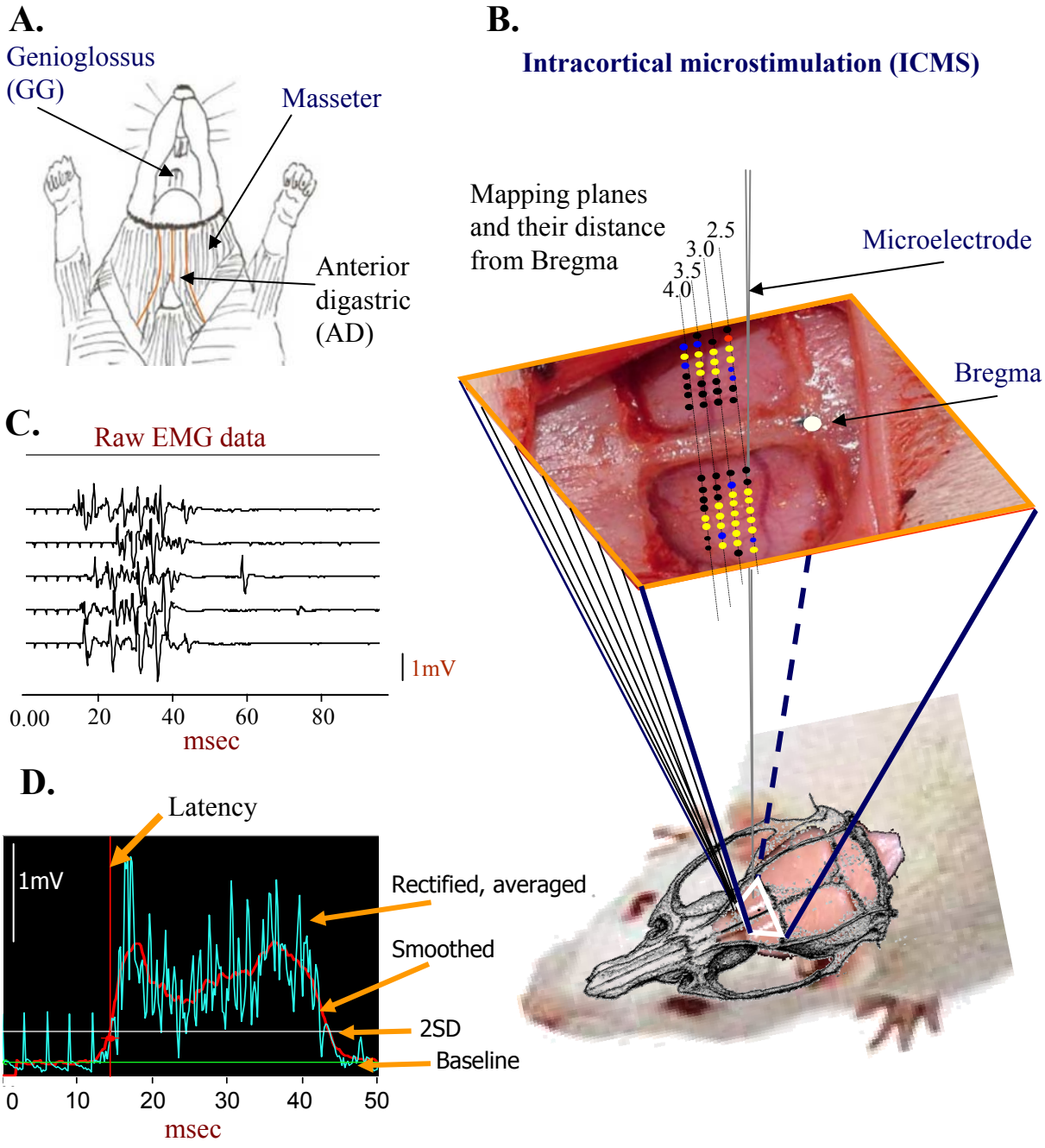


Fig. 2-4. **A.** Illustration of GG, AD and masseter muscles where EMG electrodes were inserted. **B.** The intracortical microstimulation technique (ICMS). Cortical mapping area at AP 2.5, 3.0, 3.5 and 4.0 anterior to Bregma. Each dot represents a penetration site from which ICMS evoked EMG response in AD, GG or AD+GG simultaneously (overlap). Black dots are no response sites. **C.** Example of a set of 5 ICMS-evoked EMG responses recorded from AD of a single rat. **D.** Example of data from **C** after being rectified, averaged and smoothed by a 4ms-moving average window. Any ICMS site from which at least 3 of the 5 EMG evoked responses had an amplitude larger than the baseline (green line) by 2 SD (white line) was defined as a "positive ICMS site" and the onset latency (red line) for evoking these responses was noted.

Cortical cytoarchitecture (AP 3.0)

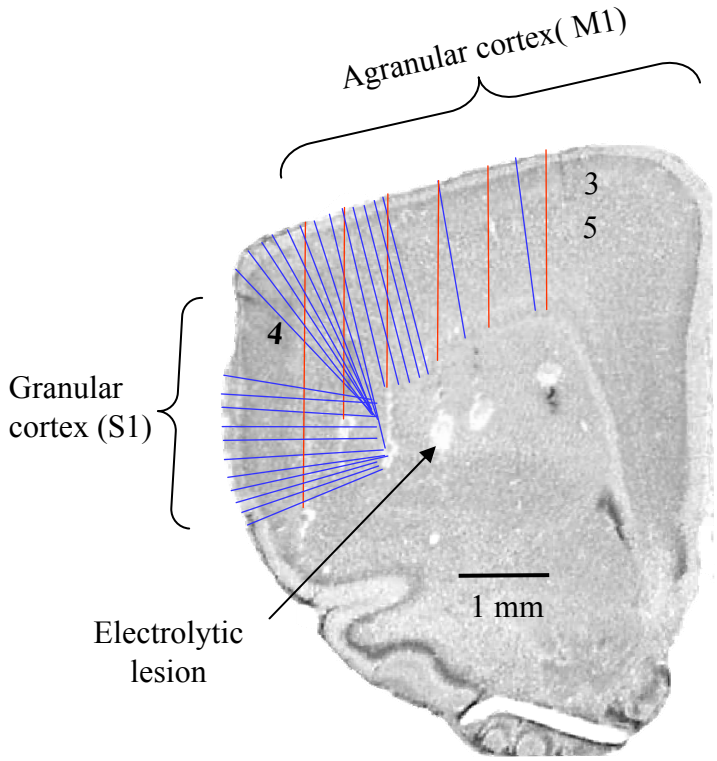


Fig. 2-5. Histological coronal section illustrating the locations of the primary motor cortex (M1) within the agranular cortex and the primary somatosensory cortex (S1) within the granular cortex at AP 3.0. Numbers illustrate the cytoarchitectonic layers. Red lines – vertical penetrations; Blue lines – penetrations perpendicular to cortical surface.

CHAPTER 3

JAW AND TONGUE MOTOR REPRESENTATIONS WITHIN FACE PRIMARY MOTOR CORTEX OF ADULT RATS: EFFECT OF DIET CONSISTENCY

1. Abstract

Changes in diet consistency are associated with changes in the pattern of mastication and alterations in peripheral sensory inputs. Although changes in motor representations within the primary motor cortex (face-M1) have been documented following alterations in sensory inputs and motor functions, it is unclear whether changes in diet consistency are associated with changes in face-M1 of rats.

Objectives: to use intracortical microstimulation (ICMS) and recordings of evoked muscle electromyographic (EMG) activity to: 1. determine the ICMS-defined motor representations of the right and left anterior digastric (RAD, LAD) and genioglossus (GG) muscles within the rat face-M1; 2. test if changes occur in the ICMS-defined motor representations of RAD, LAD and GG within face-M1 following a change in diet consistency.

Methods: Adult male Sprague-Dawley rats (200-250gr) were divided into 2 groups. The first group (n=6) was fed a hard (chow) diet ("hard diet" group). The second group (n=6) was fed a soft diet (mashed chow) ("soft diet" group). After 2-3 weeks on soft or hard diet, the rats were anaesthetised (ketamine-HCl) and ICMS (5x33.2ms train, 12x0.2ms pulses, 333Hz; ≤60μA) was applied within left and right face-M1 in a systematic series of microelectrode penetrations extending from 2.5 to 4.0 mm anterior and 1.5 to 5.5 mm lateral to Bregma, with a spatial resolution of 0.5 mm horizontally; ICMS was applied every 0.2 mm of microelectrode penetration depth. Histologically confirmed sites for which ICMS could evoke EMG activity in GG, RAD or LAD were considered to be "positive ICMS sites". Univariate analysis used a series of *t-test*s and multivariate analysis used a series of ANOVAs followed by *post-hoc* Bonferroni-adjusted pairwise comparisons where necessary, $p<0.05$.

Results: A change in diet consistency was not associated with significant changes in the number of positive ICMS sites of LAD, RAD or GG within either the left or the right face-M1. Consistent with previous findings of contralateral cortical predominance, LAD had a significantly larger number of positive ICMS sites within the right face-M1 (soft diet group: Left: 18.3 ± 4.4 (mean ± SEM), Right: 25.5 ± 3.7; hard diet group: Left: 19.2 ± 1.9, Right: 25.3 ± 1.8) and similarly RAD had a significantly larger number of positive

ICMS sites within the left face-M1 (soft diet group: Left: 23.3 ± 5.2, Right: 9.8 ± 3.2; hard diet group: Left: 24.7 ± 1.6, Right: 8.7 ± 2.2).

Conclusion: A change in diet consistency for a period of 2-3 weeks was not associated with significant changes in the AD and GG motor representations as revealed by ICMS within the rat face-M1.

2. Introduction

Electrophysiological studies that have employed intracortical microstimulation (ICMS), single neuron recordings or reversible cold block or lesioning techniques in subprimates and primates have underscored the crucial role of the primary motor cortex representing the orofacial region (face-M1) in the generation and control of elemental (*e.g.* jaw-opening, tongue-protrusion) as well as semiautomatic (*e.g.* mastication, and whisking) orofacial motor functions (for reviews, see Ebner, 2005; Murray et al., 2001; Sessle et al., 2005; Sessle et al., 1999). ICMS of face-M1 can evoke orofacial movements from an extensive area within face-M1 (Adachi et al., 2007; Burish et al., 2008; Carvell et al., 1996; Clark and Luschei, 1974; Hoffman and Luschei, 1980; Luschei et al., 1971; Luschei and Goodwin, 1975; Neafsey et al., 1986; Yamamura et al., 2002; Yao et al., 2002a; for review, see Murray et al., 2001). Single neuron recordings suggest that the neurons within these ICMS-defined motor representations receive somatosensory inputs from the orofacial tissues (including the teeth, muscles and joints) that are involved in the ICMS-evoked movements (Farkas et al., 1999; Miyashita et al., 1994; Sapienza et al., 1981; Yao et al., 2002a; for review, see Murray et al., 2001). These sensory inputs project to face-M1 neurons either directly through the thalamus (Diamond et al., 1992; Hatanaka et al., 2005; Rausell and Jones, 1995; Simonyan and Jurgens, 2005), or indirectly through the primary somatosensory cortex (face-S1)(Chakrabarti and Alloway, 2006; Hoffer et al., 2005; Iyengar et al., 2007), and can provide somatosensory feedback that is important for the distinction between soft and hard diet consistency and thus further assist in the control of tongue and jaw movements during mastication (Dubner and Sessle, 1978; Jacobs, 1998; Johansson et al., 2006; Miles et al., 2004; Murray et al., 2001; Trulsson, 2007). Since soft and hard diets have different functional demands, changes in diet consistency may be associated with different biting and chewing loads and different patterns of mastication. Indeed, it has been documented in humans and in animals that changes in jaw and tongue muscle activities and patterns of movements during mastication may occur following a change in diet consistency (Inoue et al., 2004; Okayasu et al., 2003; Proschel and Hofmann, 1988; Thexton et al., 1980).

Earlier studies of the face-M1 representing the vibrissae have demonstrated that changes in orofacial somatosensation/ somatosensory inputs as well as altered orofacial motor behaviour can be associated with changes in the vibrissal motor representations within face-M1. For example, unilateral or bilateral trimmings of the vibrissae (Huntley, 1997b; Keller et al., 1996) result in changes in the exploratory motor behaviour by the rat of its environment and is associated with reorganization of the vibrissal motor representation and the adjacent limb motor representation within limb-M1, and injury to the infraorbital nerve supplying sensory innervation to the vibrissae results in decreased vibrissal-M1 excitability (Franchi, 2001; for review, see Ebner, 2005). However, limited published data are available of the neuroplastic capabilities of face-M1 representing the oral tissues following alterations in oral sensorimotor functions, although face-M1 neuroplastic changes have been documented in association with injury to the lingual nerve supplying sensory innervation to the tongue (Adachi et al., 2007) and with modifications to the occlusion induced by tooth trimming (Lee et al., 2006)(see Chapter 4) or dental extraction in rats (see Chapter 5).

These findings indicate that face-M1 has the neuroplastic capability to adapt to changes in sensory inputs or oral motor behaviour. This led to the following hypothesis: that a change in diet consistency may also affect the ICMS-defined motor representations within face-M1 of young-adult rats. To address this hypothesis, the objectives of the present study were to use ICMS and recordings of evoked muscle electromyographic (EMG) activity to: determine if changes occur in the ICMS-defined motor representations of the left and right anterior digastric (RAD, LAD) and genioglossus (GG) muscles within the rat face-M1 following a change in diet consistency.

3. Materials and Methods

All experimental procedures were approved by the University of Toronto Animal Care Committee, in accordance with the Canadian Council on Animal Care Guidelines and the regulations of the Ontario Animals for Research Act (R.S.O. 1990). One investigator (LA-A) carried out all experimental procedures and data analysis to ensure

consistency in the experimental procedures and in the blinded data analysis. Most of the procedures have been described in detail in chapter 2 and therefore, only a brief outline follows.

3.1 Animals and study groups

Experiments were performed on young adult male Sprague-Dawley rats (150-250g on arrival, 300-400g on day of cortical mapping). The rats were housed in individual cages under similar conditions. From the day of arrival to the vivarium, water and hard (chow) diet for the hard diet group (n=6) or soft diet (mashed chow) for the soft diet group (n=6) were available *ad libitum*. Animals were monitored on a daily basis to assess body weight and food consumption to ensure continuous and similar growth rate and normal behaviour (*e.g.* exploration, freezing, rearing, jaw motion).

3.2. ICMS and EMG recordings

The ICMS technique applied in the present study is detailed in chapter 2. ICMS mapping was carried out within the face-M1 and adjacent face-S1 of rats to define the motor representations of jaw and tongue muscles. Mapping was carried out 2-3 weeks after the rats' arrival at the vivarium. Rats were maintained throughout the ICMS experiments under a stable level of general anaesthesia with ketamine HCL (Ketaset®, Ayerst Veterinary Laboratories, Ontario, Canada). EMG electrodes (40-gauge, single-stranded, Teflon-insulated stainless-steel wires) were used to record EMG activity from the left or right AD (LAD, RAD) and masseter as well as GG, vibrissal and neck muscles. Systematic mapping extended from 2.5 to 4.0 mm rostral to Bregma (*i.e.*, anteroposterior (AP) planes 2.5, 3.0, 3.5 and 4.0) and 1.5 to 5.5 mm lateral to Bregma within the left and right face-M1 and with a horizontal spatial resolution of 0.5 mm. In each penetration site, ICMS was applied every 0.2 mm of microelectrode penetration depth. Five ICMS trains (at 333 Hz, 33.2 msec, 12 pulses of 0.2 msec, 2.8 msec inter-pulses intervals) were delivered at 1 Hz with suprathreshold ICMS intensity of 60 μA. If ICMS could effectively evoke GG and/ or AD EMG responses, then a series of 5 ICMS trains at 60, 40, 20 and 60 μA was delivered. Electrolytic lesions were placed for

subsequent histological confirmation of ICMS sites within the gray matter of the S1 or M1.

3.3. Data acquisition and analysis

The data acquisition and analysis are detailed in chapter 2. For each muscle, an ICMS site was defined and counted as a "positive ICMS site" if at least 3 out of the 5 ICMS (60 μA) trains could evoke an EMG response with an onset latency ≤ 40msec and a peak activity exceeding the mean value of the initial 10 msec of the EMG response plus 2 standard deviations (SDs). The onset latency was also noted for each muscle represented at each positive ICMS site. For each muscle, an ICMS penetration was defined and counted as a "positive ICMS penetration" if it had at least 1 positive ICMS site. In addition, within each penetration, the AP and ML positions of the positive ICMS (60 μA) penetrations as well as the shortest onset latency for each of the LAD, RAD and GG muscles were noted. Cortical motor maps were used to illustrate the representation areas of LAD, RAD and GG muscles within the sensorimotor cortex of rats from each of the study groups. Positive ICMS sites for LAD, RAD and GG were plotted on a corresponding histological coronal section (AP 2.5-4.0 mm anterior to Bregma) (Fig. 3-1A). Another variation of motor maps was created by including, at each ICMS (40 or 60 μA) site, only the muscle with the shortest onset latency (Fig. 3-1B). The ML and depth positions of the centre of gravity weighted relative to the extent of the motor representations were calculated for each AP plane within face-M1.

3.4. Statistical Analyses

As detailed in chapter 2, statistical differences between groups and the effects of the independent variables (study group, cortical side, and ICMS intensity) on the dependent variables (number of positive ICMS sites or penetrations, onset latency, ML position; and the centre of gravity) were determined using a series of *t-test*, and mixed model repeated-measures (MMRM) analyses (multivariate analyses) followed by *post-hoc* Bonferroni-adjusted pairwise comparisons as appropriate. In addition, paired *t-test*

was used for within-group comparisons of the onset latencies for evoking EMG responses. A probability level of $p<0.05$ was considered statistically significant.

4. Results

Rats from the soft diet and hard diet groups were monitored on a daily basis and showed normal behaviour and a similar daily gain of body weight (see Chapter 2).

4.1. General features of AD and GG motor representations

The present study mapped a total of 4 planes within the rat sensorimotor cortex located at approximately 2.5 - 4 mm anterior to Bregma (*i.e.*, AP 2.5, 3.0, 3.5, and 4.0). Cytoarchitectonic features were used to delineate the border between the granular (S1) and agranular (M1) cortex (Donoghue and Wise, 1982). In both groups, ICMS evoked EMG activity in AD and GG muscles from an extensive area within the left and right face-M1, and positive ICMS sites were also found in the adjacent face-S1 (Fig. 3-1). In both groups, there were only few ICMS sites (<1%) from which masseter muscle EMG activity could be evoked, and only occasionally were other EMG activities (*e.g.* vibrissae) evoked by ICMS within the face-M1 mapped area. Therefore, the ICMS data for the masseter, vibrissae and neck motor representations were not included in the general data analysis.

4.2. Effects of diet consistency

4.2.1. AD and GG representations within face-M1

Statistical analyses revealed no significant differences between the soft diet and hard diet groups in any of the study measures in either the left or the right face-M1 (Tables 3-1, 3-2, 3-3; Figs. 3-2, 3-4). ICMS evoked EMG activity in AD and GG muscles from an extensive area within the left and right face-M1 and most of the positive ICMS-sites were located along the entire depth of layers V and VI (Fig. 3-1). Multivariate analyses revealed significant cortical side effects (Table 3-1). Within face M1, both LAD and RAD had a significant contralateral predominance (Fig. 3-2B, 3-2E). In addition, for RAD, there was a significant interaction between cortical side and ICMS

intensity (Table 1). There were significantly more RAD positive ICMS sites within the left than within the right face-M1 at both 40 μA ($p=0.0064$) and 60 μA ($p<0.0001$) ICMS intensities.

At a large proportion (42%) of the positive ICMS sites, ICMS evoked EMG activity simultaneously in more than 1 muscle (*i.e.*, LAD/RAD, LAD/GG, RAD/GG), *i.e.*, overlapping representations (Fig. 3-2C, 3-2F). Nonetheless, despite this extensive overlapping of motor representations, usually (>95%) only 1 muscle had the shortest onset latency of EMG activity evoked from each ICMS site (see below and Fig. 3-1B).

Analysis of the face-M1 motor representations by the number of positive ICMS penetrations from which ICMS evoked EMG activity in LAD, RAD and/or GG also revealed no significant differences between the hard diet and soft diet groups (LAD: $F=1.11$, $df=1,10$, $p=0.3159$; RAD: $F=0.14$, $df=1,10$, $p=0.7208$; GG: $F=0.03$, $df=1,10$, $p=0.8615$) (Fig. 3-2G). There were no significant differences between the groups in the mean ML or AP position of the positive ICMS penetrations in either the left or the right face-M1 (ML: $F=0.01$, $df=1,10$, $p=0.9071$; AP: $F=0.02$, $df=1,10$, $p=0.5435$) (Table 3-2). There were also no significant differences between the groups in the AP, ML or depth positions of the centres of gravity in either the left or the right face-M1 (Table 3-4; Fig. 3-4).

In all rats, ICMS (60 μA) within face-M1 (and face-S1) evoked EMG activity in LAD, RAD and/ or GG with a wide range of onset latencies (8 - 40 msec); however, within each ICMS site, only 1 muscle had the shortest onset latency of evoked EMG activity (Fig. 3-1). Although there were no significant differences between the groups in the mean onset latencies of ICMS-evoked EMG activity for LAD, RAD or GG in either the left or the right face-M1 (MMRM: LAD: $F=0.64$, $df=1,10$, $p=0.4427$; RAD: $F=0.01$, $df=1,8$, $p=0.9073$; GG: $F=0.03$, $df=1,10$, $p=0.8762$), LAD and RAD, had a significantly shorter onset latency within the contralateral face-M1 (MMRM: LAD: $F=75.00$, $df=1,10$, $p<0.0001$; RAD: $F=91.13$, $df=1,8$, $p<0.0001$) (Table 3-3A, 3-3B).

4.2.2. AD and GG representations within face-S1

ICMS also evoked AD and GG EMG activity from within layer V of the face-S1 but from a much fewer number of sites than in face-M1 (Figs. 3-1, 3-3). Statistical analyses revealed no significant differences in the number of LAD, RAD or GG positive ICMS sites between the soft diet and hard diet groups in either the left or the right face-S1 (Fig. 3-3). LAD had a significantly larger number of positive ICMS sites within the right face-S1 and RAD had a larger, but not significant, number of positive ICMS sites within the left face-S1 (MMRM: LAD: $F=16.54$, $df=1,10$. $p=0.0023$; RAD: $F=3.96$, $df=1,10$, $p=0.0748$) (Fig. 3-3). As in face-M1, LAD and RAD had a significantly shorter onset latency within the contralateral face-S1 (MMRM: LAD: $F=65.44$, $df=1,10$, $p<0.0001$; RAD: $F=24.97$, $df=1,9$, $p=0.0007$) and RAD had a significantly shorter onset latency within the face-S1 than within the face-M1. In addition, GG had a significantly shorter onset latency within the left than within the right face-S1 (MMRM: $F=6.42$, $df=1,10$, $p=0.0296$).

5. Discussion

ICMS delivered to the face sensorimotor cortex in rats was used for mapping the motor representations of the AD and GG muscles at 2.5 to 4.0 mm anterior and 1.5 to 5.5 mm lateral to Bregma. The number of sites for which ICMS could evoke EMG activity in AD and GG outlined the extent of AD and GG motor representations within the face sensorimotor cortex. The main findings in both study groups were as follows: (i) LAD, RAD and GG had extensive motor representations within the left and right face-M1 but very limited representation of the masseter muscle; (ii) LAD and RAD had a significant contralateral predominance; (iii) there was a relatively high proportion of overlapping motor representations of AD and GG; and (iv) ICMS also evoked LAD, RAD and GG EMG activity when applied to face-S1. These findings are consistent with previous findings from our laboratory and others. In addition, we found that diet consistency had no effect on these ICMS-defined motor representations within the rat face-M1.

5.1. ICMS-defined motor representations within face-M1

In both study groups, AD and GG had extensive bilateral motor representations spanning the entire depth of layers V-VI, and there was a high proportion of overlapping representations of AD and GG muscles. These findings are in accord with previous ICMS studies in rats (Adachi et al., 2007; Donoghue and Wise, 1982; Gioanni and Lamarche, 1985; Lee et al., 2006; Neafsey et al., 1986; Sanderson et al., 1984) Similar features of motor representations were also reported in ICMS studies in monkeys (Burish et al., 2008; Huang et al., 1988; Murray et al., 2001; Murray and Sessle, 1992a; Murray and Sessle, 1992b; Sessle, 2006; Sessle et al., 2007; Sessle and Wiesendanger, 1982) and fMRI and TMS studies in humans (Aziz et al., 1996; Boudreau et al., 2007; Hamdy et al., 1996; Martin et al., 2004; Svensson et al., 2003b; Svensson et al., 2006). As noted in some of these earlier studies, overlapping of motor representations may reflect shared neural networks that, along with the extensive bilateral representations of AD and GG muscles, may be important for the dynamic coordination of orofacial movements involving several muscles and may also serve as the substrate underlying M1 neuroplastic mechanisms manifested as changes in the organization of motor representation within M1 (for reviews, see Sanes and Donoghue, 2000; Sanes and Schieber, 2001).

5.2. ICMS-defined motor representations within face-S1

Bilateral, short-latency LAD, RAD and GG EMG activities could be evoked by ICMS of face-S1. These findings confirm previous findings in awake and anaesthetised rats whereby jaw and/or tongue movements can be evoked by ICMS of face-S1 (Donoghue and Wise, 1982; Neafsey et al., 1986; Sapienza et al., 1981). In awake marmosets, ICMS of S1 can also evoke lips and jaw movements but not tongue movements (Burish et al., 2008). In monkeys (Huang et al., 1989a; Lin et al., 1998; Martin et al., 1999) as well as in rabbits (Lund et al., 1984) and cats (Hiraba et al., 2007), only long-train ICMS of face-S1 can evoke semiautomatic jaw and tongue movements such as rhythmical jaw movements. These findings of face-S1 motor outputs are supported by anatomical studies in rats and monkeys showing efferent projections from

S1 to brainstem motoneurons (Grinevich et al., 2005; Jones, 1976; Rathelot and Strick, 2006; Wise and Jones, 1977a; Zhang and Sasamoto, 1990) and suggest that face-S1 plays a role in the control of orofacial movements particularly of those requiring somatosensory feedback. This is supported by other studies showing that neurons in the ICMS-defined face-S1 demonstrate movement-related activity (Hiraba, 1999; Hiraba et al., 1997; Murray et al., 2001; Yamamoto et al., 1988), receive somatosensory inputs from orofacial tissues involved in the movement evoked by the ICMS of the same face-S1 neuronal sites (Hiraba, 2004; Huang et al., 1989b; Huang et al., 1988; Lin et al., 1998; Murray and Sessle, 1992a), and lesioning or cold block of face-S1 can impair oral motor functions (Castro, 1975; Hiraba, 1999; Lin et al., 1993; Yao et al., 2002b).

It could also be argued that the observed ICMS-evoked EMG activities within face-S1 were the result of a spread of stimulating currents from face-S1 to face-M1 either directly or indirectly through axon collaterals and horizontal interneurons (Greenshaw, 1998; Keller et al., 1990; Ranck, 1975; Schwark and Jones, 1989). However, this is unlikely since many of the positive ICMS sites within face S1 had short onset latency (8-10 msec) and the mean onset latencies for LAD, RAD and GG were comparable to those of face-M1, suggesting a relatively direct projection to motoneurons rather than projections through face-M1.

5.3. Effects of diet consistency

A soft diet has different functional demands than a hard diet and therefore it has been reported that different diet consistencies may have different patterns of jaw and tongue movements during mastication (Inoue et al., 2004; Kiliaridis et al.; Lund and Kolta, 2006b; Miehe et al.; Okayasu et al.; Proschel and Hofmann, 1988). In addition, orofacial mechanoreceptors provide peripheral feedback regarding food consistency, thereby contributing to the cortical control of mastication (Jacobs, 1998; Miles et al., 2004; Trulsson, 2007). Recent studies in rats and monkeys reveal that experimental manipulations that affect somatosensory inputs and alter oral motor behaviour may be associated with neuroplastic changes within face-M1 manifested as reorganization of

motor representations (Adachi et al., 2007; Huntley, 1997b; Keller et al., 1996; Svensson et al., 2003b; Svensson et al., 2006).

However, the present study found that a change in diet consistency was not associated, over the observation period, with any effect on the ICMS-defined motor representations of AD or GG muscles within face-M1. There were no changes in the extent of AD and GG motor representations as reflected in the number of AD and/ or GG sites, in any change in face-M1 excitability as reflected in changes in the onset latencies for evoking AD or GG EMG responses, in any topographical changes as reflected in no changes in the centre of gravity of the positive ICMS sites and in any changes in the AP-ML distribution of the positive ICMS penetrations. There are several possible explanations to our findings.

First, face-M1 has the capability to adapt to significant changes in orofacial sensorimotor experience in a task-dependent manner. In rats, unilateral transection of the infraorbital nerve supplying sensory innervation to the vibrissae results, 2-3 weeks later, in decreased excitability of face-M1 representing the vibrissae (Franchi, 2001) whereas unilateral transection of the lingual nerve supplying sensory innervation to the tongue is associated 1-2 weeks later with a significantly decreased GG representation and 3-4 weeks later with a significantly increased GG representation (Adachi et al., 2007). Training humans in a novel tongue-task, results in increased excitability of face-M1 representing the tongue and increased representation of the tongue muscle involved in the training task (Boudreau et al., 2007; Svensson et al., 2003b; Svensson et al., 2006). These latter findings are in accord with limb-M1 findings following limb motor training in rats (Kleim et al., 1996) and monkeys (Nudo et al., 1996). These studies on limb-M1 demonstrate that while skilled motor exercise result in reorganization of limb-M1, non-skilled motor exercise results in angiogenesis but does not alter motor representations within limb-M1 (Kleim et al., 2002b; Remple et al., 2001). Therefore, while we could not detect reorganization of AD and GG motor representations within face-M1 we cannot rule out the possibility that other changes had occurred, or it is possible that the changes in oral motor behaviour, if they had occurred, were part of the animal's repertoire rather than development of novel oral motor skills.

Second, it is possible that neuroplastic changes may have occurred as a result of the change in diet consistency in other motor centres that we did not explore, such as the cortical masticatory area/swallow cortex (CMA) or subcortical regions (for reviews, see Dubner and Sessle, 1978; Jean, 2001; Lund and Kolta, 2006a; Sessle et al., 2005) that we did not explore that play a more prominent role than face-M1 in the control of some oral movements; changes in these areas may have accounted for the reported effects of diet consistency on oral motor patterns (Inoue et al., 2004; Lund and Kolta, 2006a; Okayasu et al., 2003; Proschel and Hofmann, 1988). It has been well documented that rhythmic masticatory movements can be generated and controlled by a central pattern generator (CPG) in the brainstem (for reviews, see Dubner and Sessle, 1978; Lund and Dellow, 1971; Lund and Kolta, 2006b; Lund et al., 1999; Sawczuk and Mosier, 2001). Afferent inputs from jaw muscles and teeth can activate the CPG (Dellow and Lund, 1971; Lund et al., 1998; Nakamura and Katakura, 1995) and modulate the activity of brainstem motoneurons (Goldberg, 1971; Lavigne et al., 1987; Sessle, 1977; Sessle and Schmitt, 1972; Tolu et al., 1993; Tolu et al., 1994a; Tolu et al., 1994b) for review, see Lowe, 1980; Lund and Kolta, 2006b; Lund et al., 1999) that control jaw and tongue movements. It has been reported that changes in diet consistency can affect central mechanisms regulating the jaw and tongue muscles reflexes which in turn can contribute to the regulation of mastication (Lund and Dellow, 1973; Lund and Kolta, 2006b; Yamamura et al., 1998). In addition, the CPG can be activated by descending projections from the CMA (Nakamura and Katakura, 1995). Studies using cortical stimulation (Hatanaka et al., 2005; Huang et al., 1989b; Martin et al., 1999; Narita et al., 2002; Yamamura et al., 2002) or single neuron recordings (Martin et al., 1997; Yamamura et al., 2002; Yao et al., 2002a) or reversible cold block or lesioning techniques (Enomoto et al., 1987; Larson et al., 1980; Narita et al., 2002) have shown that the CMA/swallow cortex may also play a crucial role in the generation and control of rhythmic jaw movements and swallowing (for reviews, see Sessle et al., 1995; Sessle et al., 2005; Sessle et al., 1999). However, changes in sensory inputs induced by transection of the mandibular and maxillary nerves do not produce CMA changes ~2 weeks later (Masuda et al., 2002).

5.4. Study limitations

The present study used the ICMS technique to delineate motor representations within the mapped area. It has been demonstrated that threshold ICMS intensity can evoke different movements within a ~0.1 mm distance (Asanuma, 1989). However, we used suprathreshold ICMS intensities of 40-60μA that are estimated to have an effective radius of current spread of approximately 0.5 mm (Andersen et al., 1975; Cheney; Cheney and Fetz, 1985; Jankowska et al., 1975; Ranck, 1975; Stoney et al., 1968a) which was also our horizontal spatial resolution. Studies using these ICMS parameters have been able to show statistically significant and specific changes within face-M1 motor representations following tooth extraction, tooth trimming and lingual nerve transection (see Chapters 4, 5 and Adachi et al., 2007; Lee et al., 2006). Nonetheless, it is possible that in the present study our mapping technique failed to detect smaller changes in motor representations that may have occurred as a result of a change in diet consistency and these changes may have been detected by experiments utilizing approaches determining threshold ICMS intensities and a smaller mapping resolution (Asanuma, 1989; Nudo et al., 1992). Another limitation is that this study did not monitor possible changes in EMG or movement patterns to determine if indeed changes in diet consistency affected the rat's oral sensorimotor behaviour. This could be addressed in future studies.

6. Conclusions

Previous studies have shown that a change in diet consistency may affect jaw and tongue muscle activities and patterns of movements during mastication in rodents, but the present study has shown that a change in diet consistency for a period of 2-3 weeks is not associated with significant changes in the jaw and tongue motor representations as revealed by ICMS within the rat face-M1. These findings suggest that any alteration in oral sensorimotor behaviour associated with a change in diet consistency may not involve changes in face sensorimotor cortex, although we cannot rule out the possibility that other forms of neuroplastic changes may occur at cortical and/ or subcortical levels and contribute to changes in oral sensorimotor behaviour.

Face-M1 positive ICMS sites

Repeated-measures ANOVA results

Muscle	Predictor	F-statistic, df, Statistical significance
AD and/ or GG	Overall Model	chi-sq=0.37, df=1, p=0.54
	Study group	F=0.45, df=1,10, p=0.52
	Cortical side	F=1.15, df=1,10, p=0.31
	Intensity	F=28.40, df=1,10, p=0.0003
	Study group * Cortical side	F<0.01, df=1,10, p=1.0
	Study group * Intensity	F=0.20, df=1,10, p=0.66
	Cortical side * Intensity	F=0.05, df=1,11, p=0.83
GG	Overall Model	chi-sq=0.30, df=1, p=0.58
	Study group	F=4.13, df=1,10, p=0.07
	Cortical side	F=1.39, df=1,10, p=0.27
	Intensity	F=7.67, df=1,10, p=0.02
	Study group * Cortical side	F=0.06, df=1,10, p=0.82
	Study group * Intensity	F=0.41, df=1,10, p=0.54
	Cortical side * Intensity	F=0.01, df=1,11, p=0.93
AD	**Overall Model**	**chi-sq=11.07, df=1, p=0.0009**
	Study group	F=0.24, df=1,10, p=0.63
	Cortical side	F=0.13, df=1,10, p=0.73
	Intensity	**F=32.51, df=1,10, p=0.0002**
	Study group * Cortical side	F=0.29, df=1,10, p=0.60
	Study group * Intensity	F=0.10, df=1,10, p=0.76
	Cortical side * Intensity	F=0.03, df=1,11, p=0.88
LAD	**Overall Model**	**chi-sq=6.42, df=1, p=0.011**
	Study group	F=0.20, df=1,10, p=0.67
	Cortical side	**F=12.03, df=1,10, p=0.006**
	Intensity	**F=25.97, df=1,10, p=0.0005**
	Study group * Cortical side	F=0.01, df=1,10, p=0.91
	Study group * Intensity	F=0.16, df=1,10, p=0.70
	Cortical side * Intensity	F=0.91, df=1,11, p=0.36
RAD	**Overall Model**	**chi-sq=20.89, df=1, p<0.0001**
	Study group	F=0.05, df=1,10, p=0.82
	Cortical side	**F=110.23, df=1,10, p<0.0001**
	Intensity	**F=19.20, df=1,10, p=0.0014**
	Study group * Cortical side	F=0.76, df=1,10, p=0.40
	Study group * Intensity	F=0.09, df=1,10, p=0.76
	Cortical side * Intensity	**F=6.89, df=1,11, p=0.024**
AD and GG	**Overall Model**	**chi-sq=7.19, df=1, p=0.0073**
	Study group	F=1.01, df=1,10, p=0.34
	Cortical side	F=0.83, df=1,10, p=0.38
	Intensity	**F=14.36, df=1,10, p=0.0035**
	Study group * Cortical side	F=0.02, df=1,10, p=0.89
	Study group * Intensity	F=0.59, df=1,10, p=0.46
	Cortical side * Intensity	F=0.14, df=1,11, p=0.72
LAD and RAD	**Overall Model**	**chi-sq=7.73, df=1, p=0.0054**
	Study group	F=0.03, df=1,10, p=0.88
	Cortical side	**F=21.81, df=1,10, p=0.0009**
	Intensity	**F=12.06, df=1,10, p=0.006**
	Study group * Cortical side	F=0.09, df=1,10, p=0.77
	Study group * Intensity	F=0.15, df=1,10, p=0.71
	Cortical side * Intensity	F=1.68, df=1,11, p=0.22
LAD and GG	**Overall Model**	**chi-sq=9.65, df=1, p=0.0019**
	Study group	F=0.67, df=1,10, p=0.43
	Cortical side	F=0.04, df=1,10, p=0.84
	Intensity	**F=16.89, df=1,10, p=0.0021**
	Study group * Cortical side	F=0.48, df=1,10, p=0.51
	Study group * Intensity	F=0.69, df=1,10, p=0.42
	Cortical side * Intensity	F=0.10, df=1,11, p=0.75
RAD and GG	**Overall Model**	**chi-sq=14.50, df=1, p=0.0001**
	Study group	F=0.56, df=1,10, p=0.47
	Cortical side	**F=16.00, df=1,10, p=0.0025**
	Intensity	**F=11.91, df=1,10, p=0.0062**
	Study group * Cortical side	F=1.00, df=1,10, p=0.34
	Study group * Intensity	F=0.74, df=1,10, p=0.41
	Cortical side * Intensity	F=2.92, df=1,11, p=0.12

Table 3-1. Mixed model repeated-measures ANOVA, followed by *post-hoc* Bonferroni-adjusted pairwise comparisons where applicable, was used in order to determine whether study group, cortical side, stimulation intensity (40 vs 60μA), or any combination of these effects significantly affected the number of positive ICMS-sites. These tests were performed separately for each muscle and each combination of muscles. (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus).

Anteroposterior-mediolateral position of the positive ICMS (60 μA) **penetrations within face-M1**
(Mean ± SEM)

Cortical Side	Measure	Hard diet group	Soft diet group	Statistical significance
Left	AP	3.1 ± 0.1	3.1 ± 0.1	p=0.48
	ML	3.2 ± 0.1	3.3 ± 0.1	p=0.45
Right	AP	3.1 ± 0.1	3.2 ± 0.1	p=0.45
	ML	3.3 ± 0.1	3.4 ± 0.1	p=0.51

Table 3-2. The mean anteroposterior (AP)-mediolateral (ML) coordinates of the ICMS penetrations within face-M1. There were no significant differences between the groups in the ML or AP mean position of the positive ICMS penetrations in either left or right face-M1.

A. Onset latencies of ICMS (60μA)-evoked EMG activities in LAD, RAD and GG within face-M1 and face-S1
(Mean ± SEM)

	Cortical Side	Muscle	Soft diet Latency	Soft diet Number of sites	Hard diet Latency	Hard diet Number of sites
M1	Left	LAD	22.6 ± 2.1	18.3 ± 4.4	20.6 ± 1.1	19.2 ± 1.9
		RAD	**14.2 ± 1.0***	**23.3 ± 5.2***	**14.5 ± 1.1***	**24.7 ± 1.6***
		GG	19.9 ± 1.9	21.3 ± 6.2	17.5± 2.2	13.5 ± 4.4
	Right	LAD	**14.2 ± 0.6***	**25.5 ± 3.7***	**13.7 ± 1.1***	**25.3 ± 1.8***
		RAD	21.2 ± 1.6	9.8 ± 3.2	22.3 ± 1.9	8.7 ± 2.2
		GG	23.1 ± 2.9	18.0 ± 3.9	21.4 ± 2.0	10.5 ± 3.2
S1	Left	LAD	23.2 ± 2.5	3.0 ± 1.7	17.1 ± 1.4	5.8 ± 2.1
		RAD	**14.2 ± 0.5***	**4.8 ± 1.3***	**13.4 ± 1.3***	**8.8 ± 4.1***
		GG	19.9 ± 1.6	10.0 ± 4.7	14.1 ± 1.3	8.2 ± 4.3
	Right	LAD	**13.3 ± 0.9***	**10.0 ± 2.7***	**15.3 ± 1.4***	**13.7 ± 4.6***
		RAD	17.4 ± 2.2	2.7 ± 1.7	17.1 ± 2.9	4.3 ±1.8
		GG	20.7 ± 2.6	11 ± 3.8	20.1 ± 2.1	7.3 ± 2.7

B. Shortest onset latencies of ICMS (60μA)-evoked EMG activities in LAD, RAD and GG within face-M1
(Mean ± SEM)

Cortical Side	Muscle	Soft diet Group	Hard diet Group
Left	LAD	16.0 ± 1.2	15.1 ± 0.9
	RAD	11.8 ± 0.5	11.5 ± 0. 4
	GG	15.7 ± 1.0	14.0 ± 0.7
Right	LAD	11.8 ± 0.5	12.0 ± 0.9
	RAD	17.2 ± 1.1	15.6 ± 0.9
	GG	18.2 ± 1.9	17.4 ± 1.2

Table 3-3. There were no significant differences in LAD, RAD or GG onset latencies between the soft and hard diet groups. LAD had significantly shorter onset latency within the right than within the left cortex (MMRM: $p<0.0001$) and RAD had a significantly shorter latency within the left than within the right cortex (MMRM: $p=0.0007$). There were no significant differences in LAD or GG onset latencies between the face-M1 and face-S1 however, RAD onset latency was significantly shorter in face-S1 than in face-M1 (MMRM: $p=0.042$).

In addition, LAD had significantly smaller number of positive ICMS sites within the right than within the left face-M1 (MMRM: $p<0.0001$) and RAD had a significantly smaller number of positive ICMS sites within the left than within the right face-S1 (MMRM: $p<0.0001$) (see Fig. 3-2).

Quite similar, LAD had significantly larger number of positive ICMS sites and shorter onset latency within the right than within the left face-S1 (*MMRM: $p=0.0023$, $p<0.0001$, respectively) and RAD had a larger (but not significant) number of positive ICMS sites and shorter onset latency within the left than within the right face-S1 (*MMRM: $p=0.075$, $p=0.0007$, respectively) (see Fig. 3-3).
(M1-primary motor cortex; S1-primary somatosensory cortex; LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus).

Centre of gravity within face-M1

(ICMS 60μA)

Repeated-measures ANOVA results

AP plane	Measure	Predictor	F-statistic, DF, Statistical Significance
2.5	ML Position	Overall Model	chi-sq=1.44, df=1, p=0.2304
		Study group	F=0.28, df=1,10, p=0.6110
		Cortical side	F=8034.98, df=1,9, p<0.0001
		Intensity	F=0.09, df=1,10, p=0.7679
		Study group * Cortical side	F=10.39, df=1,9, p=0.0104
		Study group * Intensity	F=0.16, df=1,10, p=0.7007
		Cortical side * Intensity	F=0.11, df=1,10, p=0.7430
	Depth	**Overall Model**	**chi-sq=20.51, df=1, p<0.0001**
		Study group	F=3.14, df=1,10, p=0.1068
		Cortical side	**F=6.78, df=1,9, p=0.0286**
		Intensity	F=0.85, df=1,10, p=0.3775
		Study group * Cortical side	F=1.60, df=1,9, p=0.2373
		Study group * Intensity	F=1.04, df=1,10, p=0.3324
		Cortical side * Intensity	F=0.10, df=1,10, p=0.7559
3.0	Position	**Overall Model**	**chi-sq=12.47, df=1, p=0.0004**
		Study group	F=0.41, df=1,10, p=0.5345
		Cortical side	**F=22940.6, df=1,10, p<0.0001**
		Intensity	F=1.02, df=1,10, p=0.3357
		Study group * Cortical side	**F=10.90, df=1,10, p=0.0080**
		Study group * Intensity	F=0.64, df=1,10, p=0.4415
		Cortical side * Intensity	**F=9.67, df=1,11, p=0.00099**
	Depth	**Overall Model**	**chi-sq=14.62, df=1, p=0.0001**
		Study group	F=3.87, df=1,10, p=0.0773
		Cortical side	F=0.01, df=1,10, p=0.9140
		Intensity	F=0.82, df=1,10, p=0.3856
		Study group * Cortical side	**F=6.93, df=1,10, p=0.0250**
		Study group * Intensity	F=0.24, df=1,10, p=0.6336
		Cortical side * Intensity	F=0.35, df=1,11, p=0.5649
3.5	Position	**Overall Model**	**chi-sq=5.62, df=1, p=0.0178**
		Study group	F=0.11, df=1,10, p=0.7441
		Cortical side	**F=16118.6, df=1,10, p<0.0001**
		Intensity	F=0.07, df=1,10, p=0.8001
		Study group * Cortical side	F=0.16, df=1,10, p=0.6979
		Study group * Intensity	F=0.30, df=1,10, p=0.5960
		Cortical side * Intensity	F=1.16, df=1,10, p=0.3072
	Depth	**Overall Model**	**chi-sq=45.71, df=1, p<0.0001**
		Study group	F=0.17, df=1,10, p=0.6869
		Cortical side	F=3.93, df=1,10, p=0.0756
		Intensity	F=0.03, df=1,10, p=0.8593
		Study group * Cortical side	**F=6.69, df=1,10, p=0.0271**
		Study group * Intensity	F=0.02, df=1,10, p=0.8986
		Cortical side * Intensity	F=0.07, df=1,10, p=0.7947
4.0	Position	**Overall Model**	**chi-sq=3.91, df=1, p=0.0479**
		Study group	F=0.04, df=1,8, p=0.8547
		Cortical side	**F=6108.3, df=1,5, p<0.0001**
		Intensity	F=0.07, df=1,5, p=0.8009
		Study group * Cortical side	F=0.01, df=1,5, p=0.9137
		Study group * Intensity	F<0.01, df=1,5, p=0.9709
		Cortical side * Intensity	F=0.96, df=1,2, p=0.4301
	Depth	**Overall Model**	**chi-sq=5.87, df=1, p=0.0154**
		Study group	F=0.24, df=1,8, p=0.6408
		Cortical side	F=2.20, df=1,5, p=0.1985
		Intensity	F=2.52, df=1,5, p=0.1730
		Study group * Cortical side	**F=8.88, df=1,5, p=0.0308**
		Study group * Intensity	F=1.02, df=1,5, p=0.3592
		Cortical side * Intensity	F=1.47, df=1,2, p=0.3498

Table 3-4. Mixed model repeated-measures ANOVAs revealed no significant effects of either study group, cortical side, stimulation intensity, or any combination of these effects on the ML or depth positions of the centres of gravity of the positive ICMS (60 μA) sites at AP planes 2.5, 3.0, 3.5 or 4.0 mm anterior to Bregma.

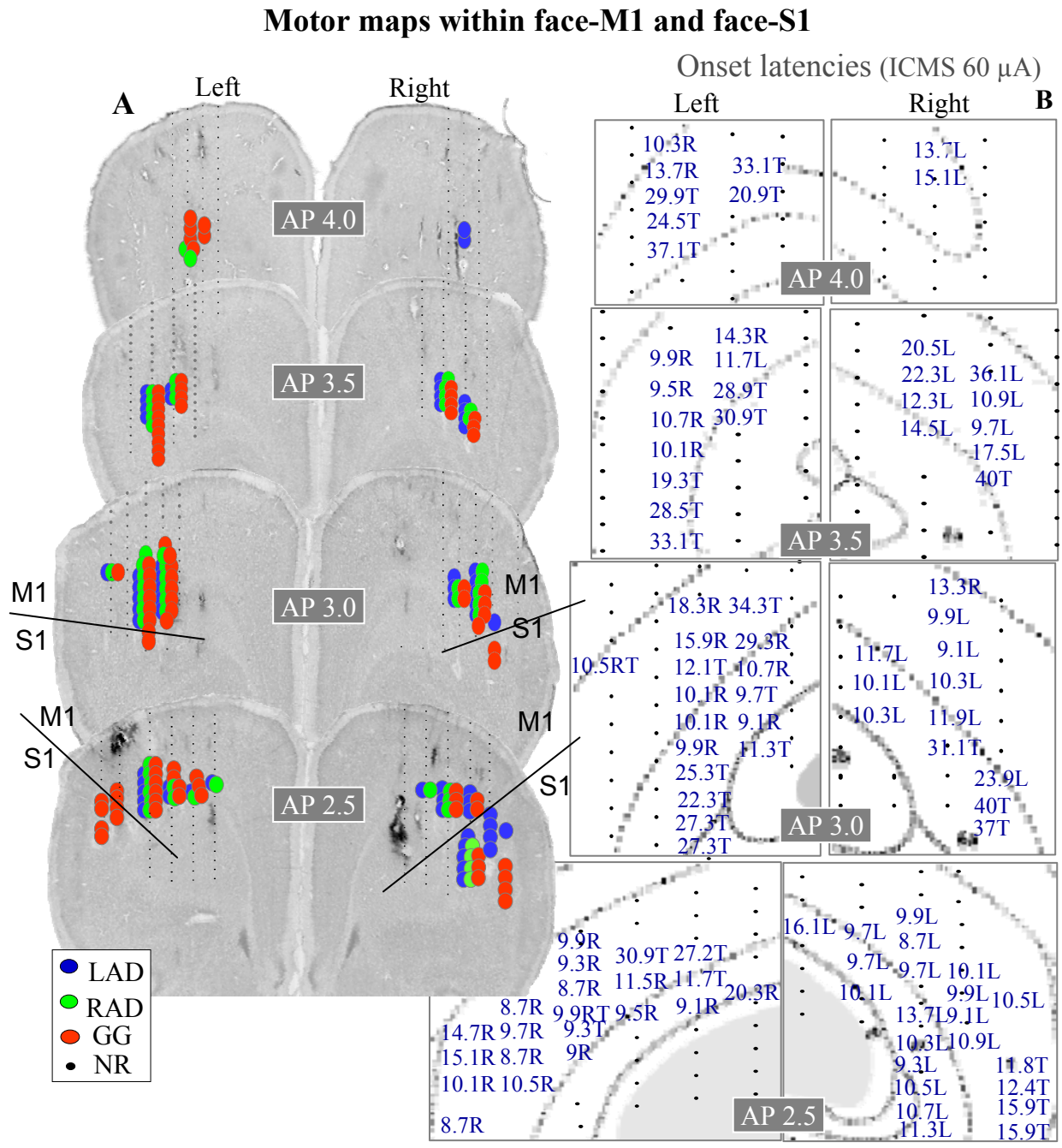


Fig. 3-1. These motor maps represent only one rat from the soft diet group, yet they do illustrate features that were common to all rats. **A.** Any site where ICMS (60 µA) evoked LAD, RAD or GG EMG activity was plotted on the corresponding cortical coronal histological sections (AP 2.5 – 4.0 mm anterior to Bregma) as follow: LAD with blue circles, RAD with green circles and GG with red circles. Black dots represent sites where ICMS could not evoke EMG activity in LAD, RAD or GG muscles. **B.** These motor maps are from the same rat as the motor maps in A. In these maps, at each ICMS site, letters correspond to the muscle with the shortest onset-latency of ICMS-evoked EMG response. Numbers are the values of the onset-latencies. Note: There was extensive, bilateral representation of the muscles with a considerable amount of sites from which ICMS could evoke EMG activity in more than one muscle; however, at each ICMS site, usually only one muscle had the shortest onset-latency of ICMS-evoked EMG response. (T or GG – genioglossus; R or RAD - right anterior digastric; L or LAD - left anterior digastric; NR – no response).

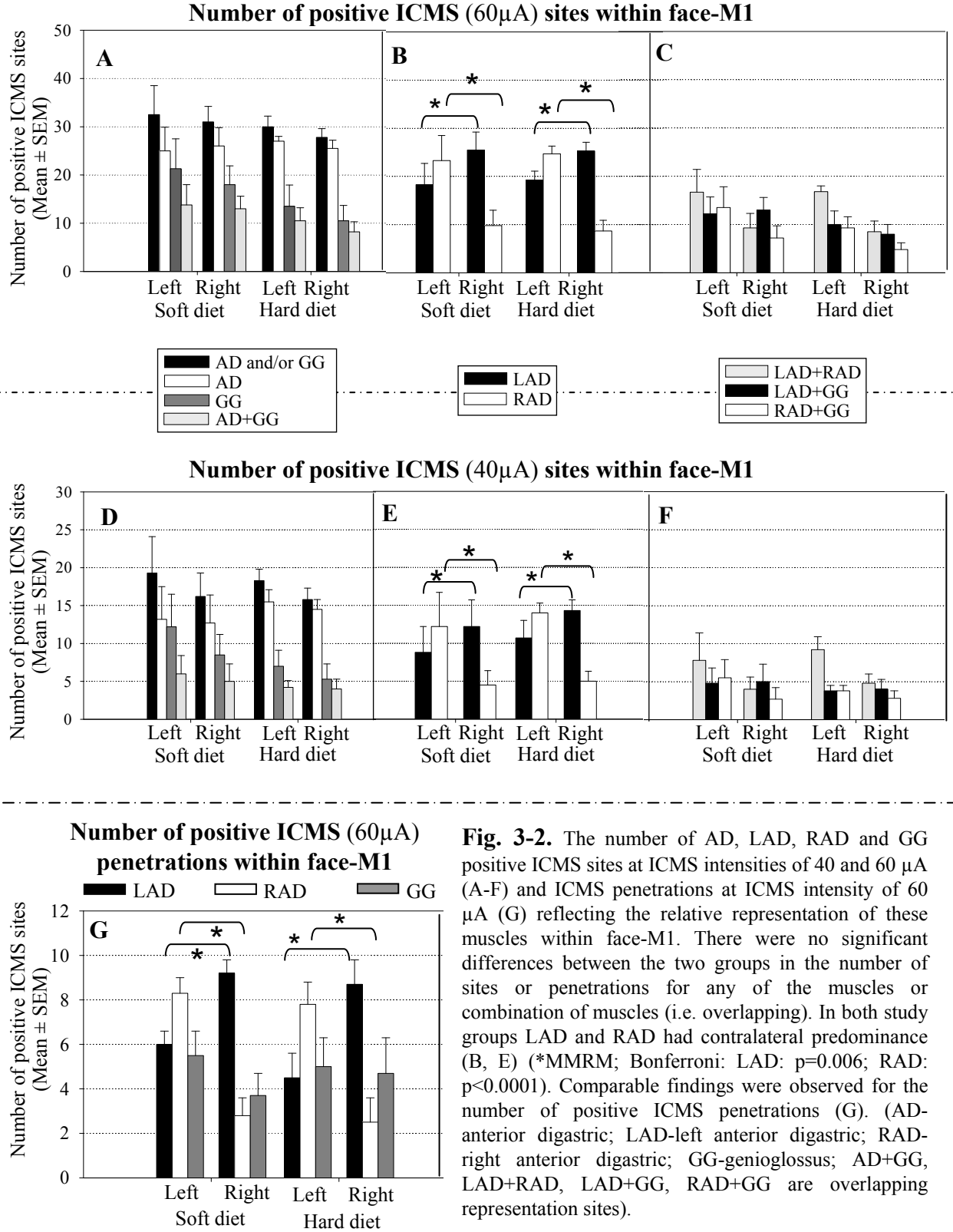


Fig. 3-2. The number of AD, LAD, RAD and GG positive ICMS sites at ICMS intensities of 40 and 60 μA (A-F) and ICMS penetrations at ICMS intensity of 60 μA (G) reflecting the relative representation of these muscles within face-M1. There were no significant differences between the two groups in the number of sites or penetrations for any of the muscles or combination of muscles (i.e. overlapping). In both study groups LAD and RAD had contralateral predominance (B, E) (*MMRM; Bonferroni: LAD: $p=0.006$; RAD: $p<0.0001$). Comparable findings were observed for the number of positive ICMS penetrations (G). (AD-anterior digastric; LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus; AD+GG, LAD+RAD, LAD+GG, RAD+GG are overlapping representation sites).

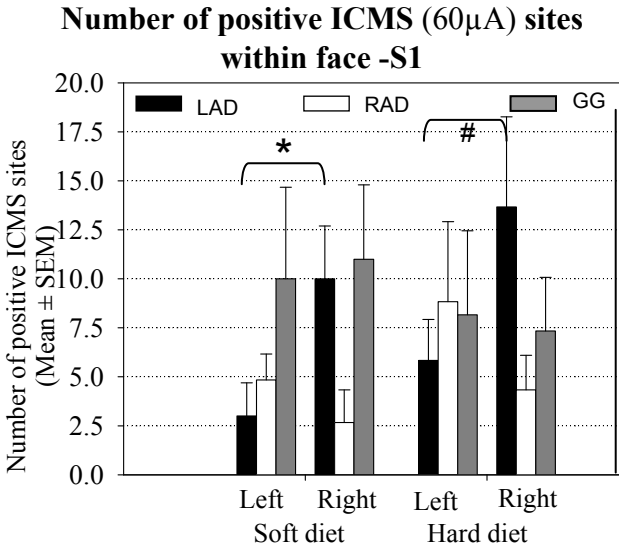


Fig. 3-3. The number of LAD, RAD and GG positive ICMS sites within face-S1 at ICMS intensity of 60 μA. There were no significant differences between the two groups (MMRM: $p>0.2$ for AD and $p>0.9$ for GG). LAD had significantly larger number of positive ICMS sites within the right than within the left face-S1 (* MMRM: LAD: $F=16.54$, $df=1,10$. $p=0.0023$) and RAD had a larger (but not significant) number of positive ICMS sites within the left than within the right face-S1 (*MMRM: $F=3.96$, $df=1,10$, $p=0.075$). (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus).

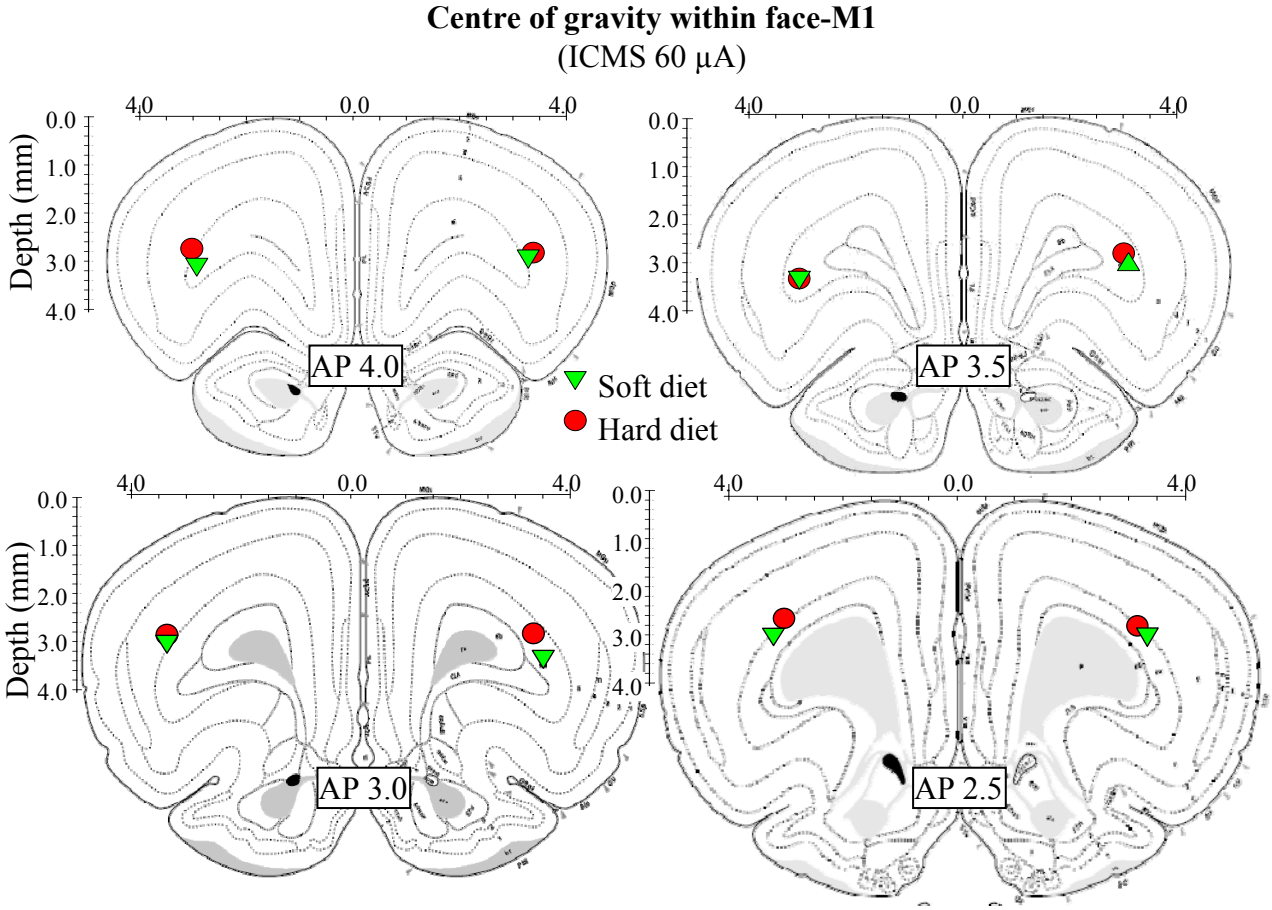


Fig. 3-4. Centre of gravity of AD and GG motor representations within each mapping plane (AP 2.5, 3.0, 3.5, 4.0). There were no significant differences between the soft and hard diet groups in the centre of gravity and within each group there were no significant differences between left and right face-M1 (Repeated ANOVA: Bonferroni: $p>0.05$). Not shown here, in all groups the AP centre of gravity was positioned between AP3.0 and AP3.5 with no significant differences between the groups or between left and right face-M1.

CHAPTER 4

JAW AND TONGUE MOTOR REPRESENTATIONS WITHIN FACE PRIMARY MOTOR COTEX OF ADULT RATS: EFFECT OF INCISOR TRIMMMING

1. Abstract

Modification to the dental occlusion induced by dental attrition, trimming or tooth loss may affect oral sensorimotor functions. It has been documented that changes in motor representations within the face primary motor cortex (face-M1) can be induced by alterations in sensory inputs and motor behaviour, but there are no published reports of whether occlusal modification is associated with changes in face-M1 of rats.

Objectives: to use intracortical microstimulation (ICMS) and recordings of evoked muscle electromyographic (EMG) activity to test if changes occur in the ICMS-defined motor representations of the right and left anterior digastric (RAD, LAD) and genioglossus (GG) muscles within the rat face-M1 following unilateral trimming of the mandibular incisor out of occlusal contact for a period of 1 week.

Methods: Adult male Sprague-Dawley rats (200-250gr) were divided into four groups. Under local and general anaesthesia, the "trim" (n=6), "trim recovered" (n=6) and "sham trim" (n=7) groups had the right lower incisor trimmed every 2 days for a period of 1 week; a naive group (n=6) had no treatment. The incisor in the trim and trim recovered groups had the incisal edge trimmed and in the sham trim group had the buccal-gingival surface trimmed without affecting the occlusal contacts. M1 mapping was carried out 1 day after the last trimming day in the trim and sham trim groups and 1 week after the last trim day in the trim recovered group. Under general anaesthesia (ketamine-HCl), ICMS (5x33.2ms train, 12x0.2ms pulses, 333Hz; ≤ 60μA) was applied within the left and right face-M1 in a systematic series of microelectrode penetrations extending from 2.5 to 4.0 mm anterior and 1.5 to 5.5 mm lateral to Bregma with a spatial resolution of 0.5 mm horizontally, and ICMS was applied every 0.2 mm of microelectrode penetration depth. Histologically confirmed sites for which ICMS could evoke EMG activity in GG, RAD or LAD were considered positive ICMS sites. Statistical analyses included *t-test* and series of ANOVAs, followed by *post-hoc* Bonferroni-adjusted pairwise comparisons, as appropriate; $p<0.05$ was considered to indicate statistical significance.

Results: Data analysis revealed no significant differences in the number of LAD, RAD and GG positive ICMS sites and onset latencies across the study groups. However, in comparison with the naïve and sham trim groups, the trim group had a smaller number of

GG positive ICMS sites within the left and right face-M1 at 1 day following the last trimming day. Also, only in the trim group was there a significant difference in the onset latency between the left and right face-M1 (paired *t-test*, $p<0.001$). In the trim recovered group, the left face-M1 had significantly larger numbers of positive sites for GG as well as AD/GG and LAD/GG overlapping sites than the right face-M1 (MMRM: $p=0.0032$, $p<0.0016$, $p=0.014$, respectively), and only in the trim recovered and sham trim groups were there significantly more RAD/GG overlapping sites within the left than within the right face-M1 (MMRM, Bonferroni: $p=0.0032$, $p=0.0016$, respectively). In addition, only in the trim recovered group, the position of the centre of gravity at AP 4.0 was significantly deeper in the left face-M1 than in the right face-M1 (MMRM, Bonferroni: $p=0.026$).

Conclusion: Unilateral trimming of the rat mandibular incisor out of occlusion may be associated with some significant neuroplastic changes in jaw and tongue motor representations within face-M1 that are evident 1-2 weeks after the trimming. These changes may be related to the animal's ability to adapt its masticatory apparatus to the altered oral state.

2. Introduction

The primary motor cortex that represents the orofacial muscles (face-M1) has a crucial role in the generation and control of orofacial motor functions (for reviews, see Ebner, 2005; Murray et al., 2001; Sessle et al., 2005; Sessle et al., 1999). The somatosensory system, including the face primary somatosensory cortex (face-S1), not only provides somatosensory feedback to further assist in the control of orofacial motor functions (Johansson et al., 2006; Paxinos, 2004; Trulsson, 2006; Trulsson, 2007; Trulsson and Essick, 2004; Woda et al., 2006) but may also participate in the generation and control of the orofacial motor functions (for reviews, see Murray et al., 2001; Sessle et al., 1999). Recent research suggests that face-M1 also plays a role in adaptation and learning processes as reflected in reorganization of motor representations following peripheral manipulations to the orofacial tissues in rats and other animals (Adachi et al., 2007; Adachi et al., 2008; Huntley, 1997b; Keller et al., 1996; Sessle et al., 2007) or following training animals and humans in an oral motor task (Boudreau et al., 2007; Svensson et al., 2003b; Svensson et al., 2006; for reviews, see Robbins et al., 2008; Sessle et al., 2007; Sessle et al., 2005). Limited data are available of the neuroplastic capabilities of face-M1 following alterations to the dental occlusion and the subsequent restoration of normal occlusion. This information is important since modifications to the dental occlusion as a result of attrition, trimming, decay or loss of teeth are common occurrences in humans that may be accompanied by altered and sometimes impaired oral sensorimotor functions (Feine and Carlsson, 2003; Johansson et al., 2006; Klineberg and Jagger, 2004; Proschel and Hofmann, 1988; Trulsson and Essick, 2004). It has also been well documented that these negative consequences can be improved following oral rehabilitation as patients adapt to a new dental prosthesis aimed at restoring function (Haraldson and Zarb, 1988; Molly et al., 2008; Trulsson and Essick, 2004). To study the possible involvement of face-M1 in orofacial motor adaptation, the present study tested whether face-M1 motor representations can be altered by experimental intraoral manipulations. The hypothesis was that modifications to the dental occlusion induced by trimming of the rat mandibular incisor would result in changes in the face-M1 motor representation. The objectives of this study were to use intracortical microstimulation

(ICMS) and recordings of evoked muscle electromyographic (EMG) activity to test if changes occur in the ICMS-defined face-M1 motor representations of the right and left anterior digastric (RAD, LAD) and genioglossus (GG) muscles following unilateral trimming of the rat mandibular incisor out of occlusal contacts for a period of 1 week.

3. Materials and Methods

Experimental procedures were approved by the University of Toronto Animal Care Committee, in accordance with the Canadian Council on Animal Care Guidelines and the regulations of the Ontario Animals for Research Act (R.S.O. 1990). To ensure consistency in the experimental procedures and data analysis, 1 investigator (LA-A) carried out all experimental procedures and data analysis in a blinded manner. Chapter 2 provides detailed description of the methods and therefore, following is only a brief description of the methods.

3.1. Animals

Young adult male Sprague-Dawley rats (150-250g on arrival, 300-400g on the day of cortical mapping) were housed in similar conditions and water and a soft diet (mashed chow) were available *ad libitum*. Animals were monitored on a daily basis to assess body weight, food consumption and general behaviour.

3.2. Study groups and dental procedures

3.2.1. Study groups

Rats were divided into 4 groups. Under general anaesthesia, the "trim" (n=6), "trim recovered" (n=6) and "sham trim" (n=7) groups had the right lower incisor trimmed every 2 days for a period of 1 week; a naive group (n=6) had neither anaesthesia nor treatment. The incisor in the trim and trim recovered groups had the incisal edge trimmed and in the sham trim group the incisor had the buccal-gingival surface trimmed without affecting the occlusal contacts (see Chapter 2, Fig. 2-3). In the trim group, ICMS mapping was carried out 1 day following the last incisor trimming day. In the trim recovered group, ICMS was carried out 1 week following the last incisor

trimming day; during this week the incisor erupted and re-gained its original length and occlusal contacts (see Chapter 2, Fig. 2-3).

3.2.2. Incisor trimming

In the trim and trim recovered groups, in order to compensate for the continuous eruption of the incisor, keeping it in a state of reduced occlusal contacts and to avoid pulp exposure, the incisal edge was trimmed carefully 1 – 2 mm every 2 days (in total 4 trims) (Figs. 2-3 A). In the sham trim group, in order to mimic the dentin exposure as the result of incisal trimming but without affecting the incisor occlusion, the labial surface of the incisor was trimmed at the gingival level to create a cavity within the dentin with a radius of ~1 mm without affecting the incisor occlusion. Thereafter, the dentin was re-exposed with the same procedure every other day, for a period of 1 week (and in total 4 times) (Figs. 2-3C, 2-3D, and 2-3E). The incisor was trimmed with a dental high-speed turbine and a diamond bur rotating at a high speed under copious saline irrigation. A dental bonding agent was applied to seal any exposed dentinal tubules.

3.3. ICMS and EMG recordings

Chapter 2 provides a detailed description of the ICMS technique applied in the present study to define the motor representations of jaw and tongue muscles within the face-M1 of rats. Mapping was carried out under a stable level of general anaesthesia with ketamine HCL (Ketaset®, Ayerst Veterinary Laboratories, Ontario, Canada). EMG electrodes (40-gauge, single-stranded, Teflon-insulated stainless-steel wires) were used to record EMG activity from the left and right LAD, RAD, masseter as well as GG, vibrissal and neck muscles. Systematic mapping was carried out within 4 AP planes located 2.5 to 4.0 mm rostral to Bregma and extended from 1.5 to 5.5 mm lateral to the midline within each of the left and right face-M1 at a horizontal spatial resolution of 0.5 mm. In each penetration site, ICMS was applied every 0.2 mm of microelectrode penetration depth. Five ICMS trains (at 333 Hz, 33.2 msec, 12 pulses of 0.2 msec, 2.8 msec inter-pulses intervals) were delivered at 1 Hz with a suprathreshold ICMS intensity of 60 μA. If ICMS could effectively evoke a GG and/ or AD EMG response, then a series of 5 ICMS trains was delivered at 60, 40, 20 and 60 μA. Electrolytic lesions were

placed for subsequent histological confirmation of ICMS sites within the gray matter of M1.

3.4. Data acquisition and analysis

Data acquisition and analysis are detailed in chapter 2. For each muscle, an ICMS site was defined and counted as a "positive ICMS site" if at least 3 out of the 5 ICMS (60 μA) trains could evoke an EMG response with onset latency ≤ 40msec and a peak activity exceeding the mean value of the initial 10 msec of the EMG response plus 2 standard deviations (SDs). For each muscle, an ICMS penetration was defined and counted as a "positive ICMS penetration" if it had at least 1 positive ICMS site. For each positive ICMS site, the AP, ML and depth coordinates as well as the onset latency for each ICMS-evoked muscle response were noted. In addition, for each positive ICMS penetration, the AP and ML coordinates as well as the shortest onset latency for each muscle were noted. Cortical motor maps were used to illustrate the representation areas of LAD, RAD and GG muscles within the sensorimotor cortex of rats from each of the study groups. Positive ICMS sites for LAD, RAD and GG were plotted on a corresponding histological coronal section (AP 2.5-4.0 mm anterior to Bregma) (Fig. 4-1A). The ML and depth positions of the centre of gravity weighted relative to the extent of the motor representations were calculated for each AP plane within face-M1.

3.5. Statistical Analyses

As detailed in chapter 2, statistical differences between groups and the effects of the independent variables (study group, cortical side, and ICMS intensity) on the dependent variables (number of positive ICMS sites or penetrations, onset latency, ML position; and the centre of gravity) were determined using a series of ANOVAs, and mixed model repeated-measures (MMRM) analyses (multivariate analyses) followed by *post-hoc* Bonferroni-adjusted pairwise comparisons as appropriate. In addition, paired *t-test* was used for within-group comparisons of the onset latencies for evoking EMG responses. A probability level of $p<0.05$ was considered statistically significant.

4. Results

Rats were monitored on a daily basis and all showed normal behaviour and a continuous gain of body weight (see Chapter 2).

4.1. General features of AD and GG motor representations

ICMS evoked EMG activity in AD and/ or GG muscles from an extensive area within the left and right face-M1 between AP coordinates 2.5 to 4.0 and ML coordinates 2.5-5.0 (Fig. 4-1). ICMS evoked EMG responses simultaneously in both AD and GG (*i.e.*, overlapping representations) from many sites. The general features of the AP–ML-depth positions and the extent of AD and GG representations, as well as the mean onset latency of evoked EMG responses (see below), were similar in the naïve and sham trim rats and not significantly different. Within the mapped area there were very few positive ICMS sites for the masseter and neck muscles (<1%) and the occasional ICMS-evoked vibrissal EMG activity was frequently confounded by the spontaneous movements of the vibrissae. Therefore, the ICMS data for masseter, vibrissae and neck muscles were not included in the data analysis of this study.

4.2. Effects of tooth trimming

4.2.1. AD and GG motor representations

The statistical analysis revealed no significant group effects on the LAD, RAD or GG number of positive ICMS sites or onset latency. However, there were significant cortical side effects (Table 4-1). The overall number of AD and/or GG positive ICMS sites was significantly larger in the left face-M1 than in the right face-M1 (MMRM, Bonferroni: $p=0.046$) (Fig. 4-2A, D). In addition, both LAD and RAD had a significant contralateral predominance (MMRM, Bonferroni: $p<0.0001$) (Fig. 4-2B).

In spite of the lack of any difference across the study groups, the trim group had a moderate decrease (but not statistically significant) in the number of GG sites within the left and right face-M1 as compared with the naive or sham trim groups, and the trim recovered group had a moderate increase (but not significant) in the number of GG sites within the left face-M1 as compared with the trim, naive and control groups (Fig. 4-2A,

C). Nevertheless, there was a significant interaction between study group and cortical side for GG (Table 4-1); only in the trim recovered group were there significantly more GG sites within the left face-M1 than within the right face-M1 (MMRM, Bonferroni: p=0.0032) (Fig 4-2A, C).

ICMS evoked EMG activity simultaneously in more than 1 muscle (any combination of LAD, RAD and GG), *i.e.*, overlapping representations, from many sites in all groups (Fig. 4-1, 4-2C.). There was a significant interaction between study group and cortical side for GG/AD, GG/LAD and GG/RAD overlapping sites (Table 4-1). While Bonferroni comparisons revealed no significant differences across the study groups, only in the trim recovered group were there significantly more positive sites within the left face-M1 than within the right face-M1 for AD/GG overlapping sites (MMRM, Bonferroni: p<0.0016), LAD/GG overlapping sites (MMRM, Bonferroni: p=0.014) and only in the sham trim and trim recovered groups were there significantly more RAD/GG overlapping sites within the left than within the right face-M1 (MMRM, Bonferroni: p=0.0032, p=0.0016, respectively).

Similar results were obtained when data from the naïve and control groups were pooled and designated as a single control group and compared with the trim and trim recovered groups (data not presented).

4.2.2. Number and distribution of positive ICMS penetrations

When the ICMS (60 μA) data were also analysed in terms of the number of positive ICMS penetrations from which ICMS evoked EMG activity in LAD, RAD or GG, there were no significant differences across the study groups in either the left or the right face-M1 (Fig. 4-2G). In addition, there were no significant differences across the groups in the mean ML and AP position of the positive ICMS penetrations (Table 4-2). Similar results were obtained when data from the naïve and sham groups were pooled and designated as a single control group and compared with the trim and trim recovered groups (data not shown).

4.2.3. Centre of gravity within face-M1

Data analysis revealed no significant differences across the study groups in the AP and ML coordinates of the centre of gravity (Fig. 4-3). However, at AP4.0 there was

a significant interaction between study group and cortical side for the depth position of the centre of gravity (MMRM: F=3.22, df=3,17, p=0.049). Bonferroni comparisons revealed that only in the trim recovered group was the depth position of the centre of gravity significantly deeper in the left face-M1 than in the right face-M1 (p=0.026) (Fig. 4-3D). Similar results were obtained when data from the naïve and control groups were pooled and designated as a single control group and compared with the trim and trim recovered groups (data not shown).

4.2.4. Onset latency of ICMS-evoked EMG activity

In all study groups, ICMS within face-M1 evoked EMG activity in LAD, RAD and/ or GG with onset latencies from 8 - 40 msec. In all study groups, the mean onset latency of ICMS-evoked EMG activity in LAD or RAD was significantly shorter in the contralateral face-M1 than in the ipsilateral face-M1 (paired *t-test*, $p<0.05$) (Fig. 4-4A). For GG, while in the control and trim recovered groups there were no significant differences between the left and right face-M1, in the trim group the onset latency was significantly shorter in the left face-M1 than in the right face-M1 (paired *t-test*, $p<0.001$). Data analysis revealed no significant differences across the study groups in the mean onset latency for evoking EMG activity in LAD, RAD or GG. However, when data from the naïve and control groups were pooled and designated as a single control group, within the right face-M1 (but not the left face-M1), GG (but not LAD or RAD) onset latency was significantly longer in the trim group than in the trim recovered group (ANOVA: p=0.022; Bonferroni: p=0.027), and showed a trend towards being significantly longer than in the control group (p=0.062) (Fig. 4-4B). There were no significant differences across the groups in LAD, RAD or GG onset latency within the left face-M1.

5. Discussion

This study has provided new evidence that modification to the dental occlusion induced by unilateral trimming of the rat incisor out of occlusion may induce some neuroplastic changes within face-M1. It also has provided data to support findings from previous studies of the ICMS-defined organizational features of AD and GG within face-

M1 (Adachi et al., 2007; Gioanni and Lamarche, 1985; Neafsey et al., 1986), with AD and GG having extensive motor representations within the left and right face-M1, and LAD and RAD having a significant contralateral predominance as reflected in the larger representation and shorter onset latency.

5.1. Effects of dental trimming

Modifications to the occlusion induced by loss of teeth, dental attrition or oral rehabilitation are common occurrences in humans. Oral tissues, including teeth, are characterised by a high innervation density (Capra, 1995; Dubner and Sessle, 1978; Hildebrand et al., 1995; Hu, 2004; Macefield, 2005; Paxinos, 2004; Svensson and Sessle, 2004; Trulsson and Essick, 2004) and while face-M1 is important for the generation and control of orofacial motor functions (Ebner, 2005; Murray et al., 2001; Sessle et al., 2005; Sessle et al., 1999), somatosensory inputs from the orofacial tissues including prominent mechanoreceptive inputs from the teeth, provide peripheral somatosensory inputs to face-M1 and face-S1 (Catania and Remple, 2002; Hatanaka et al., 2005; Henry et al., 2006; Hiraba and Sato, 2004; Iyengar et al., 2007; Remple et al., 2003; Toda and Taoka, 2004; for reviews, see Kaas et al., 2006; Murray et al., 2001) to further assist in the control of orofacial movements (for reviews, see Johansson et al., 2006; Murray et al., 2001; Sessle et al., 2005; Trulsson, 2006; Trulsson, 2007; Trulsson and Essick, 2004; Trulsson and Johansson, 2002).

It is well known that modifications to the dental occlusion in humans may induce altered patterns mastication (Johansson et al., 2006; Klineberg and Jagger, 2004; Proschel and Hofmann, 1988; Trulsson and Essick, 2004). Incisor trimming in rats has also been associated with a significant decrease in the thickness of the enamel and dentin (Michaeli et al., 1982; Risnes et al., 1995; Weinreb et al., 1985) as well as a reduction in the size and number of periodontal nerve endings (Shi et al., 2005). These data suggest that dental trimming may be associated with altered somatosensory inputs from the teeth to face-M1 and face-S1. Rats are engaged in gnawing behaviour to compensate for the continuous eruption of their incisors (Burn-Murdoch, 1999; Law et al., 2003; Ness, 1965; Risnes et al., 1995; Sessle, 1966). Occlusal modifications induced by dental

trimming or extraction are associated with morphological changes in the condyles, masticatory muscles and periodontal ligaments (Endo et al., 1998; Miehe et al., 1999; Ramirez-Yanez et al., 2004; Shi et al., 2005). Therefore, it is also possible that trimming of the rat incisors out of occlusion can modify the rat oral motor behaviour.

There is evidence to suggest that changing orofacial somatosensory inputs to the sensorimotor cortex and altered orofacial motor behaviour may result in face-M1 neuroplasticity (Adachi et al., 2007; Adachi et al., 2008; Boudreau et al., 2007; Hamdy et al., 1998; Huntley, 1997b; Keller et al., 1996; Svensson et al., 2003b; Svensson et al., 2006; for reviews, see Ebner, 2005; Robbins et al., 2008; Sessle et al., 2007; Sessle et al., 2005). However, the findings of the present study revealed no significant differences across the study groups. Nevertheless, incisor trimming did result in changes in ICMS-defined features within the trim and trim recovered groups. In the groups of naïve and control rats, GG had similar ICMS features of motor representations within the left and right face-M1, but unilateral trimming for a period of 1 week was associated with significant dissimilarities in the properties of the ICMS-defined GG motor representations between the left and right face-M1; 1 day after the last trimming day, GG onset latency was significantly longer in the right than in the left face-M1, and in addition there was a bilateral decrease (although not significant) in GG motor representations within face-M1. These changes were transient and were no longer observed 1 week later in the trim recovered group. However, 1 week after the last trimming day, there were significant differences in the GG motor representations between the left and right face-M1 despite that normal occlusal contact having been regained. GG as well as GG/AD overlapping representations increased significantly within the contralateral (left) face-M1 and the centre of gravity shifted towards a significantly deeper cortical position. These changes suggest that neuroplastic changes did occur within face-M1 as a result of the incisor trimming in the trim and trim recovered groups.

It is noteworthy that increased overlapping of motor representations has been observed also 1 week following dental extraction (see Chapter 5). Increased overlapping of motor representations has been reported to be one of the most consistent

consequences of limb motor skill training (Nudo et al., 1996). Motor skill training involves repetitions of novel coordinated movements of multiple muscles (Adams, 1984; Asanuma, 1989). Therefore, our finding of altered extent of overlapping motor representations likely reflects changes in the rat oral motor behaviour that result from a repetition of a novel oral motor behaviour (Asanuma, 1989), supporting the notion that face-M1 can be dynamically modulated in a use-dependent manner. Possible mechanisms underlying such cortical neuroplastic changes are described in Chapter 5.

In a recent study (Lee et al., 2006), bilateral trimming of the rat incisors out of occlusion for 1 week (or even 1 day) was found to be associated, 1 day later, with a non-significant decrease in the GG motor representation and a significant decrease in AD motor representation within the left and right face-M1. Furthermore, unilateral transection of the lingual nerve supplying the sensory innervation to the tongue in the rat has been associated 1-2 weeks later with a significantly decreased GG representation and 3-4 weeks later with a significantly increased GG representation (Adachi et al., 2007). Comparable with these studies in rats, 15 min of training in a novel tongue-task has resulted in increased excitability of face-M1 representing the tongue musculature (Boudreau et al., 2007), and 1 week or even 1 day of training has resulted in an increased representation of the tongue musculature (Svensson et al., 2003b; Svensson et al., 2006). Considering these findings, the present data are compatible with the following 2 notions in relation to face-M1 neuroplastic capabilities. First, that face-M1 has the capacity to adapt to significant changes in orofacial sensorimotor behaviours in a task-dependent manner as reflected in different forms (*i.e.,* changes in excitability vs reorganization of motor representations) and in different directions (*i.e.,* increased vs decreased) of neuroplastic changes associated with different intraoral manipulations. Second, the capacity for neuroplasticity is carried out in a time-dependent manner as reflected in the different forms and different directions of reorganization of motor representations at different time points following the intraoral manipulations. Assuming that the delayed GG onset latency in the trim group reflects, at least in part, decreased cortical excitability (Asanuma, 1989; Greenshaw, 1998; Ranck, 1975), it is possible that this latency shift represents an early neuroplastic change which may later develop into a functional

reorganization of motor representations, and as such, may reflect or allow for the animal to adapt to the altered oral motor behaviour (Cohen et al., 1998; Pascual-Leone et al., 1999).

5.2. Study limitations

The present study could not find significant differences across the study groups in either left or right face-M1. Yet, incisor trimming resulted in significant disparities between the left and right face-M1 in the ICMS-defined motor representations of GG muscle. Such disparities did not exist in naïve and sham rats. More substantial and significant changes have been documented following bilateral incisor trimming (Lee et al., 2006). One possible explanation for the difference found between the present study (that used unilateral incisor trimming), and the study by Lee et al. (that applied bilateral trimming) is that the changes associated with unilateral trimming were too small to be detected with our mapping technique. Replication of the present study with different ICMS parameters, such as threshold intensities or a larger sample size, might bring up evidence of neuroplasticity.

It has been reported that experimental intraoral noxious stimulation is associated with decreased face-M1 excitability (Adachi et al., 2008; Boudreau et al., 2007). Therefore, it could have been argued that the observed changes in this study were confounded by the exposed dentin tubules and perhaps exposed pulp that could have rendered the tooth more sensitive to mechanical, thermal and perhaps noxious stimuli (Hu, 2004). However, this is unlikely for several reasons. First, our trimming was limited to 1-2 mm of supragingival trimming and it has been reported that in continuously erupting incisors in rats the pulpal innervation is generally relatively scarce (Naftel et al., 1999) and that pulpal axons can rapidly adapt by terminating 2 mm away from the incisal edge (for reviews, see Hildebrand et al., 1995; Paxinos, 2004) and can be found neither in the lingual odontoblastic layer of the pulp nor within the dentinal tubules (Zhang et al., 1998; for review, see Paxinos, 2004). Second, we did not observe pulp exposure, moreover, at the termination of each trimming session we applied a dentin bonding agent to seal the opened dentin tubules. Third, tooth injury in the rat that

results in pulp exposure, changes spontaneous behaviours indicating dental pain (Chudler and Byers, 2005). However, we observed no gross behavioural modifications in the rats undergoing incisal trimming and there were no significant changes in the mean body weights across the study groups. Nonetheless, we did not record EMG or movement parameters during the animal's normal oral sensorimotor behaviour; future studies could monitor EMG and movement patterns to determine if incisal trimming is associated with alterations in oral sensorimotor behaviour.

6. Conclusions

Unilateral trimming of the rat mandibular incisor out of occlusion for a period of 1 week may be associated with differential and time-dependent neuroplastic changes within face-M1 that are evident 1 day and 1 week later. These changes may be related to the animal's ability to adapt to the altered oral state as it adopts an altered oral motor behaviour to compensate for the altered oral state.

Face-M1 positive ICMS sites

Repeated-measures ANOVA results

Muscle	Predictor	F-statistic, df, Statistical significance
AD and/or GG	**Overall Model**	**chi-sq=25.38, df=1, p<0.0001**
	Study group	F=1.08, df=3,21, p=0.38
	Cortical side	**F=4.51, df=1,21, p=0.046**
	Intensity	**F=55.32, df=1,21, p<0.0001**
	Study group * Cortical side	F=0.75, df=3,21, p=0.53
	Study group * Intensity	F=0.05, df=3,21, p=0.98
	Cortical side * Intensity	F=0.14, df=1,24, p=0.71
GG	**Overall Model**	**chi-sq=23.44, df=1, p<0.0001**
	Study group	F=0.83, df=3,21, p=0.49
	Cortical side	**F=13.95, df=1,21, p=0.0012**
	Intensity	**F=18.88, df=1,21, p=0.0003**
	Study group * Cortical side	**F=3.52, df=3,21, p=0.033**
	Study group * Intensity	F=0.12, df=3,21, p=0.94
	Cortical side * Intensity	F=0.21, df=1,24, p=0.65
AD	**Overall Model**	**chi-sq=34.44, df=1, p<0.0001**
	Study group	F=1.23, df=3,21, p=0.32
	Cortical side	F=3.27, df=1,21, p=0.085
	Intensity	**F=73.87, df=1,21, p<0.0001**
	Study group * Cortical side	F=0.59, df=3,21, p=0.63
	Study group * Intensity	F=0.16, df=3,21, p=0.92
	Cortical side * Intensity	F=0.18, df=1,24, p=0.68
LAD	**Overall Model**	**chi-sq=38.59, df=1, p<0.0001**
	Study group	F=1.39, fd=3,21, p=0.27
	Cortical side	**F=30.52, df=1,21, p<0.0001**
	Intensity	**F=49.84, df=1,21, p<0.0001**
	Study group * Cortical side	F=0.68, df=3,21, p=0.57
	Study group * Intensity	F=0.10, df=3,21, p=0.96
	Cortical side * Intensity	F=3.89, df=1,24, p=0.06
RAD	**Overall Model**	**chi-sq=39.08, df=1, p<0.0001**
	Study group	F=0.99, df=3,21, p=0.41
	Cortical side	**F=176.05, df=1,21, p<0.0001**
	Intensity	**F=58.30, df=1,21, p<0.0001**
	Study group * Cortical side	F=0.79, df=3,21, p=0.51
	Study group * Intensity	F=0.49, df=3,21, p=0.69
	Cortical side * Intensity	**F=15.03, df=1,24, p=0.0007**
AD and GG	**Overall Model**	**chi-sq=28.66, df=1, p<0.0001**
	Study group	F=0.89, df=3,21, p=0.46
	Cortical side	**F=21.16, df=1,21, p=0.0002**
	Intensity	**F=30.91, df=1,21, p<0.0001**
	Study group * Cortical side	**F=4.60, df=3,21, p=0.013**
	Study group * Intensity	F=0.12, df=3,21, p=0.95
	Cortical side * Intensity	F=0.39, df=1,24, p=0.54
RAD and LAD	**Overall Model**	**chi-sq=27.89, df=1, p<0.0001**
	Study group	F=1.18, df=3,21, p=0.34
	Cortical side	**F=23.45, df=1,21, p<0.0001**
	Intensity	**F=31.50, df=1,21, p<0.0001**
	Study group * Cortical side	F=2.06, df=3,21, p=0.14
	Study group * Intensity	F=0.14, df=3,21, p=0.93
	Cortical side * Intensity	F=1.26, df=1,24, p=0.27
LAD and GG	**Overall Model**	**chi-sq=25.84, df=1, p<0.0001**
	Study group	F=0.87, df=3,21, p=0.47
	Cortical side	F=2.98, df=1,21, p=0.099
	Intensity	**F=30.42, df=1,21, p<0.0001**
	Study group * Cortical side	**F=4.55, df=3,21, p=0.013**
	Study group * Intensity	F=0.06, df=3,21, p=0.98
	Cortical side * Intensity	F=0.04, df=1,24, p=0.84
RAD and GG	**Overall Model**	**chi-sq=46.42, df=1, p<0.0001**
	Study group	F=0.74, df=3,21, p=0.54
	Cortical side	**F=84.07, df=1,21, p<0.0001**
	Intensity	**F=32.71, df=1,21, p<0.0001**
	Study group * Cortical side	**F=4.01, df=3,21, p=0.021**
	Study group * Intensity	F=0.12, df=3,21, p=0.95
	Cortical side * Intensity	**F=7.19, df=1,24, p=0.013**

Table 4-1. Mixed model repeated-measures ANOVA, followed by *post-hoc* Bonferroni-adjusted pairwise comparisons where applicable, was used in order to determine whether study group, cortical side, stimulation intensity (40 vs 60μA), or any combination of these effects significantly affected the number of positive ICMS-sites. These tests were performed separately for each muscle and each combination of muscles. (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus)

Anteroposterior-mediolateral position of the positive ICMS (60μA) penetrations within face-M1

(Mean ± SEM)

Cortical Side	Measure	Naive	Sham trim	Trim	Trim recovered	ANOVA
Left	AP	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	p=0.60
	ML	3.3 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	p=0.32
Right	AP	3.2 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	p=0.47
	ML	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	p=0.88

Table 4-2: The anteroposterior (AP) - mediolateral (ML) position of the ICMS penetrations. There were no significant differences across the groups in the mean AP or ML position in either left or right face-M1.

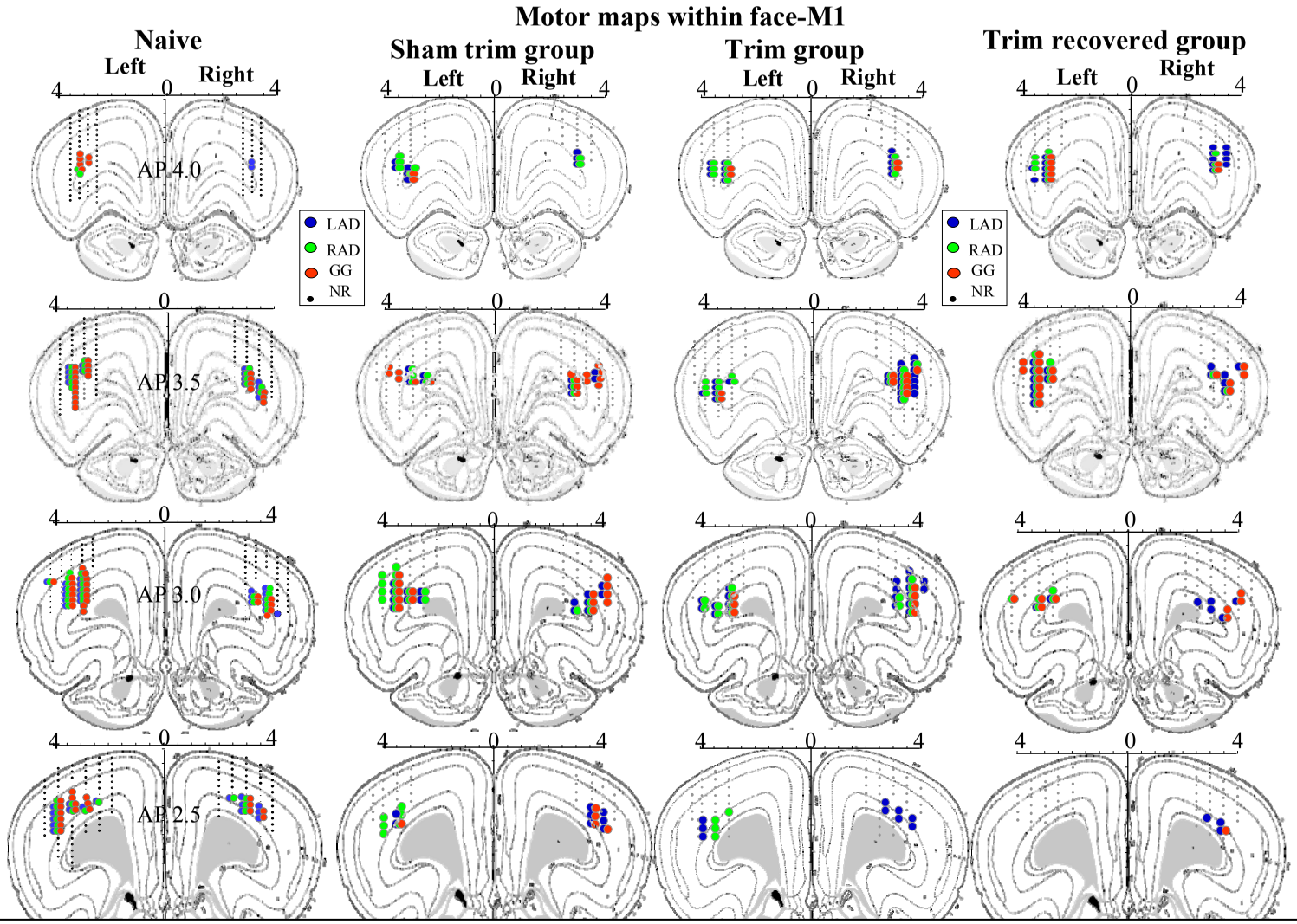
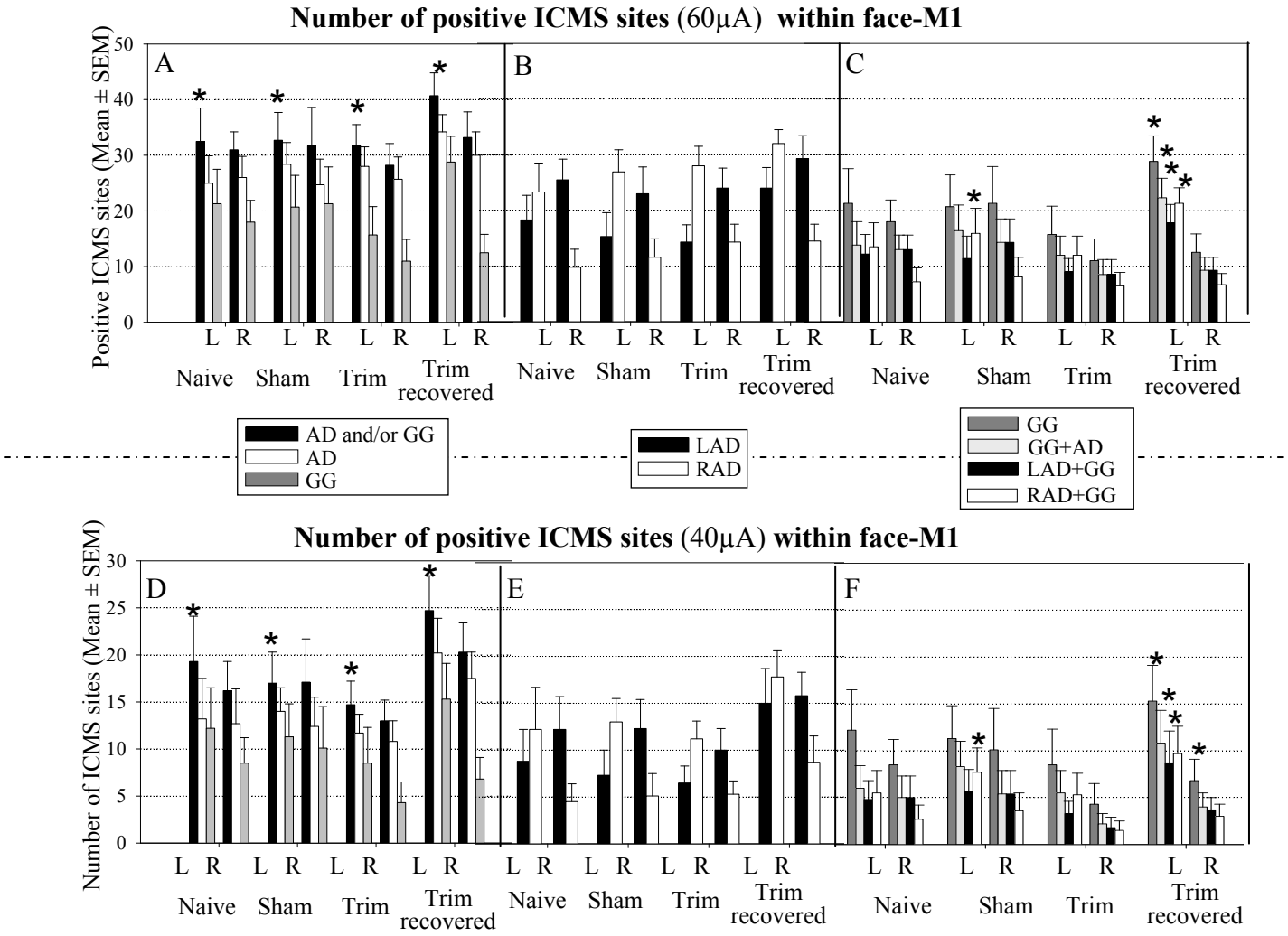


Fig. 4-1: Representative motor maps of LAD, RAD and GG in a rat from the naïve group as compared with a rat from the trim, sham trim and trim recovered groups, each having a mean number of positive ICMS-sites close to the mean of its group. Any site where ICMS could evoke LAD, RAD or GG EMG activity was plotted on the corresponding cortical coronal section, from AP 2.5 to AP 4.0 mm anterior to Bregma: LAD in blue circles, RAD in green circles and GG red circles. Black dots represent sites where ICMS could not evoke EMG activity in LAD, RAD or GG muscles. Note the extensive, bilateral representation of LAD, RAD and GG.



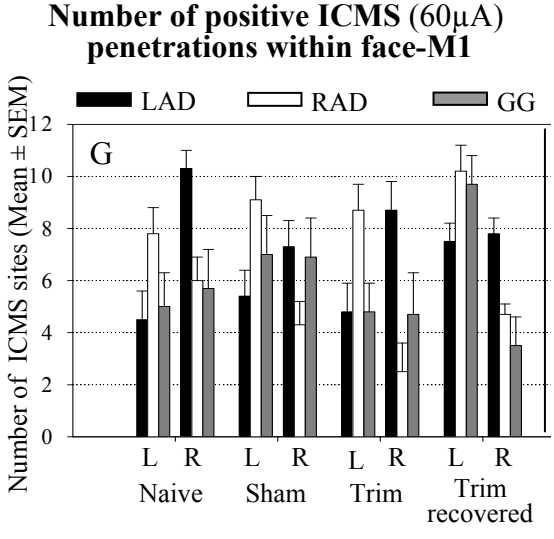


Fig. 4-2. Positive ICMS sites at ICMS intensities of 60 (**A-C**) and 40μA (**D-F**) and penetrations at ICMS intensity of 60 μA (**G**). There were no significant differences across the study groups in the number of LAD, RAD or GG positive ICMS sites or penetrations in either left or right face-M1. **A and D:** The overall number of AD and GG positive ICMS sites was significantly larger in the left face-M1 than in the right face-M1 (*MMRM, Bonferroni: $p=0.047$). **B and E:** LAD and RAD had a significant contralateral predominance (*MMRM, Bonferroni: $p<0.0001$). **C and F:** only in the trim recovered group in comparison with the right face-M1, the left face-M1 had significantly more positive sites for GG (*MMRM, Bonferroni: $p=0.0027$), AD/GG overlapping sites (*MMRM, Bonferroni: $p<0.0001$), LAD/GG overlapping sites (* MMRM, Bonferroni=0.0081) and only in the trim recovered and control groups were there significantly more RAD/GG overlapping sites within the left than within the right face-M1 (*MMRM, Bonferroni: $p=0.084$, $p<0.0001$, respectively).

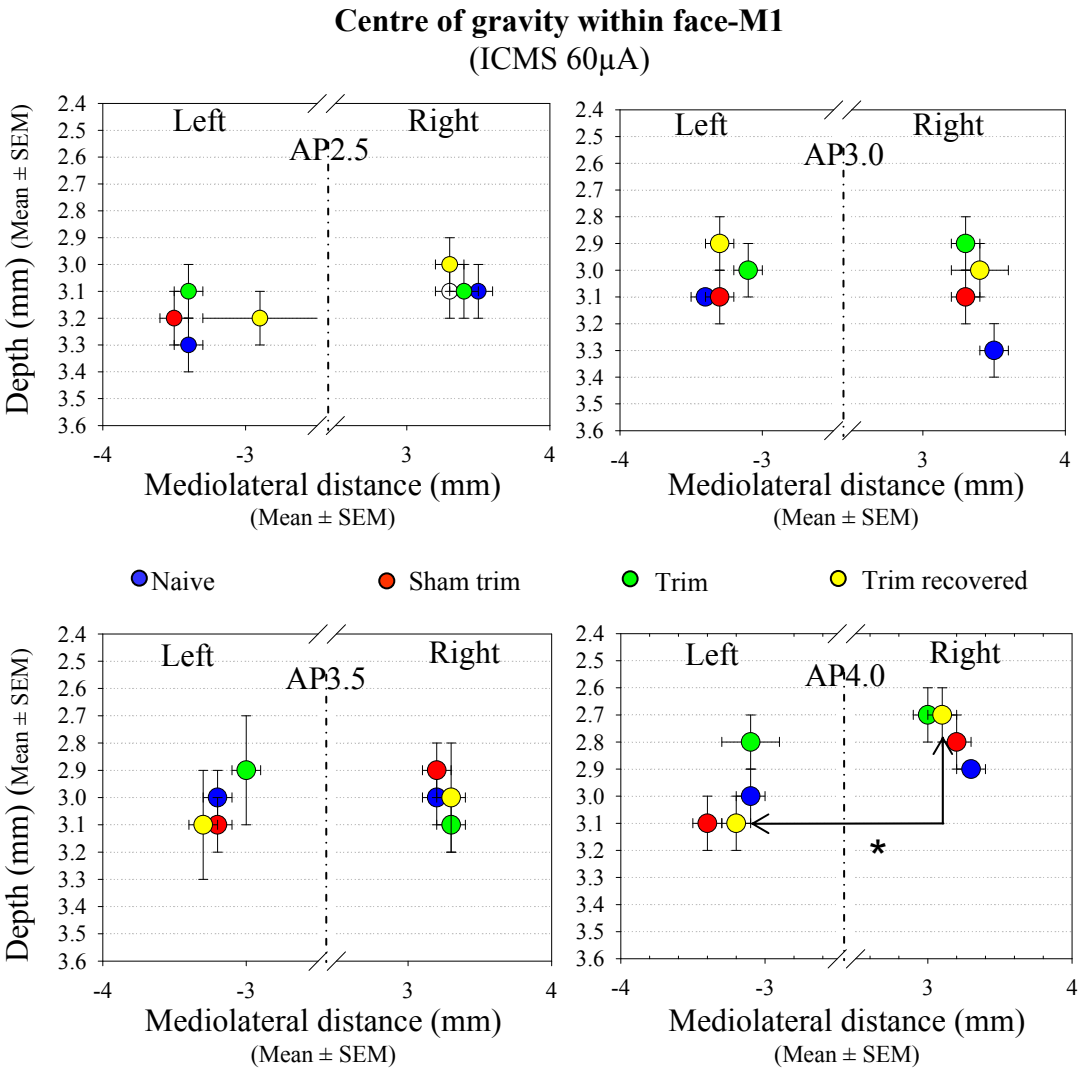


Fig. 4-3. The centre of gravity (Mean ± SEM) of the overall representation of AD and GG at each of the AP mapping planes (AP 2.5, 3.0, 3.5, 4.0). There were no significant differences across the study groups. At AP4.0, only in the trim recovered group, the depth position was significantly deeper in the left face-M1 than in the right face-M1 (* MMRM: p=0.049; Bonferroni: p=0.026).

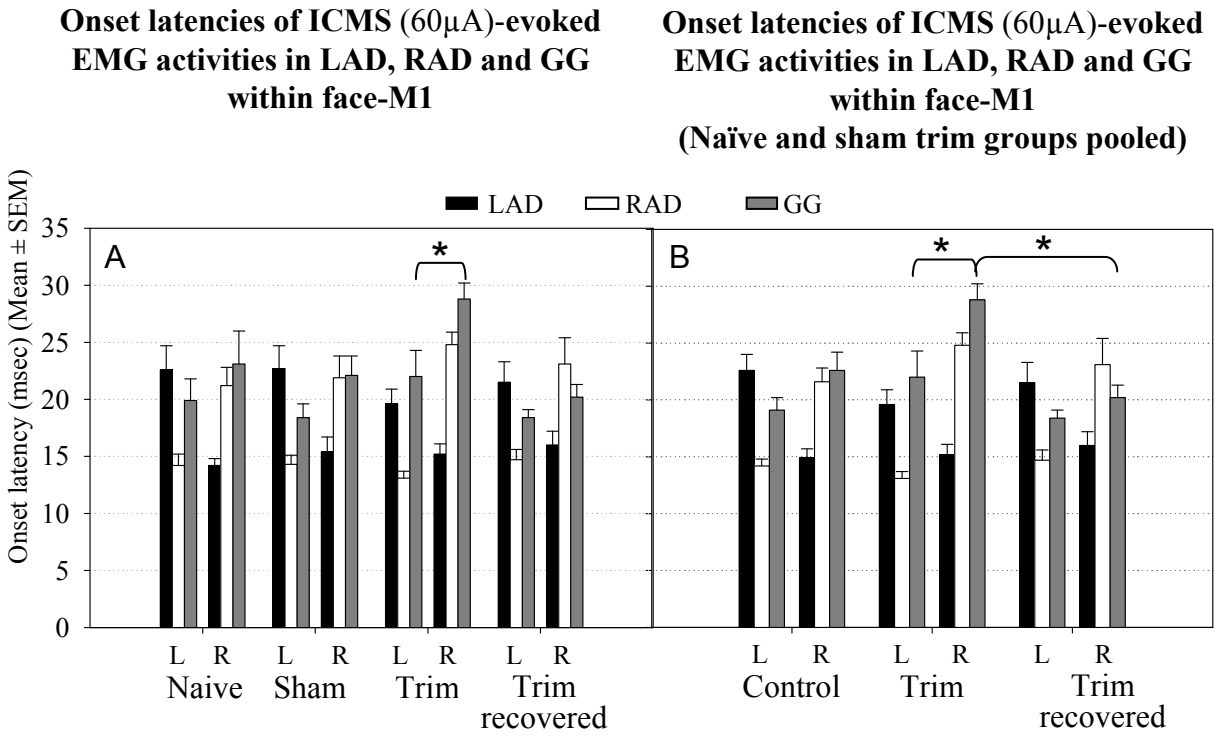


Fig. 4-4. A: Onset latencies of ICMS-evoked EMG responses in LAD, RAD and GG. There were no significant differences across the study groups. In all groups, LAD had a significantly shorter onset latency within the right face-M1 and RAD had a significantly shorter onset latency within the left face-M1 (paired *t-test*, $p<0.05$). Only in the trim group did GG have a significantly longer onset latency within the right face-M1 than within the left face-M1 (*paired *t-test*, $p<0.001$). **B.** Similar results were obtained following pooling the naïve and sham trim groups into one control group, except that after pooling, within the right face-M1, GG onset latency was significantly longer in the trim group than in the trim recovered group (*ANOVA: $p=0.022$; Bonferroni: $p=0.027$) and showed a trend towards being significantly longer than in the control group (*$p=0.062$) (Fig. 4-4B). There were no significant differences across the groups in LAD, RAD and GG onset latency within the left face-M1.

CHAPTER 5

JAW AND TONGUE MOTOR REPRESENTATIONS WITHIN FACE PRIMARY MOTOR CORTEX OF ADULT RATS: EFFECT OF INCISOR EXTRACTION

1. Abstract

Numerous studies have documented sensorimotor cortical neuroplasticity following peripheral tissue injury but limited data are available of the neuroplastic capabilities of the face primary motor cortex (face-M1) following intraoral manipulations and no study has addressed whether neuroplastic changes may occur in face-M1 following the loss of teeth.

Objectives: to use intracortical microstimulation (ICMS) and recordings of evoked muscle electromyographic (EMG) activity to test if changes occur in the ICMS-defined motor representations of the right and left anterior digastric (RAD, LAD) and genioglossus (GG) muscles within the rat face-M1 and adjacent face primary somatosensory cortex (face-S1) following unilateral extraction of the mandibular incisor tooth.

Methods: Adult male Sprague-Dawley rats (200-250gr) were divided into 3 groups. Under local and general anaesthesia, an "extraction" group (n=8) received muco-alveolar bone surgery and extraction of the mandibular right incisor; and a "sham extraction" group (n=6) received surgery with no extraction. A "naïve" group (n=6) had neither surgery nor extraction. One week later, under general anaesthesia (ketamine-HCl), ICMS (5x33.2ms train, 12x0.2ms pulses, 333Hz; ≤60μA) was applied within the left and right face-M1 and adjacent face-S1 in a systematic series of microelectrode penetrations extended from 2.5 to 4.0 mm anterior and 1.5 to 5.5 mm lateral to Bregma with a spatial resolution of 0.5 mm horizontally and ICMS was applied every 0.2 mm of microelectrode penetration depth. Histologically confirmed sites for which ICMS could evoke EMG activity in GG, RAD or LAD were considered to be "positive ICMS sites". Statistical analyses included a series of ANOVAs followed by *post-hoc* Bonferroni-adjusted pairwise comparisons where necessary, $p<0.05$.

Results: Extraction of the right mandibular incisor was associated with significant neuroplastic changes in the ICMS features of the jaw and tongue motor representations within the contralateral left face-M1 and adjacent face-S1, reflected in an increased number of positive ICMS sites for which RAD EMG responses could be evoked (M1: Sham-23.2±4.2 (mean±SEM), naïve-23.3±5.2, extraction-51.3±4.6; S1: sham-6.7±1.7,

naïve-4.8±1.3, extraction-21.3±3.0) as well as a lateral shift of the LAD and RAD centre of gravity in face-M1 (RAD: sham-3.3mm±0.1mm (mean±SEM), naïve-3.2±0.1, extraction-3.7±0.1; LAD: sham-3.2±0.1, naïve-3.2±0.1, extraction-3.6±0.1).

Conclusion: Unilateral dental extraction may be associated with significant neuroplastic changes in the AD motor representation within the rat's contralateral face-M1 and adjacent face-S1 that are evident by 1 week after the extraction. These changes may be related to the animal's ability to adapt to the altered oral state.

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2. Introduction

The primary motor cortex representing the orofacial region (face-M1) plays a crucial role in the generation and control of orofacial motor functions (*e.g.* jaw opening, tongue protrusion, mastication). This role is evident from electrophysiological studies in subprimates and primates employing intracortical microstimulation (ICMS), single neuron recordings, reversible cold block or lesioning techniques (for reviews, see Ebner, 2005; Murray et al., 2001; Sessle, 2006; Sessle et al., 1999). Analogous studies have revealed that the somatosensory system including the primary somatosensory cortex (face-S1) may also play a role in the control of orofacial movements (for reviews, see Ebner, 2005; Murray et al., 2001; Sessle et al., 1999). This is supported by the existence of 2 parallel projections of direct (through the thalamus) (Hatanaka et al., 2005; Miyashita et al., 1994; Rausell and Jones, 1995; Simonyan and Jurgens, 2005) and indirect (through face-S1) (Chakrabarti and Alloway, 2006; Hoffer et al., 2005; Hoffer et al., 2003; Iyengar et al., 2007; Izraeli and Porter, 1995; Miyashita et al., 1994) somatosensory inputs to face-M1 that provide important peripheral feedback from the orofacial tissues including the teeth (Farkas et al., 1999; Murray et al., 2001; Murray and Sessle, 1992a).

One striking finding of these studies is that ICMS can evoke movement or electromyographic (EMG) activity in orofacial muscles from a large area of M1, indicating an extensive orofacial motor representation (Adachi et al., 2007; Burish et al., 2008; Carvell et al., 1996; Clark and Luschei, 1974; Hoffman and Luschei, 1980; Luschei et al., 1971; Luschei and Goodwin, 1975; Neafsey et al., 1986; Yamamura et al., 2002; for reviews, see Ebner, 2005; Murray et al., 2001). Numerous studies conducted primarily in that part of M1 representing the vibrissae have revealed its neuroplastic capabilities, whereby its motor representations can be altered by peripheral manipulations of vibrissal sensory inputs or motor outputs (Donoghue et al., 1990; Franchi, 2000a; Franchi, 2000b; Franchi, 2001; Franchi et al., 2006; Franchi and Veronesi, 2004; Huntley, 1997a; Huntley, 1997b; Jacobs and Donoghue, 1991; Keller et al., 1996; Sanes et al., 1990; Sanes et al., 1988; Toldi et al., 1996; for reviews, see Buonomano and Merzenich, 1998; Ebner, 2005; Sanes and Donoghue, 2000).

Neuroplastic changes may also occur in that part of face-M1 representing the tongue following training humans and monkeys in a novel tongue protrusion task (Boudreau et al., 2007; Svensson et al., 2003b; Svensson et al., 2006; Yao et al., 2002c; for reviews, see Sessle et al., 2007; Sessle and Yao, 2002). These studies suggest that face-M1 has the capacity to undergo neuroplastic changes and be remodelled throughout life. Very limited published data are available, however, on the neuroplastic capabilities of face-M1 following intraoral manipulations (Adachi et al., 2007) and no study has addressed whether loss of teeth induces neuroplastic changes in the ICMS features of face-M1 and adjacent face-S1. Yet loss of teeth is a common clinical occurrence in humans that may be associated with changes in sensorimotor behaviour (Carlsson, 1984; Feine and Carlsson, 2003; Fontijn-Tekamp et al., 2000; Haraldson and Zarb, 1988). In rats, tooth extraction or unilateral trimming of a tooth out of occlusion produces after 1-2 weeks morphological changes in the mandibular condyles, masticatory muscles and periodontal ligaments which may lead to alterations in masticatory motor functions (Endo et al., 1998; Miehe et al., 1999; Ramirez-Yanez et al., 2004; Shi et al., 2005). Since it is possible that such alterations in motor functions may be associated with neuroplastic changes within face-M1 that may reflect the animal's ability to adapt its motor functions to the altered oral state, the objective of this study was to use ICMS and recordings of evoked muscle EMG activity to test if changes occur in the ICMS-defined motor representations of right and left jaw (anterior digastric, RAD, LAD) and tongue (genioglossus, GG) muscles within the rat face-M1 and adjacent face-S1 following unilateral extraction of the mandibular incisor tooth.

3. Materials and Methods

All experimental procedures were approved by the University of Toronto Animal Care Committee, in accordance with the Canadian Council on Animal Care Guidelines and the regulations of the Ontario Animals for Research Act (R.S.O. 1990). All experimental procedures and data analysis were carried out by 1 investigator to ensure consistency in the experimental procedures and data analysis. Most of the procedures

have been described in detail in chapter 2 and therefore, only an abbreviated outline follows.

3.1. Animals

Experiments were performed on young adult male Sprague-Dawley rats (150-250g on arrival, 300-400g on day of cortical mapping) housed in similar conditions and receiving water and mashed diet (Rodent diet #2018M, Harlan Teklad) *ad libitum*. Animals were monitored on a daily basis to assess body weight and food consumption, general behaviour (Chudler and Byers, 2005) and any post-operative complication such as bleeding or inflammation. Buprenorphine hydrochloride 0.05 mg/kg was given S.C. every 8-12 hours during the first post-operative day.

3.2. Study groups and dental procedures

Rats were divided into 3 groups. Under general anaesthesia supplemented by local anaesthesia, an "extraction" group (n=8) received mucoalveolar bone surgery and extraction of the right mandibular incisor (for details, see Chapter 2). A "sham extraction" group (n=6) received the same extraction protocol as the extraction group but the tooth was not actually extracted. Rats in a "naïve" group (n=6) received neither surgical treatment nor tooth extraction.

3.3. ICMS and EMG recordings

The ICMS technique applied in the present study is detailed in chapter 2. ICMS mapping was carried out within the face-M1 and adjacent face-S1 of rats to define the motor representations of jaw and tongue muscles. Mapping was carried out 1 week following the incisor extraction. Rats were maintained throughout the ICMS experiments under a stable level of general anaesthesia with ketamine HCL (Ketaset®, Ayerst Veterinary Laboratories, Ontario, Canada). EMG electrodes (40-gauge, single-stranded, Teflon-insulated stainless-steel wires) were used to record EMG activity from the LAD, RAD, left or right masseter as well as GG, vibrissal and neck muscles. Systematic mapping extended from 2.5 to 4.0 mm rostral to Bregma (*i.e.*, anteroposterior (AP)

planes 2.5, 3.0, 3.5 and 4.0) and 1.5 to 5.5 mm lateral to Bregma within the left and right face-M1 and with a horizontal spatial resolution of 0.5 mm. In each penetration site, ICMS was applied every 0.2 mm of microelectrode penetration depth. Five ICMS trains (at 333 Hz, 33.2 msec, 12 pulses of 0.2 msec, 2.8 msec inter-pulses intervals) were delivered at 1 Hz with suprathreshold ICMS intensity of 60 μA. If ICMS could effectively evoke GG and/ or AD EMG responses, then a series of 5 ICMS trains was delivered at 60, 40, 20 and 60 μA. Electrolytic lesions were made for subsequent histological confirmation of ”positive ICMS sites” (see below) within the gray matter of the S1 or M1.

3.4. Data acquisition and analysis

The data acquisition and analysis are detailed in chapter 2. ICMS sites were defined as a “positive ICMS sites” if at least 3 out of the 5 ICMS (40 or 60 μA) trains evoked an EMG response with onset latency ≤ 40msec and a peak activity exceeding the mean value of the initial 10 msec of the EMG response plus 2 standard deviations (SDs). For each muscle the number of positive ICMS sites was counted and the onset latency for evoking the EMG responses was noted. An ICMS penetration was defined and counted as a “positive ICMS penetration” if it had at least 1 positive ICMS site. In addition, within each penetration, for each muscle, the AP and ML positions of the positive ICMS (60 μA) penetrations were noted. In addition, within each penetration the shortest onset latency was noted for each muscle. Cortical motor maps were used to illustrate the representation areas of LAD, RAD and GG muscles within the sensorimotor cortex of rats from each of the study groups. Positive ICMS sites for LAD, RAD and GG were plotted on a corresponding histological coronal section (AP 2.5-4.0 mm anterior to Bregma) (Fig. 5-1). The AP, ML and depth positions of the centre of gravity weighted relative to the extent of the motor representations or relative to the shortest onset latency within each penetration were calculated for each of the LAD, RAD or GG muscles.

3.5. Statistical Analyses

As described in chapter 2, statistical differences between groups and the effects of the independent variables (study group, cortical side, and ICMS intensity) on the dependent variables (number of positive ICMS sites or penetrations, onset latency, ML position and the centre of gravity) were determined using a series of ANOVAs, and mixed model repeated-measures (MMRM) analyses (multivariate analyses) followed by *post-hoc* Bonferroni-adjusted pairwise comparisons as appropriate. In addition, paired Student's *t-test* was used for within-group comparisons of the onset latencies between left and right sensorimotor cortex and of the body weight before and after tooth extraction. A probability level of $p<0.05$ was considered statistically significant.

4. Results

Rats were monitored on a daily basis and demonstrated normal behaviour and continuous gain of body weight (see Chapter 2 for details).

4.1. General features of AD and GG motor representations

Mapping extended from 2.5 to 4.0 mm anterior and 1.5 to 5.5 mm lateral to Bregma with a spatial resolution of 0.5 mm horizontally and every 0.2 mm of depth (Fig. 5-1). For each study group, the calculated mean numbers of positive ICMS-sites that could evoke EMG activity in AD and/or GG reflect the muscles' motor representations within the mapped area (Fig. 5-1).

ICMS of the left and right face-M1 evoked EMG activity in ipsilateral and contralateral AD and/ or GG muscles. AD and GG EMG activity could also be evoked by ICMS of the left and right face-S1 (Fig. 5-1). ICMS evoked EMG responses simultaneously in both AD and GG (*i.e.,* overlapping representation) from many sites. The general features of the AP–ML-depth positions and the extent of AD and GG representations, as well as the mean onset latency of evoked responses (see below), were similar in the naïve and sham extraction rats.

Within the mapped area there were very few positive ICMS sites for the masseter (extraction: 0.55 ± 0.33 (mean ± SEM), Sham: 1.17 ± 0.75, Naïve: 0.00) and neck (extraction: 3.88 ± 1.67, Sham: 4.75 ± 1.84, Naïve: 3.5 ± 1.37). ICMS could also occasionally evoke vibrissal EMG activity which was confounded by frequent spontaneous movements of the vibrissae. Therefore, the ICMS data for the masseter, vibrissae and neck ICMS data were not included in the general data analysis.

4.2. Effects of tooth extraction

4.2.1. AD and GG motor representations

There were no significant differences between the sham extraction and naïve groups in any of the study measures, and there was no significant effect of tooth extraction on the number of AD positive ICMS sites within the right face-M1 or on GG sites within the left or right face-M1. However, multivariate analyses revealed a significant interaction between study group and cortical side for AD (Table 5-1). In the left face-M1, the number of AD sites was significantly larger in the extraction group as compared with the sham extraction and naive groups (MMRM, Bonferroni: $p=0.0009$ and $p=0.0036$ respectively). Univariate analysis revealed that this was the case evident at both 40 and 60 µA ICMS intensities (Figs. 5-2A, 5-2D). In addition, only in the extraction group was the number of AD positive ICMS sites significantly larger in the left than in the right face-M1 (MMRM, Bonferroni: $p=0.0018$).

In all study groups, there was a significant cortical side effect for both LAD and RAD (Table 1); LAD and RAD had bilateral representations within face-M1, with a contralateral predominance (MMRM, Bonferroni: $p<0.0001$). In addition, there was a significant interaction between study group and cortical side for RAD sites, but not for LAD sites, within the left face-M1, but not the right face-M1 (Table 5-1); within the left face-M1, the number of RAD positive ICMS sites was significantly larger in the extraction group than in the sham extraction and naïve groups (MMRM: Bonferroni: $p<0.0001$). This was the case at both 40 and 60 µA ICMS intensities (Figs. 5-2B, 5-2E). However, tooth extraction had no significant effect on the number of LAD sites across the study groups in either the left or the right face-M1 (Table 5-1, Figs. 5-2B, 5-2E).

In all groups, at a large proportion (42-45%) of the overall positive ICMS sites, ICMS evoked EMG activity in more than 1 muscle (any combination of LAD, RAD and GG), *i.e.,* overlapping representations (Figs. 5-1, 5-2C, 5-2F). Consequently, the number of RAD sites included sites for which ICMS activated only the 1 muscle (*i.e.,* RAD-only) and sites for which ICMS activated additional muscles (*i.e.,* RAD and LAD, or RAD and GG). The number of RAD-only sites was significantly larger in the extraction group than in the sham extraction and naïve groups at both 40 and 60 µA ICMS intensities (40 µA - ANOVA: p=0.0036, Bonferroni: p=0.0049 and 0.031, respectively; 60 µA - ANOVA: p=0.0004; Bonferroni: p=0.0065 and 0.0042, respectively). There was a significant study group effect for RAD/GG overlapping sites (Table 5-1). The number of RAD/GG overlapping sites was significantly larger in the extraction group than in the naïve group but there was only a trend in comparison with the sham extraction group (MMRM: Bonferroni: p=0.027 and 0.077, respectively). Univariate analysis revealed no significant differences across the study groups for RAD/GG overlapping sites at either 40 or 60 µA ICMS intensities (Fig. 5-2C, 5-2F).

Tooth extraction also affected the number of AD (but not GG) positive ICMS sites within the left face-S1 but not the right face-S1. While multivariate analysis revealed no study group effects, univariate analysis revealed that in the left face-S1, the numbers of AD sites and RAD sites were significantly larger in the extraction group than in the sham extraction and naïve groups at 60 µA ICMS intensity (Figs. 5-4A, 5-4B).

4.2.2. Number and distribution of positive ICMS penetrations

When the ICMS (60 µA) data were also analysed in terms of the number of positive ICMS penetrations from which ICMS evoked EMG activity in LAD, RAD or GG, this analysis also revealed no significant differences between sham extraction and naïve groups in any of the study measures. However, tooth extraction significantly affected the number of RAD (but not LAD or GG) penetrations within the left face-M1 but not the right face-M1 (MMRM: F=5.95, df=2,17, p=0.011). In the left face-M1, RAD had a significantly larger number of positive ICMS penetrations in the extraction group as compared with the sham extraction and naïve groups (Bonferroni p=0.014 and 0.021, respectively) (Fig. 5-3).

The ML distributions of the positive ICMS penetrations are illustrated in Fig. 5-5. In the left face-M1, but not the right face-M1, the mean ML position of all positive ICMS penetrations (irrespective of the muscle and the AP position) was significantly more lateral in the extraction group than in the sham extraction group but not the naïve group (3.6mm ± 0.1mm, 3.2 ± 0.1, 3.3 ± 0.1 respectively; ANOVA: p=0.025; Bonferroni: p=0.029 and 0.19 respectively). In addition, the position of the most lateral positive ICMS penetration was significantly more lateral in the extraction group as compared with the sham extraction and naïve groups (4.8mm ± 0.1mm, 3.9 ± 0.2, 4.3 ± 0.1 respectively; ANOVA: p=0.0001, Bonferroni: p=0.0001 and 0.025 respectively). There were no significant differences across the groups in the AP position of the positive ICMS penetrations (extraction: 3.1 ± 0.0, Sham: 3.1 ± 0.1, Naïve: 3.1 ± 0.1, respectively; ANOVA: p=0.41).

4.2.3. Centre of gravity within face-M1

Tooth extraction significantly affected the position of the centre of gravity within the left face-M1 but not the right face-M1 (Fig. 5-6). The RAD and LAD centres of gravity weighted against the number of positive ICMS (60 μA) sites occurred significantly more lateral in the extraction group as compared with the sham extraction and naïve groups (MMRM: RAD: p=0.0007, LAD: p=0.0002; Bonferroni: RAD: p=0.049 and 0.009, respectively; LAD: p=0.023 and 0.0009, respectively). The RAD and LAD centres of gravity in the left face-M1 also had a trend towards a more superficial position (MMRM: p=0.0015, Bonferroni: p=0.077 and 0.1 respectively). For each muscle, the mean AP position of the centre of gravity was between AP 3.0 and AP 3.5, with no significant differences across the groups or between left and right face-M1. There were no significant differences across the groups for the AP, ML and depth positions of the GG centre of gravity. However, when the centre of gravity was weighted against the mean onset latency, the GG as well as the RAD and LAD centres of gravity occurred significantly more lateral in the extraction group as compared with the sham extraction and naïve groups (MMRM: p=0.0001, Bonferroni: p<0.0001).

4.2.4. Onset latency of ICMS-evoked EMG activity

In all rats, ICMS within face-M1 evoked EMG activity in LAD, RAD and/ or GG with a wide range of onset latencies (8 - 40 msec). There were no significant differences across the study groups in the mean onset latencies of evoked EMG responses in LAD, RAD or GG in either the left or the right face-M1, although, the mean onset latency for LAD or RAD responses were significantly shorter in the contralateral face-M1 as compared with the ipsilateral face-M1 (paired *t-test*, $p<0.05$)(Table 5-2). In addition, many of S1 positive ICMS sites had short onset latencies of < 10msec, comparable to those in face-M1 (t-test, $p>0.05$).

5. Discussion

The novel finding of the present study is that extraction of the right mandibular incisor of adult rats was associated, 1 week later, with significant neuroplastic changes in the AD motor representation within the contralateral face-M1 and adjacent face-S1. Tooth extraction resulted in a significant increase in the number of positive ICMS sites and penetrations for RAD, RAD-only and RAD/ GG overlapping sites. There was a significant lateral shift in the mediolateral position of the centre of gravity of the LAD and RAD positive ICMS sites, and the mean ML position of the positive ICMS penetrations. Some analogous changes were also documented in the adjacent face-S1. These findings collectively suggest that face-M1 has the capability to undergo neuroplastic changes in association with dental extraction and that these changes may contribute to sensorimotor behavioural adjustments to tooth loss.

The ICMS mapped area within the left and right face-M1 extended between 2.5 – 4.0 mm anterior and 1.5 – 5.5 mm lateral to Bregma. The sham extraction and naïve groups did not differ in the ICMS features within this mapped area. There was a large bilateral motor representation of AD and GG within face-M1 and a significant contralateral predominance for RAD and LAD. These findings are consistent with previous studies in rats (see also chapter 3 and 4; Adachi et al., 2007; Donoghue and Wise, 1982; Gioanni and Lamarche, 1985; Lee et al., 2006; Neafsey et al., 1986; Sanderson et al., 1984), monkeys (Burish et al., 2008; Clark and Luschei, 1974; Huang et al., 1989b; Huang et al., 1988; Murray and Sessle, 1992a; Murray and Sessle, 1992b;

Murray and Sessle, 1992c) and in humans (Boudreau et al., 2007; Gooden et al., 1999; Hamdy et al., 1996; Hamdy et al., 1999; Martin et al., 2004; Meyer et al., 1997; Nordstrom, 2007; Svensson et al., 2003b; Svensson et al., 2006). AD and GG had motor representations also within face-S1, consistent with earlier findings in rats (Donoghue and Wise, 1982; Neafsey et al., 1986; Sapienza et al., 1981) and marmosets (Burish et al., 2008). These findings of face-S1 motor outputs are also supported by anatomical studies in rats and monkeys showing efferent projections from S1 to brainstem motoneurons (Grinevich et al., 2005; Jones, 1976; Rathelot and Strick, 2006; Wise and Jones, 1977a; Zhang and Sasamoto, 1990) and support the view that face-S1 may play a role in the control of orofacial movements (Farkas et al., 1999; Inoue et al., 1989; Murray et al., 2001; Yao et al., 2002b).

In addition, ICMS evoked AD and GG EMG activities simultaneously from many sites within the face-M1. Such overlapping of LAD, RAD and GG motor representations within face-M1 has been reported in other studies in rats and monkeys and is considered to be important for the dynamic bilateral coordination of orofacial movements involving the action of several muscles (Burish et al., 2008; Gioanni and Lamarche, 1985; Huang et al., 1988; Murray and Sessle, 1992a; Murray and Sessle, 1992b; Neafsey et al., 1986; Sessle and Wiesendanger, 1982) (see Chapters 3 and 4).

5.1. Effects of tooth extraction

5.1.1. Neuroplasticity associated with altered somatosensory inputs

The orofacial tissues including teeth and their periodontal tissues are characterized by a high tactile sensitivity attributed to a rich innervation density (for reviews, see Macefield, 2005; Miles, 2005; Paxinos, 2004), and prominent representation within face-S1 (Catania and Remple, 2002; Iyengar et al., 2007; Kaas et al., 2006; Remple et al., 2003). Face-M1 also receives somatosensory inputs from orofacial tissues including the teeth (Miyashita et al., 1994; Murray and Sessle, 1992a; Yao et al., 2002b), either directly through the thalamus (Hatanaka et al., 2005; Henry and Catania, 2006; Rausell and Jones, 1995; Simonyan and Jurgens, 2005), or indirectly through face-S1 (Chakrabarti and Alloway, 2006; Hoffer et al., 2005; Huffman and

Krubitzer, 2001b; Huntley, 1997a; Iyengar et al., 2007; Izraeli and Porter, 1995; Keller et al., 1996; Lin et al., 1993; Lin et al., 1998; Porter, 1996; Yao et al., 2002b; for review, see Kaas et al., 2006), and these inputs may provide peripheral feedback needed for the control of orofacial motor functions (Miyashita et al., 1994; Murray et al., 2001; Murray and Sessle, 1992a; Murray and Sessle, 1992b; Murray and Sessle, 1992c; Yao et al., 2002b).

Earlier studies in rats and in humans have shown that alterations in somatosensory inputs induced by deafferentation may result in changes in face-M1 motor representations. In rats, somatosensory deafferentation induced by transection of the lingual nerve supplying the tongue results, 1- 4 weeks later, in significant time-dependent changes of the GG representation within face-M1 (Adachi et al., 2007). Furthermore, deafferentation of the infraorbital nerve supplying sensory innervation of the vibrissae results, 2-3 weeks later, in a significant decreased excitability (increased threshold) of face-M1 representing vibrissal movements (Franchi, 2001). In humans, TMS (transcranial magnetic stimulation) studies have reported that peripheral deafferentation induced by lingual nerve anaesthesia is associated with decreased excitability of face-M1 representing the tongue (Halkjaer et al., 2006), although local anaesthesia to lower facial skin produces increased excitability of face-M1 representing the peri-oral muscles (Yildiz et al., 2004).

Tooth extraction is associated with irreversible deafferentation of pulp and periodontal ligament afferents. Therefore, dental extraction may conceivably alter the exteroceptive, proprioceptive and perhaps nociceptive inputs from the oral cavity to face-S1 and face-M1 that could account for the documented changes in the motor representations within face-M1. Since it is indeed well known that dental and other types of intraoral surgery are associated with post-operative pain (Labanc, 1992; Robinson et al., 2004), the possible confounding effect of post-operative pain in the extraction group cannot be excluded as a factor in the neuroplastic changes observed in the present study. However, this is unlikely since the sham extraction group, which also underwent intraoral surgery, did not show any changes within face-M1 and its ICMS features did not differ from those of the naïve group. Furthermore, intraoral pain induced by injection

of the algesic glutamate into the tongue in rats and application of capsaicin to the tongue in healthy humans (Adachi et al., 2008; Boudreau et al., 2007) is associated with decreased face-M1 excitability. In contrast, the present study documented that tooth extraction induced an increased RAD motor representation that is suggestive of increased face-M1 excitability (Monfils et al., 2005; Ridding and Rothwell, 1997).

5.1.2. Neuroplasticity associated with altered sensorimotor functions

Somatosensory inputs from the orofacial tissues to face-M1 and face-S1 provide important peripheral feedback that is crucial for the control of orofacial motor functions (Johansson et al., 2006; for reviews, see Haas and Lennon, 1995; Kaas et al., 2006; Murray et al., 2001; Trulsson and Essick, 2004). For example, it is well know in humans that changes in sensory inputs as a result of mandibular (sensory) nerve block are associated with motor deficits reflected in drooling, tongue biting and difficulties with speaking (Haas and Lennon, 1995) and in rabbits bilateral transection of the mandibular and maxillary (sensory) nerves results in altered patterns of mastication (Inoue, 1989). Therefore, alterations in peripheral somatosensory inputs from the teeth and periodontium can induce changes in oral motor behaviour and altered pattern of mastication that may in turn affect proprioceptive as well as exteroceptive inputs/feedback from the orofacial tissues involved in the altered orofacial movements and thereby may indirectly (through the altered somatosensory inputs) contribute to the observed changes in face-M1 motor representations following dental extraction.

In addition to changes in oral motor functions induced by altered peripheral somatosensory inputs/ feedback, changes in the dental occlusion may also alter patterns of jaw movements in humans (for review, see Johansson et al., 2006; Klineberg and Jagger, 2004; Proschel and Hofmann, 1988; Trulsson and Essick, 2004). In rats, dental extraction results in morphological changes in the mandibular condyles and masticatory muscles (Endo et al., 1998; Miehe et al., 1999) that suggest alterations had occurred in oral motor functions. Such changes in oral motor functions may also contribute to the changes in motor representations within face-M1. Indeed, numerous studies conducted primarily in limb-M1 have revealed use-dependent changes in the limb motor representations following training in a novel limb motor skill (Kleim et al., 1998; Nudo

et al., 1996; Remple et al., 2001; for review, see Barbay et al., 2005). Consistent with these studies, training monkeys (Sessle et al., 2007; Sessle et al., 2005) and humans (Boudreau et al., 2007; Svensson et al., 2003b; Svensson et al., 2006) in a novel tongue protrusion task is associated with a significantly increased tongue motor representation within face-M1.

One of the most consistent findings involving limb-M1 studies is increased overlapping representations of the movements involved in the acquisition of a limb motor skill (Nudo et al., 1996). Such increased overlapping of motor representation is considered to be crucial for coordinating movements involving more than 1 muscle (Nudo et al., 1996; Sanes et al., 1995). It is interesting to note that, in the present study, dental extraction resulted in not only an increased RAD representation but also increased overlapping representations of RAD and GG. Based on the concept of use-dependent neuroplasticity, it is possible that such increased overlapping following tooth extraction reflects or allows for coordinated jaw and tongue movements to adapt to the altered oral state. The specific changes in the motor representations within face-M1 suggest that unilateral extraction of the mandibular incisor may have induced changes in oral motor behaviour as the rat adapted to the altered oral state and perhaps adopted a novel oral motor behaviour for chewing, gnawing and/ or other oral functions. Such adaptation requires repetition of the novel motor movement/s similar to the occurrence in learning a novel motor skill. Consistent with the concept of use-dependent neuroplasticity, it is possible that these changes in the rat's oral motor behaviour could have induced changes in the motor representation within face-M1. On the other hand, it is possible that the extraction first induced changes in sensory inputs to the face-M1 which then resulted in the face-M1 neuroplastic changes in jaw and tongue motor representations that led to altered oral motor behaviour. Further studies are planned to address these possibilities.

5.2. Changes in other cortical or subcortical areas

Although this study documented changes in face-M1, we cannot rule out the possibility that tooth extraction also induced changes in subcortical relays of descending motor outputs. First, many of the corticobulbar projections are multisynaptic, and

therefore ICMS-evoked responses may involve subcortical relays such as the basal ganglia and red nucleus (Hatanaka et al., 2005; Satoh et al., 2006b; Takada et al., 1999; Takada et al., 1994; Zhang and Sasamoto, 1990). Second, afferent inputs from the orofacial region including the teeth can activate the central pattern generator (CPG) (for review, see Dubner and Sessle, 1978; Lund and Dellow, 1971; Lund and Kolta, 2006b; Lund et al., 1999 2001) and rapidly modulate the activity of brainstem motoneurons controlling tongue and jaw muscles (*e.g.* Goldberg, 1971; Lavigne et al., 1987; Sessle, 1977; Sessle and Schmitt, 1972; Tolu et al., 1993; Tolu et al., 1994a; Tolu et al., 1994b; for review, see Lowe, 1980; Lund and Kolta, 2006b). Third, it has been reported that transection of the facial (motor) nerve induces motor reorganization not just within face-M1 (Toldi et al., 1996) but also within brainstem VII motor and sensory V nuclei (Kis et al., 2004). Nonetheless, intracortical changes are likely the major contributors to the present study findings of neuroplastic changes in the RAD motor representations since increased subcortical synaptic efficacy is expected to be associated with decreased onset latency (and decreased ICMS thresholds) (Asanuma et al., 1976; Butovas and Schwarz, 2003; Ranck, 1975; Ridding and Rothwell, 1997; Stoney et al., 1968b; Tehovnik, 1996; Tehovnik et al., 2006) that was not evident in the extraction group.

Studies involving limb amputation report that neuroplastic changes can occur in limb-M1 motor representations as well as in limb S1 and in subcortical relays of somatosensory information (Dettmers et al., 2001; Lotze et al., 1999; Manger et al., 1996). There is also evidence to suggest that alterations in orofacial somatosensation may induce neuroplastic changes at subcortical as well as cortical levels of the ascending trigeminal somatosensory system (*e.g.* S1, thalamus, brainstem and peripheral nerves) (for review, see Kaas et al., 2008). For example, sensory perturbation induced by capsaicin injection into the lip induces reorganization of the vibrissae and orofacial receptive fields at both thalamic and S1 levels in rats (Katz et al., 1999). Similarly, sensory deprivation induced by perioral or intraoral local anaesthesia in rats induces reorganization of the orofacial receptive fields at both thalamic and S1 levels (Faggin et al., 1997; Nicolelis et al., 1993), and local anaesthesia of the oral or perioral tissues in humans results in neuroplastic changes within face-M1 (Halkjaer et al., 2006; Yildiz et

al., 2004). Dental deafferentation (*i.e.,* tooth extraction, pulp extirpation) is also associated with changes within the trigeminal mesencephalic nucleus (Linden and Scott, 1989), and trigeminal brainstem sensory nuclei (Hu et al., 1986; Hu et al., 1999; Kwan et al., 1993) as well as in face-S1 (Henry et al., 2005). Since there is evidence to suggest that face-M1 receives somatosensory inputs either directly through the thalamus or indirectly through S1 (Henry and Catania, 2006; Miyashita et al., 1994; Welker, 1976), the changes in face-M1 observed in the present study may reflect changes having occurred within face-S1, thalamic or subthalamic relays.

The possible involvement of face-S1 in our observed changes in face-M1 is supported by our novel findings of neuroplastic changes of motor outputs within face-S1 following the peripheral alterations. While the present study is the first to demonstrate ICMS-evoked EMG activities in AD and GG within face-S1, ICMS-evoked jaw and tongue movements were observed in other studies applying ICMS within face-S1 in rats (Donoghue and Wise, 1982; Neafsey et al., 1986; Sapienza et al., 1981) and in marmosets (Burish et al., 2008). While there is anatomical evidence for efferent projections from S1 to motoneurons (Jones, 1976; Wise and Jones, 1977a; Zhang and Sasamoto, 1990), it is also possible that the observed ICMS-evoked EMG activities within face-S1 were the result of spread of stimulating currents from face-S1 to face-M1 either directly or indirectly through axon collaterals (Greenshaw, 1998; Ranck, 1975). However, this is an unlikely explanation of our findings since dental extraction had a significant effect specifically on the RAD representation within the contralateral face-S1 (and face-M1) and RAD representation expanded within face-S1 only in the extraction group but not in the sham extraction and naïve groups.

5.3. Mechanisms underlying face-M1 neuroplasticity

Although the present study did not directly address the mechanisms underlying the observed neuroplastic changes, some insights into possible mechanisms are provided from studies focussing on M1 representing the limbs or vibrissae. The observed changes in the extent of the RAD motor representation in conjunction with a lateral shift of the centre of gravity and the mean position of the ICMS penetrations within 1 week

following tooth extraction, suggest that at least part of the reorganization of motor representations within face-M1 could have been due to asymmetric lateral expansion of RAD motor representation (Abbruzzese and Trompetto, 2002; Cohen et al., 1998) that may have resulted from cortical neuroplastic changes involving mechanisms such as potentiation of existing latent synapses through disinhibition of horizontal inhibitory connections (Farkas et al., 2000; Farkas and Toldi, 2001; Huntley, 1997b; Jacobs and Donoghue, 1991; Keller, 1993) or through long-term potentiation of existing active synapses of axon collaterals (Monfils and Teskey, 2004b; Monfils et al., 2004) for review, see (Buonomano and Merzenich, 1998; Rioult-Pedotti and Donoghue, 2003). This is in distinction to a symmetric expansion of motor representation with no apparent shift of the centre of gravity where ICMS cannot distinguish between changes at the cortical level and changes at subcortical relays (Abbruzzese and Trompetto, 2002; Ridding et al., 2000). However, increased subcortical synaptic efficacy is expected to be associated with decreased onset latency (and decreased ICMS thresholds) (Asanuma et al., 1976; Butovas and Schwarz, 2003; Ranck, 1975; Ridding and Rothwell, 1997; Stoney et al., 1968b; Tehovnik, 1996; Tehovnik et al., 2006). The lack of significant changes in onset latency of RAD (or LAD or GG) responses across the study groups further supports the likelihood that at least some of the observed face-M1 changes in the present study were a result of cortical neuroplastic changes rather than subcortical changes in the excitability of corticofugal projections.

5.4. Clinical implications

Loss of teeth is a common dental occurrence that may be accompanied by impaired oral motor functions, sometimes making the most vital functions of eating and speaking difficult and thereby jeopardizing the patient's quality of life (Brennan et al., 2008; Feine and Carlsson, 2003; Muller et al., 2007; Johansson et al., 2006; Sheiham et al., 2001). In the present study, dental extraction was associated 1 week later with significant neuroplastic changes of motor representations within face-M1. In humans, peripheral deafferentation induced by nerve injury or local anaesthesia of the orofacial tissues is associated with neuroplastic changes in the TMS-defined motor representations

within face-M1 (Halkjaer et al., 2006; Yildiz et al., 2004), and modification to the dental occlusion can alter face-M1 neuronal activity as revealed by fMRI (Kordass et al., 2007). These findings raise the possibility that after loss of teeth, changes in oral somatosensory inputs as well as changes in functional motor behaviour may be associated with neuroplastic changes within face-M1 also in humans. Such cortical changes may allow for or reflect functional adaptation (or maladaptation) of the masticatory system to the altered oral state. In recent years, animal and human models have been used to develop treatment approaches that take advantage of M1 neuroplastic mechanisms to improve functional recovery following neurological disorders (for reviews, see Kaas et al., 2008; Robbins et al., 2008). Thus, further clarification of the cortical effects of oral manipulations and their underlying neuroplastic mechanisms may provide improved therapeutic strategies to ensure the restoration of oral functions and consequently improved quality of life of patients experiencing loss of teeth or other undesirable oral alterations.

Face-M1 Positive ICMS sites
Repeated-measures ANOVA results

Muscle	Predictor	F-statistic, DF, Statistical Significance
AD and/or GG	**Overall Model**	**chi-sq=21.47, df=1, p<0.0001**
	Study group	**F=3.70, df=2,17, p=0.046**
	Cortical side	**F=6.5-0, df=1,17, p=0.021**
	Intensity	**F=31.50, df=1,17, p<0.0001**
	Study group * Cortical side	F=1.70, df=2,17, p=0.21
	Study group * Intensity	F=0.87, df=2,17, p=0.43
	Cortical side * Intensity	F=0.39, df=1,19, p=0.54
LAD	**Overall Model**	**chi-sq=28.40, df=1, p<0.0001**
	Study group	F=3.00, df=2,17, p=0.077
	Cortical side	**F=56.72, df=1,17, p<0.0001**
	Intensity	**F=45.49, df=1,17, p<0.0001**
	Study group * Cortical side	**F=4.68, df=2,17, p=0.024**
	Study group * Intensity	F=0.89, df=2,17, p=0.43
	Cortical side * Intensity	**F=4.60, df=1,19, p=0.045**
RAD	**Overall Model**	**chi-sq=21.00, df=1, p<0.0001**
	Study group	**F=9.24, df=2,17, p=0.0019**
	Cortical side	**F=136.05, df=1,17, p<0.0001**
	Intensity	**F=39.45, df=1,17, p<0.0001**
	Study group * Cortical side	**F=24.55, df=2,17, p<0.0001**
	Study group * Intensity	F=2.81, df=2,17, p=0.088
	Cortical side * Intensity	**F=14.38, df=1,19, p=0.0012**
GG	**Overall Model**	**chi-sq=22.31, df=1, p<0.0001**
	Study group	F=0.68, df=2,17, p=0.52
	Cortical side	F=3.70, df=1,17, p=0.071
	Intensity	**F=9.18, df=1,17, p=0.0076**
	Study group * Cortical side	F=0.06, df=2,17, p=0.94
	Study group * Intensity	F=0.02, df=2,17, p=0.98
	Cortical side * Intensity	F=0.26, df=1,19, p=0.62
AD and GG	**Overall Model**	**chi-sq=16.61, df=1, p<0.0001**
	Study group	**F=4.27, df=2,17, p=0.031**
	Cortical side	F=2.63, df=1,17, p=0.12
	Intensity	**F=21.89, df=1,17, p=0.0002**
	Study group * Cortical side	F=0.75, df=2,17, p=0.49
	Study group * Intensity	F=0.28, df=2,17, p=0.76
	Cortical side * Intensity	F=0.60, df=1,19, p=0.45
AD	**Overall Model**	**chi-sq=22.31, df=1, p<0.0001**
	Study group	**F=9.87, df=2,17, p=0.0014**
	Cortical side	**F=4.67, df=1,17, p=0.045**
	Intensity	**F=53.58 df=1,17, p<0.0001**
	Study group * Cortical side	**F=7.87, df=2,17, p=0.0038**
	Study group * Intensity	F=2.41, df=2,17, p=0.12
	Cortical side * Intensity	F=0.64, df=1,19, p=0.43
RAD and LAD	**Overall Model**	**chi-sq=21.53, df=1, p<0.0001**
	Study group	F=1.97, df=2,17, p=0.17
	Cortical side	**F=8.33, df=1,17, p=0.01**
	Intensity	**F=29.67, df=1,17, p<0.0001**
	Study group * Cortical side	F=2.16, df=2,17, p=0.15
	Study group * Intensity	F=0.89, df=2,17, p=0.43
	Cortical side * Intensity	F=1.39, df=1,19, p=0.25
LAD and GG	**Overall Model**	**chi-sq=16.46, df=1, p<0.0001**
	Study group	F=2.65, df=2,17, p=0.099
	Cortical side	**F=5.10, df=1,17, p=0.037**
	Intensity	**F=26.03, df=1,17, p<0.0001**
	Study group * Cortical side	F=1.78, df=2,17, p=0.20
	Study group * Intensity	F=0.18, df=2,17, p=0.84
	Cortical side * Intensity	F<0.01, df=1,19, p=0.98
RAD and GG	**Overall Model**	**chi-sq=5.60, df=1, p=0.018**
	Study group	**F=5.21, df=2,17, p=0.017**
	Cortical side	**F=23.50, df=1,17, p=0.0002**
	Intensity	**F=19.00, df,1,17, p=0.0004**
	Study group * Cortical side	F=3.18, df=2,17, p=0.067
	Study group * Intensity	F=0.45, df=2,17, p=0.65
	Cortical side * Intensity	F=3.10, df=1,19, p=0.094

Table 5-1. Mixed model repeated-measures ANOVA, followed by *post-hoc* Bonferroni-adjusted pairwise comparisons where applicable, was used in order to determine whether study group, cortical side, stimulation intensity (40 vs 60μA), or any combination of these effects significantly affected the number of positive ICMS-sites. These tests were performed separately for each muscle and each combination of muscles.

Onset latencies of ICMS (60μA) **-evoked EMG activities in LAD, RAD and GG within face-M1**
(Mean ± SEM)

Group	Muscle	Left face-M1	Right face-M1
Extraction	RAD	**12.3 ± 0.6***	21.0 ± 2.6
	LAD	20.5 ± 2.2	**13.3 ± 1.1***
	GG	18.1 +/- 1.7	18.9 +/- 0.6
Sham	RAD	**13.6 ± 0.6***	21.50 ± 1.5
	LAD	18.8 ± 1.5	**14.6 ± 1.5***
	GG	23.8 +/- 2.1	**22.4 +/- 2.9**
Naïve	RAD	**14.2 ± 1.0***	21.20 ± 3.47
	LAD	22.5 ± 2.1	**14.2 ± 0.6***
	GG	19.9 +/- 1.9	23.1 +/- 2.9

Table 5-2. There were no significant differences across the groups in the mean onset latency for ICMS-evoked EMG activities in LAD or RAD in either left or right face-M1. Within each study groups LAD and RAD had significantly shorter onset latency within the contralateral face-M1. There were no significant differences in GG onset latency across the groups or between left and right face-M1.

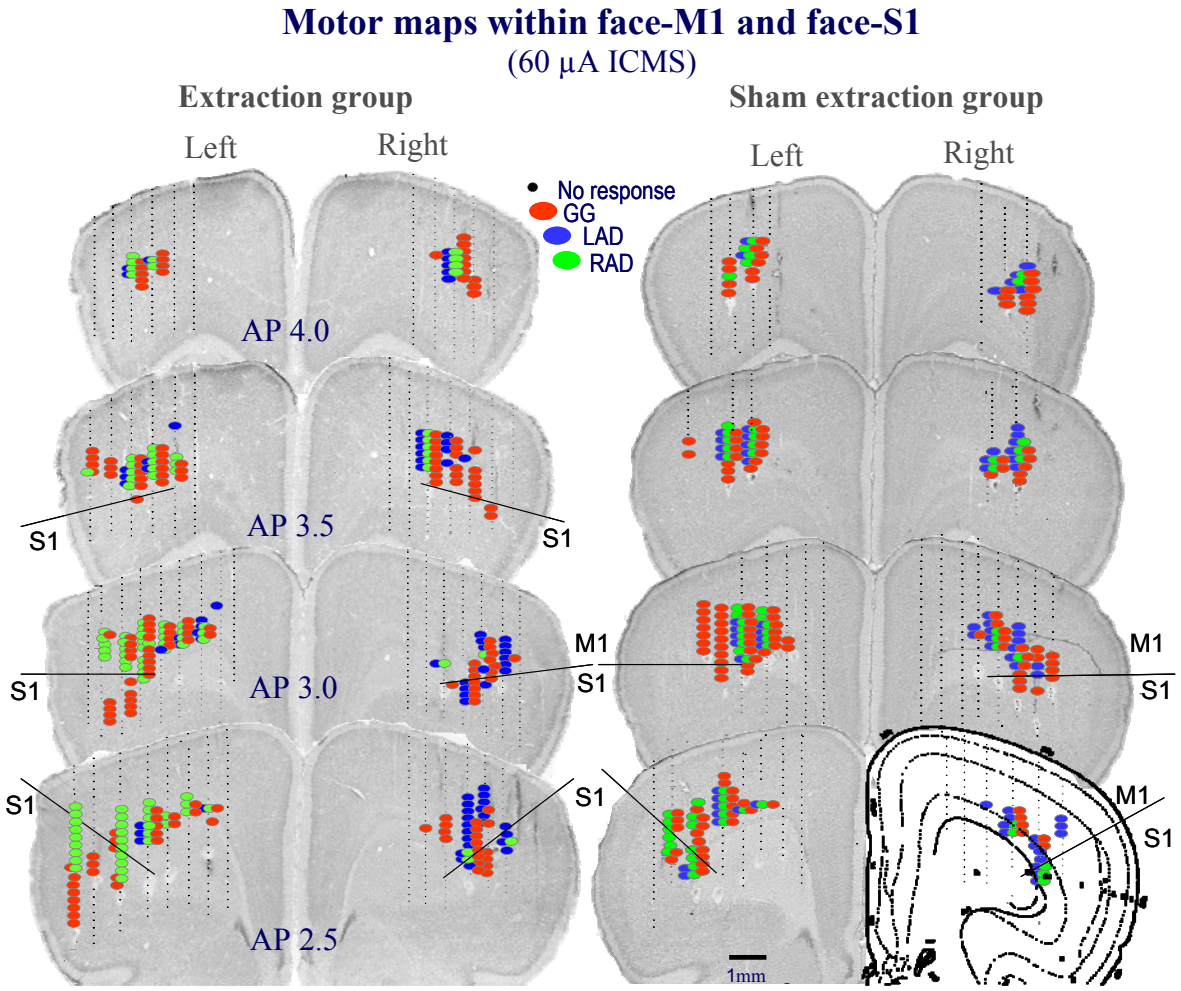

Fig. 5-1. Representative motor maps of LAD, RAD and GG in a rat from the extraction group as compared with a rat from the sham extraction group each are having a mean number of positive ICMS sites close to the mean of its group. Any site where ICMS could evoke LAD, RAD or GG EMG activity was plotted on the corresponding cortical coronal histological section (AP 2.5 – 4.0 mm anterior to Bregma): LAD with blue circles, RAD with green circles and GG with red circles. Black dots represent sites where ICMS could not evoke EMG activity in LAD, RAD or GG muscles. Note the extensive, bilateral representation of LAD, RAD and GG. (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus; S1-primary somatosensory cortex; M1- primary motor cortex).

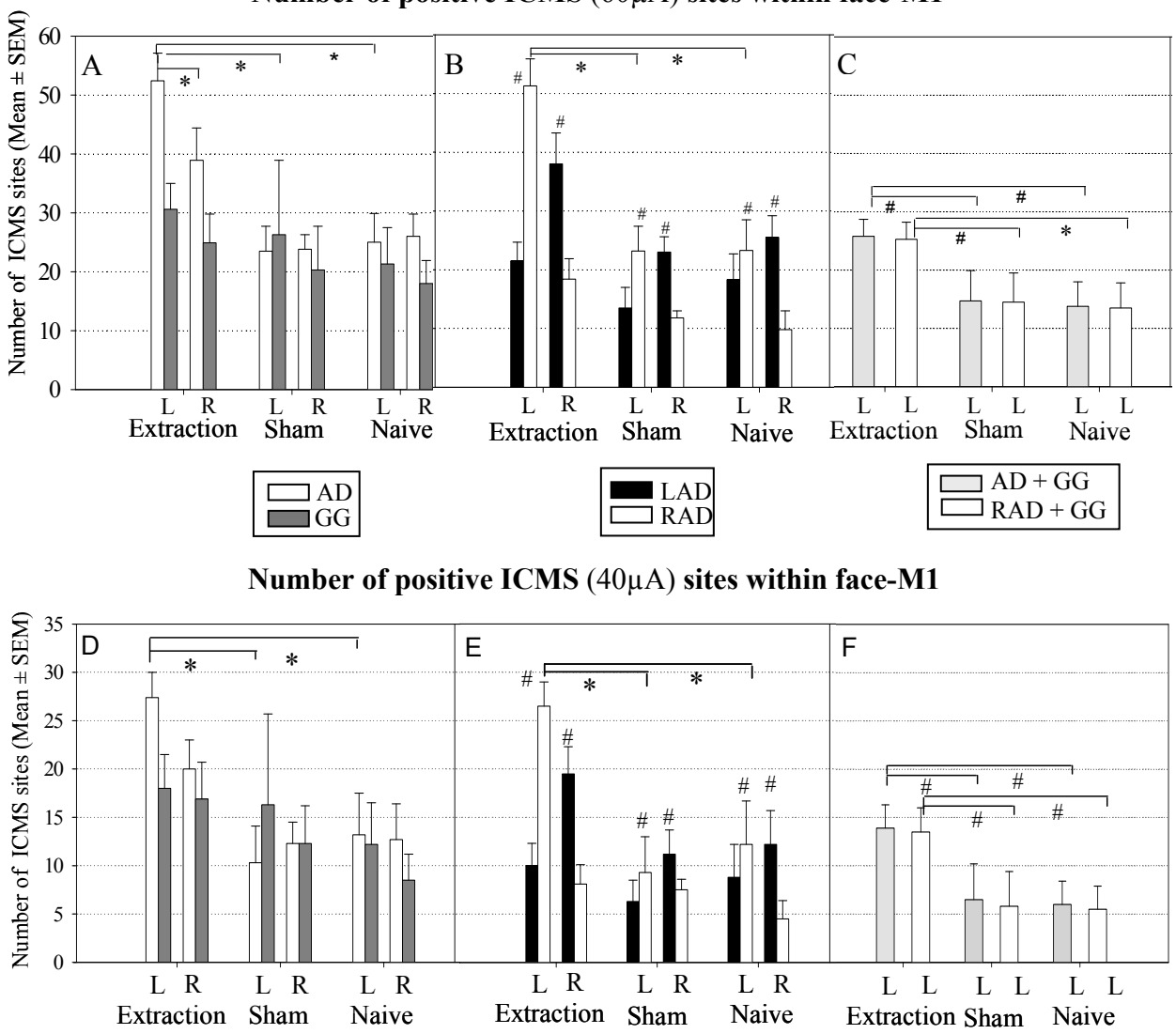

Fig. 5-2. The number of positive ICMS sites within face-M1 at ICMS intensity of 60 μA (A-C) and 40 μA (D-F). **A and D:** Within the left face-M1, the number of AD sites was significantly larger in the extraction group than in the sham and naive groups (***60 μA**: ANOVA: p=0.0004, Bonferroni: p=0.0011 and 0.0019, respectively. **40 μA**: ANOVA: p=0.0051, Bonferroni: p=0.0083 and 0.029, respectively). **B and E:** LAD and RAD had a significantly larger number of positive ICMS sites within the contralateral face-M1 (*MMRM, Bonferroni: p<0.0001). In all groups, at ICMS intensities of **40** and **60 μA,** LAD and RAD had a significantly larger number of sites in the contralateral face-M1 (#paired t-test, p<0.05). Within the left face-M1, the number of RAD sites was significantly larger in the extraction group than in the sham and naïve groups (***60 μA**: ANOVA: p=0.0004, Bonferroni: p=0.0015 and 0.0016, respectively. **40 μA**: ANOVA: p=0.005, Bonferroni: p=0.0082 and 0.029, respectively). **C and F:** In the left face-M1, at ICMS intensity of **60 μA,** the number of RAD/GG overlapping sites was significantly larger in the extraction group than in the naïve group (*ANOVA, Bonferroni, p<0.05); and the number of AD/GG overlapping sites was larger, but not significant, in the extraction group than in the sham and naïve groups (#ANOVA, Bonferroni, p<0.1); at ICMS intensity of **40 μA,** the number of AD/GG and RAD/GG overlapping sites was larger, but not significantly in the extraction group than in the sham and naïve groups (#ANOVA, Bonferroni, p<0.1). (AD - anterior digastric, LAD - left anterior digastric, RAD - right anterior digastric, and GG - genioglossus, R – right face-M1, L – left face-M1).

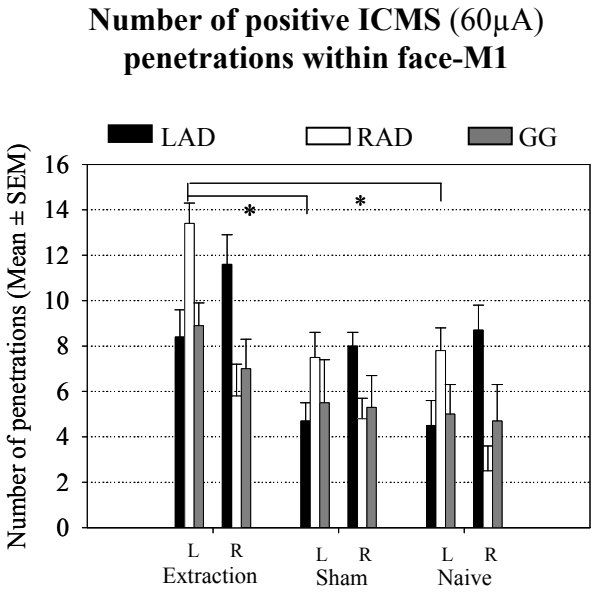

Fig 5-3. The number of positive ICMS (60 μA) penetrations for LAD (left anterior digastric), RAD (right anterior digastric) and GG (genioglossus) within the left and right face-M1. In the left face-M1 RAD had significantly larger number of positive ICMS penetrations in the extraction group than in the sham and naïve groups (*MMRM ANOVA, $p<0.05$) (R-right, L-left).

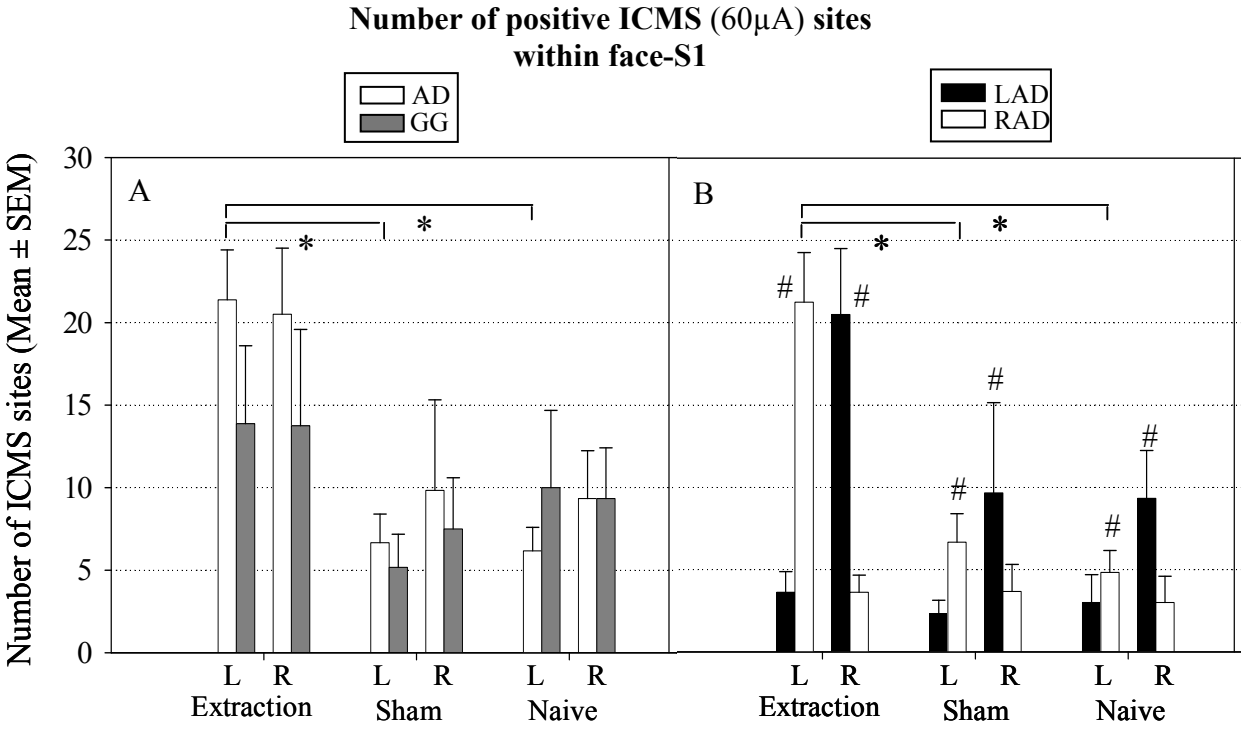

Fig. 5-4. **A**. The number of positive ICMS sites within face-S1 at ICMS intensity of **60 μA**. **A**. In the left face-S1, the number of AD positive ICMS (60 μA) sites was significantly larger in the extraction group than in the sham and naive groups (*ANOVA: p=0.0003, Bonferroni: p=0.0013 and p=0.0009, respectively). **B.** In all groups LAD and RAD had significantly larger number of positive ICMS (60 μA) sites in the contralateral face-S1 (# paired t-test, $p<0.05$). In the left face-S1, the number of RAD sites was significantly larger in the extraction group than in the sham and naïve groups (* ANOVA: p=0.0002; Bonferroni: p=0.0013 and 0.0004, respectively. (AD - anterior digastric; LAD-left anterior digastric; RAD-right anterior digastric and GG-genioglossus; R –right face-S1, L-left face-S1).

Mediolateral distribution of positive ICMS (60 µA) **penetrations within face-M1**

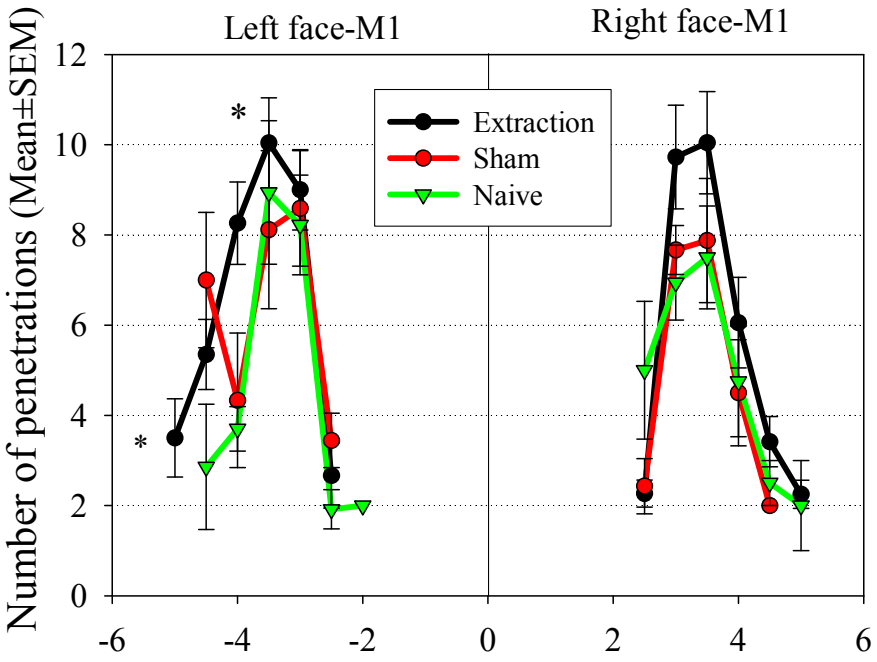

Fig. 5-5: Mediolateral (ML) distribution of the positive ICMS penetrations from which ICMS (60µA) evoked EMG activities in AD or GG within the left and right face-M1 irrespective of the anteroposterior position. In the left face-M1, the mean ML position was significantly more lateral in the extraction than in the sham group (*ANOVA: $p=0.026$, Bonferroni, $p<0.05$) and the most lateral position was significantly more lateral in the extraction group than in the sham and naïve groups (*ANOVA: $p=0.0001$, Bonferroni: $p=0.0001$ and 0.025 respectively).

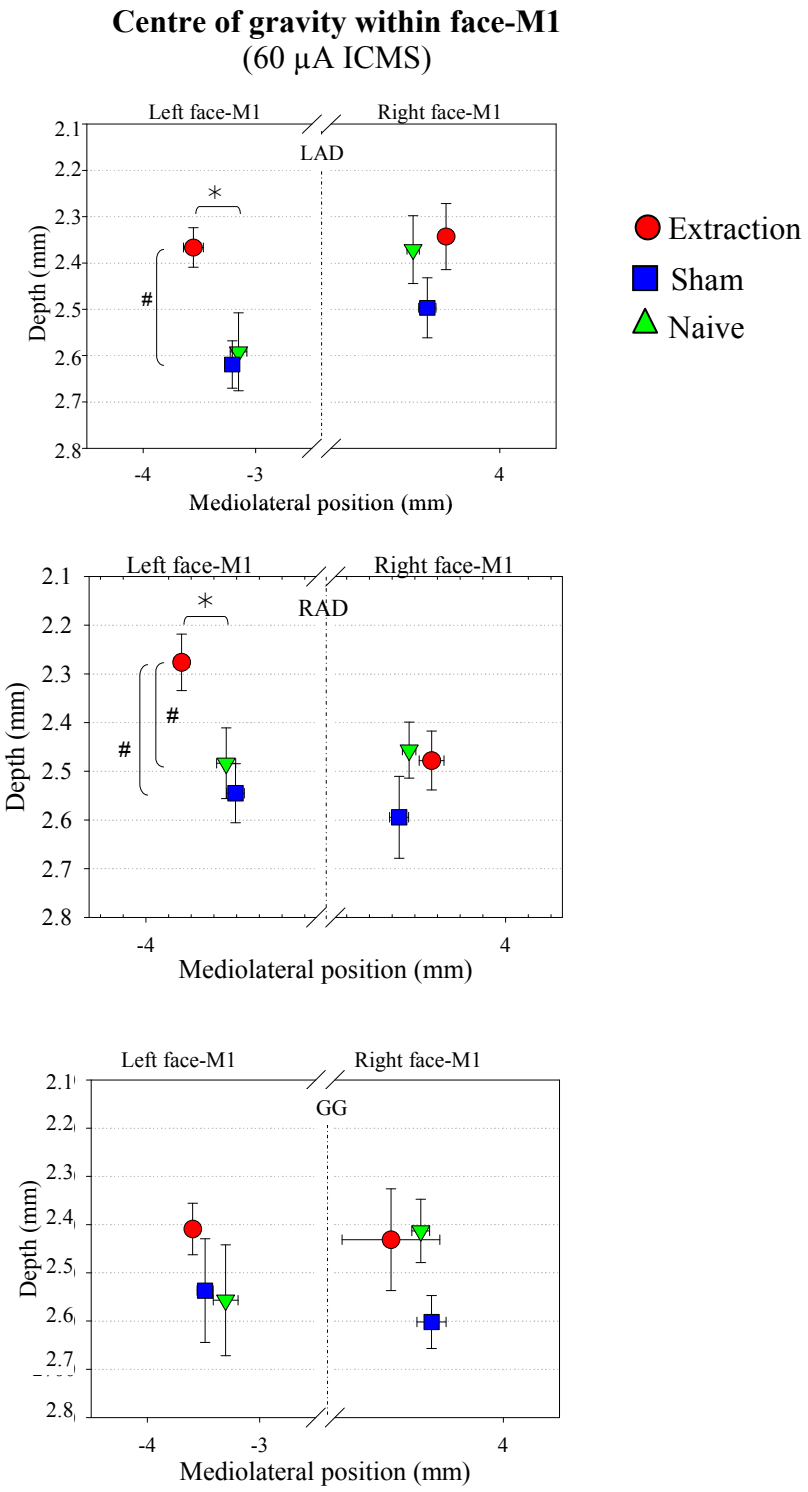

Fig. 5-6: Centre of gravity by muscle irrespective to AP position. In the extraction group, the centre of gravity for LAD (left anterior digastric) and RAD (right anterior digastric) was located significantly more lateral (*MMRM: $p<0.001$, Bonferroni: $p<0.05$) and had a trend towards a more superficial position (#MMRM: $p=0.0015$, Bonferroni: $p=0.077$ and 0.1 respectively). In all study groups the centre of gravity was positioned between AP3.0 and AP3.5 with no significant differences across the study groups or between left and right face-M1. There were no significant differences across the study groups in GG centre of gravity in either left or right face-M1.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

1. General features of jaw and tongue motor representations in control rats

The present ICMS studies have documented the general organizational features of the jaw-opening (anterior digastric, AD) and tongue-protrusion (genioglossus, GG) motor representations within the face sensorimotor cortex of male Sprague Dawley rats. ICMS mapping extended from 2.5 to 4.0 mm anterior and 1.5 to 5.5 mm lateral to Bregma with a spatial resolution of 0.5 mm horizontally and at 0.2 mm steps of cortical depth. Cortical maps were constructed by stimulating these sites at a constant ICMS intensity of 40 or 60 μA. The number of sites for which ICMS evoked EMG activity in AD and/ or GG outlined the extent of AD and GG motor representations within the face sensorimotor cortex.

In the 3 studies of this thesis, experimental and sham groups were compared to the same naïve (soft diet) group. In each of the studies there were no significant differences between the sham and naïve groups in any of the study measures. AD and GG had large motor representations within the left and right face-M1 and LAD and RAD had a significant contralateral predominance. AD and GG representations span the entire depth of layers V-VI and were characterised by multiple neighbouring and intermingled representations that often overlapped. These characteristics of jaw and tongue motor representations are consistent with previous studies in rats (Adachi et al., 2007; Neafsey et al., 1986) and monkeys (Burish et al., 2008; Huang et al., 1989b; Huang et al., 1988; for reviews, see Murray et al., 2001; Sessle et al., 1999). Overlapping motor representations have also been reported in mapping studies of limb-M1 and this has been thought to reflect the convergence and divergence of motor outputs on brainstem motoneurons (for reviews, see Sanes and Donoghue, 2000; Sanes and Schieber, 2001; Schieber, 2000; Schieber, 2001; Tehovnik et al., 2006). In addition, the rich network of intracortical connectivity (for reviews, see Keller, 1993; Mountcastle, 1997; Schieber, 2001) may suggest a "*shared neural substrate*" (Sanes et al., 1995) whereby neurons within different representation areas are interconnected. Such overlapping has been considered important for the spatiotemporal coordination of several muscles during movements (Aroniadou and Keller, 1993; Kwan et al., 1987; Nudo et al., 1996; Sanes et al., 1995) and may explain observations in monkey studies where long-duration ICMS

trains delivered to face-M1 can evoke coordinated rhythmic jaw movements (Hatanaka et al. 2005; Huang et al., 1989a; Martin et al., 1999; Satoh et al., 2006b; Yamamura et al., 2002; Zhang and Sasamoto, 1990) and long-duration ICMS trains, matching the time course of the motor function being studied, can evoke coordinated and complex movements in space (Graziano et al., 2002b).

While ICMS within the mapped area evoked AD and GG EMG activity, masseter (jaw-closing) EMG activity was usually not observed. This is consistent with studies in rats and monkeys demonstrating a marked paucity of jaw-closing motor representation (Clark and Luschei, 1974; Huang et al., 1988; McGuinness et al., 1980; Murray et al. 2001; Neafsey et al., 1986). This finding suggests that face-M1 plays an important role in the generation of some but not all orofacial movements. However, cold block of face-M1 has resulted in a significantly increased spontaneous EMG activity of the masseter muscles in monkeys suggesting that while ICMS of face-M1 facilitates the spontaneous and reflex-induced activity of the motoneurons supplying the anterior digastric muscle (an agonist that functions in jaw opening), it may inhibit the activity of the motoneurons supplying the masseter muscle (an antagonist muscle that acts during jaw closing) (Yamamura et al., 2002). Therefore, it is possible that the face-M1 neurons have an inhibitory effect on masseter motoneurons (Monkeys: Chase et al., 1973), yet it is also possible that the masseter representation is masked by an inhibitory effect of intracortical interneurons which has been shown in limb-M1 (Cats: Ethier et al., 2007) but not tested as of yet for face-M1.

Noteworthy are our findings of LAD, RAD and GG motor representations within face-S1. These are in accord with findings from earlier studies showing in awake and anaesthetised rats, as well as in marmosets, that jaw and/or tongue movements can be evoked by ICMS of face-S1 (Burish et al., 2008; Donoghue and Wise, 1982; Neafsey et al., 1986; Sapienza et al., 1981). Although it could be argued that the observed ICMS-evoked EMG activities within face-S1 in the present studies were the result of activation of distant face-M1 neurons through current spread and transynaptic interactions (Greenshaw, 1998; Keller et al., 1990; Ranck, 1975; Schwark and Jones, 1989), there are several lines of evidence that this is unlikely and that face-S1 does possess motor outputs

and plays an important role in the control of orofacial movements. First, the distance between some of the positive ICMS sites within S1 and M1 was larger than the estimated current spread of ~0.5mm at an ICMS intensity of 50 μA (Cheney, 2002). Second, many of the positive ICMS sites within face S1 had short onset latencies (8-10 msec) and the mean onset latencies for LAD, RAD and GG were comparable to those of face-M1, suggesting relatively direct projections to motoneurons. Third, under similar stimulation parameters, only dental extraction (but not trimming or changes in diet consistency) had a specific and significant effect on the motor representation of the RAD within the contralateral face-S1 (as well as face-M1). Thus, the neuroplastic changes in face-S1 were not a generalised feature of any oral manipulation. Fourth, the finding of motor outputs from face-S1 is in agreement with anatomical studies in rats and primates, showing efferent projections from S1 to brainstem motoneurons (Grinevich et al., 2005; Jones, 1976; Rathelot and Strick, 2006; Wise and Jones, 1977a; Zhang and Sasamoto, 1990). Fifth, studies in monkeys and rabbits have shown that rhythmic jaw and tongue movements, as well as swallowing movements, can be evoked by long-train ICMS of face-S1 (Huang et al., 1989a; Lin et al., 1998; Lund et al., 1984; Martin et al., 1999). These same face-S1 sites show orofacial movement-related neuronal activity (Hiraba, 1999; Hiraba et al., 1997; Murray et al., 2001; Yamamoto et al., 1988), and receive somatosensory inputs from the same orofacial region involved in the movement evoked by the ICMS of the same face-S1 sites (Huang et al., 1989b; Huang et al., 1988; Lin et al., 1998; Murray and Sessle, 1992a). Sixth, cold block or lesions of face-S1 can impair oral motor functions in monkeys and rats (Castro, 1975; Murray et al., 2001; Yao et al., 2002b).

2. Effects of intraoral manipulation on face-M1 and face-S1 motor representations

It has been well documented that face-M1 and face-S1 play a crucial role in sensorimotor integration and control of elemental and semiautomatic orofacial motor functions (for reviews, see Ebner, 2005; Murray et al., 2001; Sessle et al., 2005; Sessle et al., 1999). Furthermore, many studies have shown that experimental modifications in somatosensory inputs and/or altered motor behaviours induced by motor training,

peripheral nerve deafferentation or cortical stimulation, are associated with neuroplastic changes within limb-M1 and vibrissal-M1 manifested as reorganization of limbs and vibrissal motor representations (for reviews, see Butefisch, 2006; Ebner, 2005). Furthermore, recent studies involving training of awake monkeys and humans in a novel tongue-protrusion task have documented neuroplastic changes within face-M1 (Boudreau et al., 2007; Robbins et al., 2008; Sessle et al., 2007; Sessle et al., 2005; Sessle and Yao, 2002; Svensson et al., 2003b; Svensson et al., 2006). In 2 other studies, acute noxious stimulation of the tongue in rats decreased face-M1 excitability, and injury to the lingual (sensory) nerve resulted in time-dependent changes in the GG motor representation within face-M1 (Adachi et al., 2007; Adachi et al., 2008). However, up to the present thesis, no study had addressed the question of whether neuroplastic changes may occur in face-M1 following modifications to the dental occlusion, loss of teeth, or a change in diet consistency. Nevertheless, it has been documented in animals and in humans that injury to the sensory nerves supplying the oral tissues, and modification to the dental occlusion, induced by dental extraction or trimming, as well as a change in diet consistency, may affect oral sensorimotor functions (Endo et al., 1998; Haas and Lennon, 1995; Inoue et al., 2004; Inoue et al., 1989; Klineberg and Jagger, 2004; Miehe et al., 1999; Okayasu et al., 2003; Proschel and Hofmann, 1988).

In light of these considerations, the hypothesis of the present studies was that dental trimming or extraction, or a change in diet consistency, would be associated with significant changes in the ICMS-defined motor representations in face-M1. However, within the observation period, this hypothesis was not fully supported by the data. The present studies found that a change in diet consistency for a period of 2-3 weeks had no significant effects on the extent or topographical organization of jaw (AD) and tongue (GG) motor representations within face-M1 and face-S1. Incisor trimming resulted, 1 day and 1 week later, in significant disparities in GG onset latency or GG representation between left and right face-M1 that did not exist in the naïve or sham trim rats. Unilateral extraction of the lower incisor resulted, 1 week later, in a significant increase in the RAD motor representation within the contralateral face-M1 along with a substantial lateral shift of the centre of gravity of the LAD and RAD positive ICMS sites

and the mean ML position of the positive ICMS penetrations. These findings provide the first documentation that dental manipulations can be associated with neuroplastic changes within the face-M1 that are manifested as asymmetric, directionally selective expansion of the AD motor representation. Moreover, dental extraction was also associated with a significantly increased RAD motor representation within the contralateral face-S1, documenting for the first time that face-S1 motor outputs also have the capacity to undergo neuroplastic changes following peripheral manipulations.

3. Implications of findings to sensorimotor behaviour

The novel findings of this thesis project of the neuroplastic capabilities of face-M1 as well as face-S1 motor outputs may shed light on how animals adapt to alterations in orofacial sensorimotor functions induced by intraoral manipulations. The oral tissues including the teeth and their periodontal tissues are characterized by a rich innervation density (for reviews, see Macefield, 2005; Miles et al., 2004; Paxinos, 2004) and somatosensory inputs to face-M1 and face-S1 from the orofacial tissues, including the teeth, provide peripheral feedback that is needed for the control of orofacial motor functions (Burish et al., 2008; Inoue et al., 1989; Iyengar et al., 2007; for reviews, see Haas and Lennon, 1995; Kaas et al., 2006; Murray et al., 2001). Earlier studies in subprimates and in primates have shown that alteration in somatosensory inputs induced by somatosensory deafferentation (Adachi et al., 2007; Franchi, 2001; Halkjaer et al., 2006; Yildiz et al., 2004), a sustained somatosensory stimulation to the pharynx (Hamdy et al., 1998), as well as experimental noxious stimuli (Adachi et al., 2008; Boudreau et al., 2007), may result in neuroplastic changes within face-M1, as reflected in an altered cortical excitability and/ or altered motor representations. Therefore, it is possible that changes to the dental occlusion induced by either dental extraction or trimming in the present study conceivably altered the somatosensory inputs from the teeth to face-S1 and face-M1 and contributed to the observed changes within face-M1.

There is however, another possible factor to consider. Rats use their incisors for feeding, fighting and other oral motor functions. Yet, gnawing is a unique motor behaviour of rodents to compensate for the continuous eruption of their incisors (Law et

al., 2003; Ness, 1965; Risnes et al., 1995; Sessle, 1966). It is well known that modification to the dental occlusion in humans and rodents, induced by dental extraction or trimming, can alter oral motor functions (Endo et al., 1998; Miehe et al., 1999; Ramirez-Yanez et al., 2004; for review, see Klineberg and Jagger, 2004). Therefore, it is possible that dental trimming or extraction of the incisor also modified the rat's gnawing motor behaviour. Changes in diet consistency may also be associated with altered motor behaviour such as altered biting and chewing forces and different patterns of mastication (Inoue et al., 2004; Okayasu et al., 2003; Proschel and Hofmann, 1988). It has been shown in monkeys that alterations in motor functions can affect the somatosensory inputs from the orofacial tissues involved in the altered orofacial movements (for review, see Murray et al., 2001). Such changes in somatosensory input may contribute to changes within face-M1 motor representations (see above). In addition, changes in oral motor behaviour may also contribute directly to the changes within face-M1, consistent with the concept of use-dependent neuroplasticity whereby motor representations are altered by motor experience (Boudreau et al., 2007; Sessle et al., 2007; Sessle et al., 2005; Svensson et al., 2003b; Svensson et al., 2006). These considerations raise the question why dental extraction, and to a lesser extent dental trimming, induced significant changes in motor representations within face-M1 while a change in diet consistency had a little effect.

One possible explanation may be related to the specific finding of increased overlapping motor representations within face-M1 of the extraction and trim recovered groups. It has been reported that limb motor skill training, but not non-skilled training, induces cortical reorganization within limb-M1 (Kleim et al., 2002b; Remple et al., 2001) and one of the most consistent findings involving limb motor skill training studies is an increased overlapping representation of the movements involved in the acquisition of a limb motor skill (Nudo et al., 1996). Such organization is consistent with the notion that the M1 is organized to control movement rather than contraction of individual muscles (Asanuma, 1989; Graziano et al., 2002b; Kakei et al., 1999). It is interesting to note that dental extraction resulted in not just a significant increased RAD representation, but also significant increased overlapping representations of RAD and GG, and dental trimming

was associated with a significant disparity in AD/GG overlapping representations between the left and right face-M1. Therefore, one possible explanation for the differences in our findings between the study groups is that a change in diet consistency involved changes in non-skilled motor functions such as changes in the amount of forces applied during biting. In contrast, tooth extraction or trimming would likely have necessitated adoption of novel oral motor skills (such as unilateral biting) and novel coordination of jaw and tongue movements. This could explain why only dental extraction or trimming had a significant effect on face-M1 motor representations. This explanation is consistent with the occurrence of face-M1 neuroplasticity in the acquisition of a novel oral motor skill (Boudreau et al., 2007; Sessle et al., 2007; Sessle et al., 2005; Svensson et al., 2003b; Svensson et al., 2006).

Another possible explanation for the differential effects could be the capability of face-M1 to be modelled in a task-dependent manner. It has been demonstrated that different forms of neuroplastic changes are linked to different modes of peripheral manipulations. In the present study, unilateral trimming of the mandibular incisor for a period of 1 week resulted, 1 day later, in no apparent changes in face-M1 motor representations, whereas bilateral trimming of the mandibular incisors has resulted in a decreased AD representation that reversed once the teeth were allowed to re-erupt back into occlusion (Lee et al., 2006). Bilateral trimming of the vibrissae for 5 days also resulted in changes in the vibrissal motor representations that reversed once the vibrissae were allowed to re-grow back to normal length (Keller et al., 1996). Sensory denervation of the lingual nerve has resulted in reorganization of the tongue motor representation within face-M1 (Adachi et al., 2007), although sensory denervation of the infraorbital nerve has resulted in a decreased excitability of the vibrissal-M1, but no apparent changes in motor representations (Franchi, 2001). In humans, lingual nerve block is associated with decreased excitability of face-M1 representing the tongue (Halkjaer et al., 2006), but local anaesthesia of the facial skin has resulted in increased excitability of face-M1 representing the orofacial muscles (Yildiz et al., 2004). In addition, while training in a novel tongue or limb motor skill has resulted in reorganization of tongue or limb motor representations, respectively, non-skilled limb training has induced

angiogenesis but not altered limb motor representation within limb-M1 (Kleim et al., 2002b; Svensson et al., 2003b; Svensson et al., 2006; Swain et al., 2003). Thus, face-M1 may be modelled in a specific task-dependent manner whereby different modes of peripheral manipulation are associated with different forms (motor representation, cortical excitability) and directions (increase, decrease or no change) of neuroplastic changes, consistent with our findings of differential effects of various intraoral manipulations.

Yet another explanation for the differential effects across the study groups may relate to time-dependent neuroplasticity, as it has been shown that different but specific forms of neuroplastic changes can occur at different points of time following a peripheral manipulation. Unilateral transection of the lingual nerve is associated with a significantly decreased GG representation 1-2 weeks later, and with a significantly increased GG representation 3-4 weeks later (Adachi et al., 2007). The mechanisms underlying cortical neuroplasticity may also vary over time. For example, early stages (3 days) of training in a limb-motor skill have been associated with enhanced gene expression in rats (Kleim et al., 1996), while synaptogenesis and reorganization of motor representations have occurred only later, after10 days of training (Kleim et al., 2004). These findings raise the possibility that face-M1 has the capacity to adapt to significant changes in orofacial sensorimotor experience and can be modelled in a specific time-dependent manner. Therefore, although the present ICMS study could not detect reorganization of motor representations following a change in diet consistency, we cannot rule out the possibility that other forms of neuroplastic changes did occur or that the underlying mechanisms required more time in order to manifest as reorganization of motor representations.

4. Possible role of neuroplastic changes outside face-M1 and face-S1

Dental denervation (*i.e.,* tooth extraction or pulp extirpation) has been associated with reorganization of the mechanoreceptive fields within the V mesencephalic nucleus (Linden and Scott, 1989), V brainstem nuclei (Hu et al., 1986; Hu et al., 1999; Kwan et al., 1993) and face-S1 (Henry et al., 2005). Face-M1 receives a large amount of peripheral somatosensory inputs directly through the thalamus (Hatanaka et al., 2005;

Rausell and Jones, 1995; Simonyan and Jurgens, 2005), or indirectly through face-S1 (Chakrabarti and Alloway, 2006; Hoffer et al., 2005; Iyengar et al., 2007; Izraeli and Porter, 1995; Miyashita et al., 1994). It has been demonstrated that cortical disinhibition and unmasking of latent inputs from S1 to M1 may contribute to face-M1 neuroplasticity (see below) (Farkas et al., 2000). Therefore, it may be suggested that some of the neuroplastic changes observed within face-M1 in the extraction, trim and trim recovered groups were the result of altered sensory inputs and changes within face-S1 or subcortical relay stations.

ICMS of M1 evokes EMG responses through activation of brainstem motoneurons; however, many of the corticobulbar projections are multisynaptic involving subcortical relay stations (Hatanaka et al., 2005; Satoh et al., 2006b; Takada et al., 1999; Takada et al., 1994; Zhang and Sasamoto, 1990). Motoneuronal synaptic efficacy can be rapidly modulated by a large number of inputs (*e.g.* (oldberg, 1971; Lavigne et al., 1987; Sessle, 1977; Sessle and Schmitt, 1972; Tolu et al., 1993; Tolu et al., 1994a; Tolu et al., 1994b). Furthermore, it has been documented that alteration in motor outputs induced by transection of the facial nerve induces motor reorganization not just within face-M1 but also within brainstem motor nuclei (Kis et al., 2004). Therefore, although the present study had demonstrated neuroplastic changes within face-M1 and face-S1 following dental extraction or trimming, we cannot rule out the possibility that changes had also occurred at other subcortical or cortical areas and that the altered motor representations (AD in the extraction group and GG in the trim recovered group) are, at least in part, a reflection of altered subcortical synaptic efficacy. However, this is unlikely since altered subcortical synaptic efficacy could be expected to be associated with altered onset latency and altered ICMS thresholds for evoking AD or GG activity (Asanuma et al., 1976; Butovas and Schwarz, 2003; Ranck, 1975; Ridding and Rothwell, 1997; Stoney et al., 1968b; Tehovnik et al., 2006), but such changes could not be observed in either the dental extraction group or the trim recovered group. Nevertheless, decreased subcortical synaptic efficacy could explain, at least in part, the disparity in GG onset latency between left and right face-M1 observed in the trim group.

In addition, the lack of observed neuroplastic changes within face-M1 following a change in diet consistency cannot rule out the possibility that changes may have occurred in other cortical and subcortical areas. Other studies have shown that trimming of teeth and a change in diet consistency may be associated with an altered pattern of mastication (see above) and it has been well documented that the cortical masticatory area (CMA) (for review, see Sessle et al., 2005) and brainstem central pattern generator (for reviews, see Lund and Kolta, 2006b; Sessle, 2006) play an important role in the generation and control of masticatory movements. Therefore, it is possible that these areas played an important role in the altered oral motor behaviour that may have occurred as a result of the change in diet consistency. Nevertheless, transection of the mandibular and maxillary branches supplying sensory innervation of the teeth and other orofacial tissues did not result, ~2 weeks later, in a significant change in the rabbit CMA (cortical masticatory area) motor outputs (Masuda et al., 2002).

5. Mechanisms underlying face-M1 neuroplasticity

There is a clear lack of studies related to the mechanisms underlying face-M1 neuroplasticity following manipulations in the oral environment. Tooth extraction and trimming may be associated with altered afferent inputs to face-M1 and face-S1 as a result of peripheral denervation, reduced occlusal contacts and possibly pain. Such changes in sensory inputs can alter the balance between sensory inputs and motor outputs (Buonomano and Merzenich, 1998; Rioult-Pedotti and Donoghue, 2003) and contribute to disinhibition (or inhibition) and unmasking (or masking) of latent excitatory connections. Consequently, ICMS can excite neighbouring neurons that previously were non-responsive, thereby increasing the ICMS-defined motor representations (Farkas et al., 2000; Huntley, 1997a; Jacobs and Donoghue, 1991) for reviews, see (Chen et al., 2002; Jones, 1993; Navarro et al., 2007).

Occlusal modifications induced by dental extraction or trimming may result in altered oral motor behaviour (see above). Face-M1 and face-S1 receive somatosensory inputs from oral tissues involved in orofacial movements (for review, see Murray et al., 2001). Adapting to an altered pattern of mastication conceivably requires repetition of

the novel motor movements which may be somewhat analogous to learning a novel motor skill (Adams, 1984). Consequently, repeated jaw or tongue movements may result in sustained somatosensory inputs to face-S1 and face-M1 that may induce enhanced synaptic efficacy (Asanuma, 1989; Hamdy et al., 1998; Jones, 1993). Enhanced synaptic efficacy can facilitate the ability for ICMS of face-M1 areas to evoke movements that previously could not be evoked by a similar stimulus to these areas; this may thereby increase motor representation. Similarly, a decreased motor representation may reflect decreased synaptic efficacy associated with decreased somatosensory inputs and decreased motor function (Monfils and Teskey, 2004a; Rioult-Pedotti and Donoghue, 2003). These assumptions may explain, in part, our observation of increased RAD representation within left face-M1, 1 week following extraction of the rat right mandibular incisor. However, trimming the rat right mandibular incisor out of occlusal contacts was associated 1 week later with a decreased (but not significant) GG representation. The differences between the 2 studies may be related to differences in the extent of intervention since in the extraction group there was a complete loss of unilateral incisal contacts including loss of periodontal tissues while in the trim group there was only a partial loss of occlusal contacts with no loss of periodontal ligament. Therefore, it is possible that the changes induced by dental trimming were not significant enough to result in changes consistent with the notion that face-M1 has the capability to adapt to significant changes in orofacial sensorimotor experience.

6. Study limitations

The ICMS is considered to be an appropriate technique for mapping the functional properties of motor outputs within the sensorimotor cortex (for reviews, see Asanuma, 1989; Taylor and Gross, 2003). The extent of motor representations is inferred from measuring the ICMS-evoked EMG responses in target muscles. The ICMS parameters such as the cortical depth at which the ICMS is applied, state of anaesthesia, previous stimulation, muscle posture as well as individual variations can all have an effect on the features of the ICMS-evoked EMG responses and thereby may influence the overall extent of motor representations (Asanuma, 1989; Donoghue and Wise, 1982;

Graziano et al., 2002b; Greenshaw, 1998; Huntley and Jones, 1991b; Nudo et al., 1992; Sessle and Wiesendanger, 1982; Tandon et al., 2008; Tehovnik et al., 2006). Therefore, in the present studies we attempted to control these possible sources of variability as much as possible and the main focus of the study was not the absolute extent of motor representations but rather the changes in the ICMS features and motor representations across the study groups. We applied similar stimulation parameters in all experiments and all rats were kept at a similar narrow window of anaesthetic state. The use of general anaesthesia is itself a potential confound but the general feature of the face-M1 motor maps were comparable to those defined in anaesthetized animals (Huang et al., 1989b; Tandon et al., 2008) and we ensured comparable anaesthetic and experimental conditions applied to all study groups.

Another related matter is the reliability of the ICMS technique to delineate functional boundaries for analysing motor representations within our mapping area (Huntley and Jones, 1991b; Nudo et al., 1992). As noted above, it is possible that the changes induced by a change in diet consistency or unilateral trimming of the incisor were small and within the range of normal variability and thus could not be detected (Nudo et al., 1992; Nudo et al., 1996). Alternatively, it is also possible that changes in motor representations were too small to be detected by the ICMS mapping technique. It has been demonstrated that at threshold ICMS intensities, the movement of different muscles can be evoked within ~100 μm displacement of the microelectrode position (Asanuma, 1989); however, the horizontal spatial resolution in our study was 500 μm. Therefore, our mapping could have explored only large changes in motor representations and could have missed smaller changes if they had occurred. On the other hand, we used suprathreshold ICMS intensities of 40-60μA that may have activated additional distant pyramidal neurons through direct current spread or indirectly through axon collaterals thereby resulting in overestimated motor representations (Sessle and Wiesendanger, 1982; for reviews, see Asanuma, 1989; Cheney, 2002; Tehovnik et al., 2006) that could have masked changes that could have been detected by mapping at threshold ICMS intensities.

The possible confounding effects of post-operative pain in the extraction group may also be a factor in the observed neuroplastic changes. However, this is unlikely since the extraction and sham extraction groups showed normal general behaviour (Chudler and Byers, 2005) and a continuous gain in body weight, plus the sham extraction did not show any changes within face-M1 and did not differ from the naïve group. Furthermore, experimentally induced pain has been associated with decreased face-M1 excitability (Adachi et al., 2008; Boudreau et al., 2007) while we observed increased RAD motor representation suggestive of increased face-M1 excitability (Monfils et al., 2004; Ridding and Rothwell, 1997).

7. Significance of the findings and future directions

The novel findings of this thesis along with recent findings from our studies in awake monkeys and humans provide evidence for the neuroplastic capabilities of the face-M1 as well as face-S1. Such cortical changes may reflect or allow for functional adaptation (or maladaptation) of the masticatory system to the altered oral state or altered oral motor behaviour and may contribute to the mechanisms whereby patients undergoing oral rehabilitation can (or cannot) restore the lost orofacial sensorimotor functions. This information is important since injuries to the oral tissues and modifications to the dental occlusion induced by dental extraction, attrition or trimming are common occurrences in humans that may sometimes be accompanied by impaired oral sensorimotor functions (Haas and Lennon, 1995; Johansson et al., 2006; Klineberg and Jagger, 2004; Svensson et al., 2003b; Trulsson and Essick, 2004). Furthermore, impaired oral motor functions are common in many neurological disorders (*e.g.* brain injury, stroke, Parkinson disease), sometimes making the most vital functions of eating, swallowing and speaking difficult and thereby jeopardizing the patient's quality of life (Brennan et al., 2008; Feine and Carlsson, 2003; Johansson et al., 2006; Sheiham et al., 2001). Therefore, understanding the mechanisms underlying orofacial sensorimotor functions is important for the development of new treatment strategies to facilitate recovery of such patients suffering from sensorimotor deficits and improve their quality of life. Indeed, in recent years, based on animal and human study models, principles of

M1 neuroplasticity have been translated to novel evidence-based practices that induce cortical neuroplasticity or reverse redundant plastic changes in order to enhance the effectiveness of rehabilitation of patients suffering from sensorimotor disorders (Butefisch, 2006; Hummel and Cohen, 2005; Kaas et al., 2008; Lotze et al., 1999; Miles, 2005; Robbins et al., 2008). For example, in limb amputees there is a positive correlation between phantom limb pain and the levels of cortical reorganization of limb and tongue muscles as defined by fMRI, and treatment with myoelectric prostheses results in less phantom limb pain and re-reorganization of limb and lip representations (Lotze et al., 1999). Recent studies in rats (Adkins et al., 2008), monkeys (Frost et al., 2003) and humans (Brown et al., 2006) have shown that pairing rehabilitative training with cortical electrical stimulation induces more behavioral improvement than training alone. Regrettably, these principles, and in particular as they relate to face-M1 and orofacial sensorimotor disorders, have not been thoroughly investigated in animals or humans. Our animal model has proven to be appropriate for further studies of face-M1 neuroplastic capabilities in rats and complementary studies can be designed for monkeys and humans.

A number of questions arise from the present ICMS studies that warrant further exploration in future studies. This thesis project used anaesthetised male rats as the animal model. There are obvious differences at the functional, anatomical and molecular levels between brains of primates and subprimates (Johansson and Lansner, 2007). Rats have a specialised dental apparatus and engage in gnawing motor behaviour (Burn-Murdoch, 1999; Hildebrand et al., 1995; Michaeli et al., 1974). Furthermore, cortical neuroplasticity may be modulated by gender effects (Hattemer et al., 2007; Jonasson, 2005) and state of anaesthesia (Huang et al., 1989b; Sapienza et al., 1981; Tandon et al., 2008). Therefore the findings of the present studies cannot be automatically extrapolated to the human dentition and its relations to sensorimotor function and underlying mechanism. These can be addressed in future studies that can be designed to be undertaken in awake rats as well as in monkeys and in humans of both genders.

Although the ICMS technique had successfully addressed the objectives of the present thesis and provided novel findings related to the neuroplastic capabilities of face-

M1 and face-S1, it will be interesting to complement the ICMS studies with other electrophysiological (*e.g.* somatosensory evoked potentials and recordings of single neuronal activity), anatomical and pharmacological methods as well as contemporary neuroimaging techniques. The ICMS technique has good spatial and temporal resolution but can provide detailed information related to a relatively small segment of the brain. The approach could be complemented in future studies by fMRI that despite its poorer spatial and/or temporal resolutions can provide a functional picture of the whole brain at one point of time (for review, see Cheney, 2002).

Confirmation of our findings of the existence and role of face-S1 motor outputs can be addressed by cold block techniques of face-M1 to test if short-latency ICMS-evoked EMG responses from S1 are not affected by the cold block. Other electrophysiological studies could apply other manipulations to the teeth (*e.g.* orthodontic, dental implants) to test whether they produce neuroplastic changes similar or different to those observed following dental extraction. Anatomical studies could be carried out to reveal whether face-S1 has characteristic features similar to face-M1 such as efferent projections from the ICMS-defined motor output neurons to brainstem motoneurons and afferent projections from the thalamic VL nucleus to the same face-S1 neurons. Studies of orofacial motor behaviour and detailed kinematics of jaw and tongue movements may be useful in understanding the contribution of altered motor functions to face-M1 neuroplasticity following the peripheral manipulations. Future studies could also address the time course of M1 and S1 neuroplasticity by testing changes occurring at different time intervals following the intraoral manipulation. It would also be interesting to extend our current finding and test if intraoral manipulations are associated with neuroplastic changes within other orofacial motor centers such as brainstem and CMA/swallow cortex.

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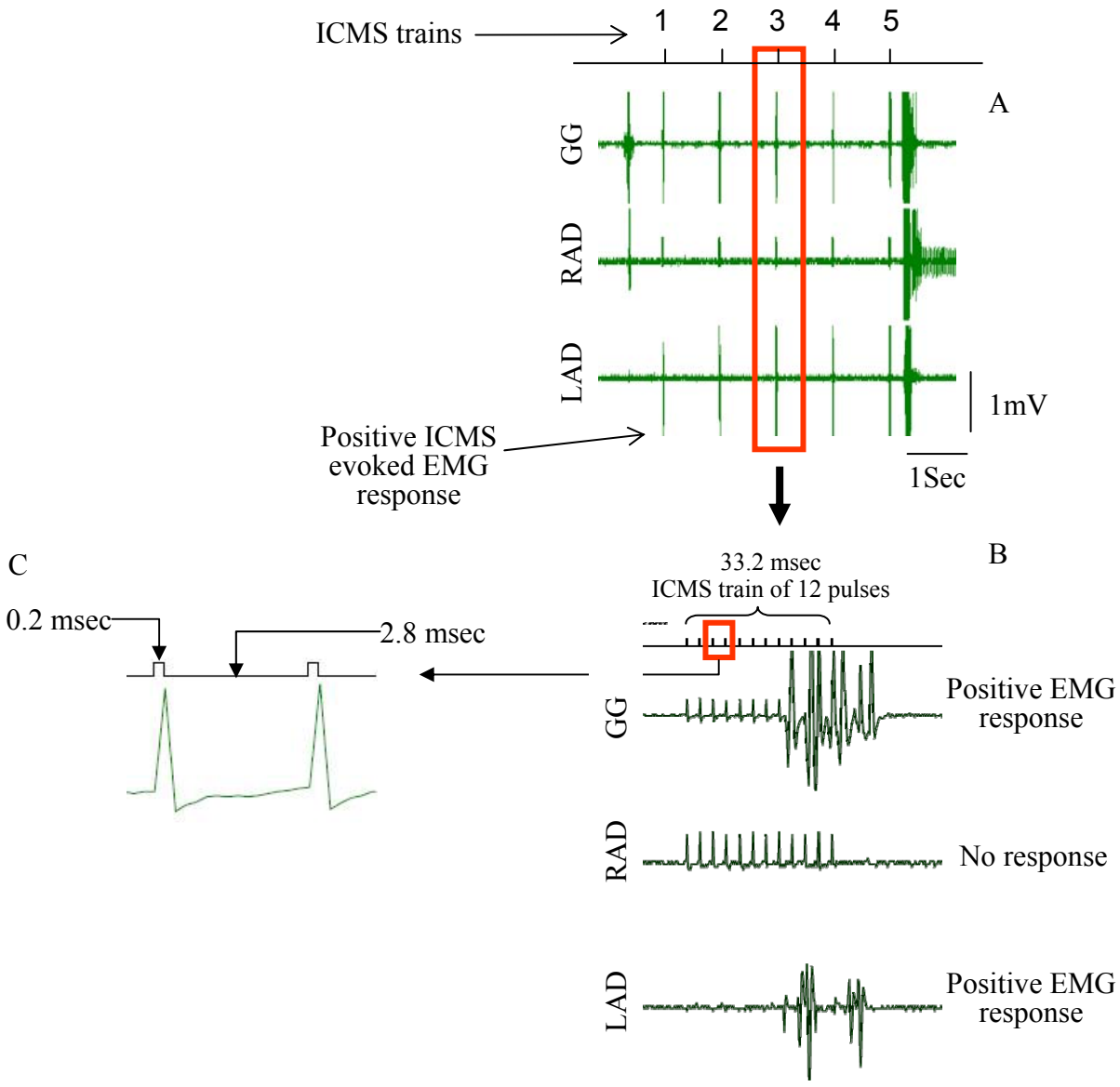
Appendix 2-1

ICMS train and EMG evoked responses



(A) Five ICMS trains (at 1HZ) and evoked EMG responses recorded from LAD (left anterior digastric), RAD (right anterior digastric) and GG (genioglossus). (B) Each ICMS train was a 33.2 msec and consisted 12 pulses at 333 Hz. (C). Each pulse was 0.2 msec long, with 2.8 msec inter-pulse intervals.

Appendix 2-2

Dependent and independent variables

Independent variables:

1. Study groups:

Experimental Groups:

Trim group (n=6)
Trim recovered group (n=6)
extraction group (n=8)
Soft diet group (n=6)
Hard diet group (n=6)

Sham control groups:

Sham trim group (n=7)
Sham extraction group (n=6)

2. Cortical side:

Left face-M1 or face-S1
Right face-M1 or face-S1

3. ICMS intensity:

ICMS intensity 40 μA
ICMS intensity 60 μA

4. Muscle

a. LAD;
b. RAD;
c. GG

Dependent variables:

1. Number of positive ICMS sites for each muscle (LAD, RAD, GG) or combination of these muscles
2. Number of positive ICMS penetration for LAD, RAD or GG
3. Onset latency (the onset time of the ICMS-evoked EMG response)
4. AP, ML poison of the positive ICMS penetrations
5. Centre of gravity of positive ICMS sites

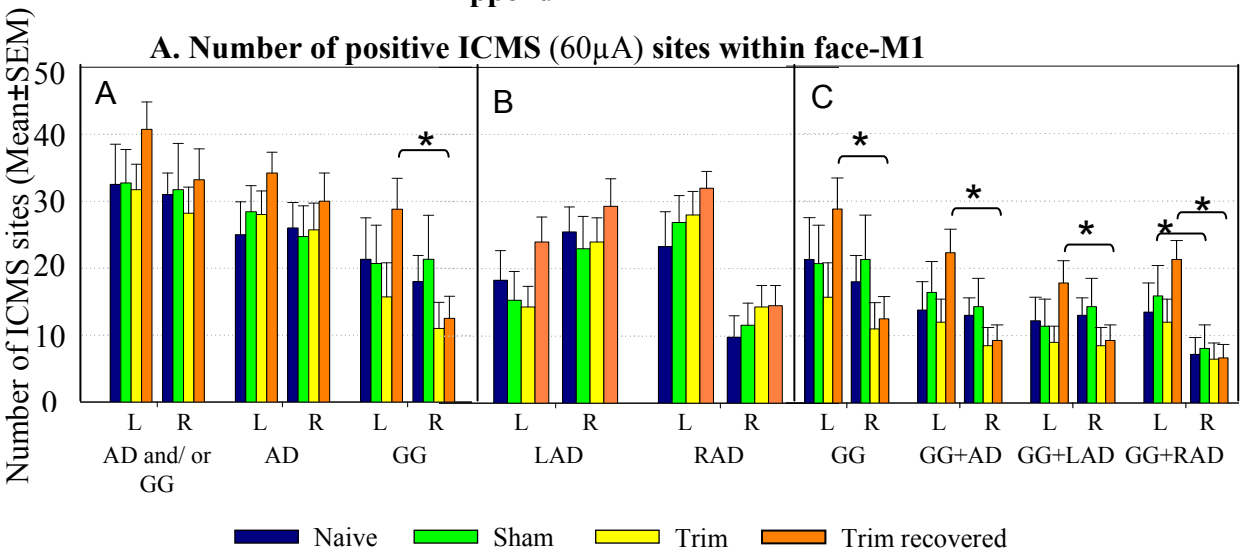
Appendix 3-1
Number of positive ICMS sites within face-M1
Summary of univariate group comparisons
(Mean ± SEM)

Stimulus Intensity	Cortical Side	Muscle	Soft diet group	Hard diet group	*t-test*
40 µA	Left	AD and/ or GG	19.3 ± 4.8	18.3 ± 1.5	p=0.85
		GG	12.2 ± 4.3	7.0 ± 2.1	p=0.31
		AD	13.2 ± 4.3	15.5 ± 1.6	p=0.63
		LAD	8.8 ± 3.4	10.7 ± 2.3	p=0.67
		RAD	**12.2 ± 4.5**	**14.0 ± 1.3**	p=0.71
		AD+ GG	6.0 ± 2.4	4.2 ± 0.9	p=0.51
		LAD + RAD	7.8 ± 3.6	9.2 ± 1.7	p=0.75
		LAD + GG	4.8 ± 2.0	3.8 ± 0.7	p=0.64
		RAD + GG	5.5 ± 2.4	3.8 ± 0.7	p=0.54
	Right	AD and/ or GG	16.2 ± 3.1	15.8 ± 1.5	p=0.93
		GG	8.5 ± 2.7	5.3 ± 2.0	p=0.37
		AD	12.7 ± 3.7	14.5 ± 1.3	p=0.66
		LAD	**12.2 ± 3.5**	**14.3 ± 1.4**	p=0.58
		RAD	4.5 ± 1.9	5.0 ± 1.3	p=0.84
		AD+ GG	5.0 ± 2.3	4.0 ± 1.3	p=0.72
		LAD + RAD	4.0 ± 1.6	4.8 ± 1.2	p=0.69
		LAD + GG	5.0 ± 2.3	4.0 ± 1.3	p=0.72
		RAD + GG	2.7 ± 1.5	2.8 ± 1.0	p=0.93
60 µA	Left	AD and/ or GG	32.5 ± 6.0	30.0 ± 2.2	p=0.71
		GG	21.3 ± 6.2	13.5 ± 4.4	p=0.33
		AD	25.0 ± 4.9	27.0 ± 1.0	p=0.70
		LAD	18.3 ± 4.4	19.2 ± 1.9	p=0.87
		RAD	**23.3 ± 5.2**	**24.7 ± 1.6**	p=0.82
		AD+ GG	13.8 ± 4.2	10.5 ± 2.7	p=0.52
		RAD + LAD	16.7 ± 4.7	16.8 ± 1.2	p=0.97
		LAD + GG	12.2 ± 3.5	10.0 ± 2.8	p=0.64
		RAD + GG	13.5 ± 4.3	9.3 ± 2.3	p=0.42
	Right	AD and/ or GG	31.0 ± 3.2	27.8 ± 1.8	p=0.40
		GG	18.0 ± 3.9	10.5 ± 3.2	p=0.17
		LAD	**25.5 ± 3.7**	**25.3 ± 1.8**	p=0.97
		RAD	9.8 ± 3.2	8.7 ± 2.2	p=0.77
		AD	26.0 ± 3.8	25.5 ± 1.7	p=0.91
		AD+ GG	13.0 ± 2.6	8.2 ± 2.1	p=0.18
		RAD + LAD	9.3 ± 3.0	8.5 ± 2.2	p=0.83
		LAD + GG	13.0 ± 2.6	8.0 ± 2.0	p=0.16
		RAD + GG	7.2 ± 2.5	4.8 ± 1.4	p=0.43

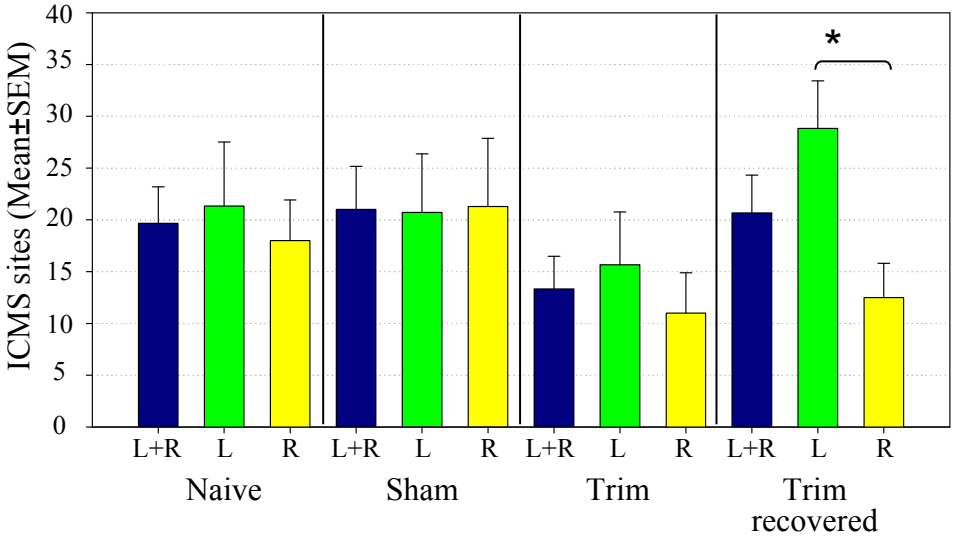
The number of positive ICMS sites obtained for each muscle or group of muscles within left and right face-M1 of each of the study groups at ICMS intensities of 40 and 60µA. There were no significant differences between the soft and hard diet groups (*t-test*, $p>0.05$). Within each group, LAD had significantly more sites within the right face-M1 and RAD had significantly more sites within the left face-M1 (*t-test*: $p<0.05$). (AD-anterior digastric; LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus).

Appendix 4-1

A. Number of positive ICMS (60μA) sites within face-M1



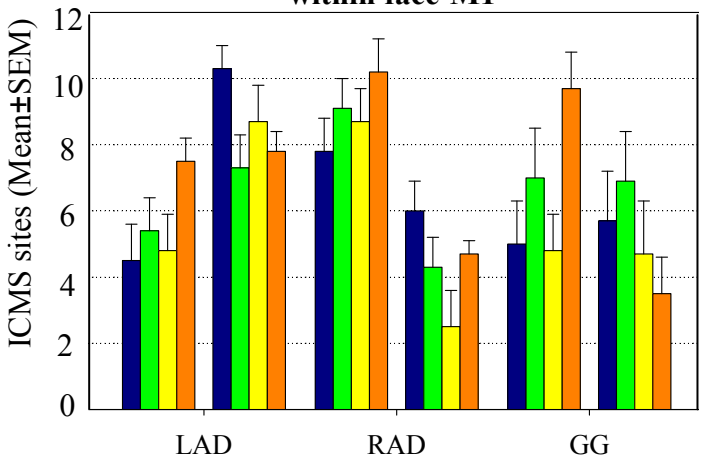
B. Number of GG positive ICMS (60μA) sites within face-M1



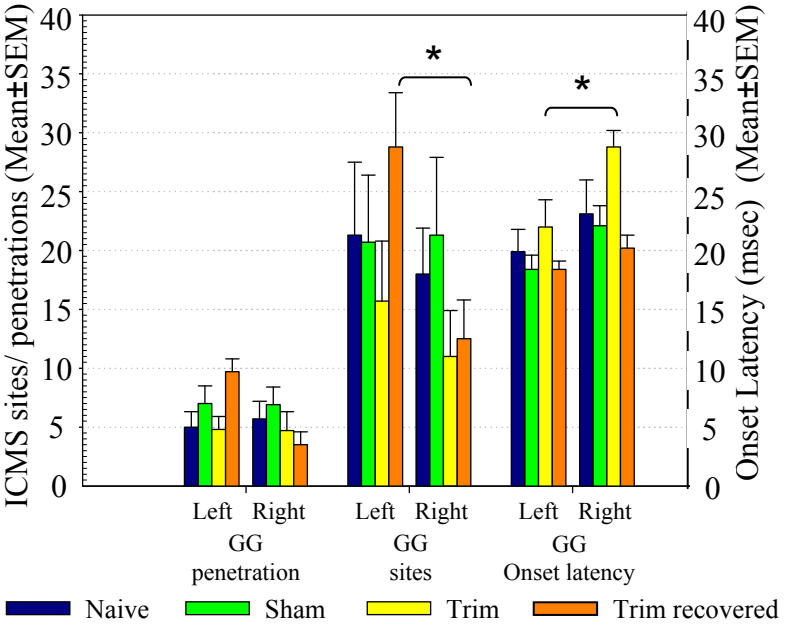
Positive ICMS sites at ICMS intensities of 60μA. There were no significant differences across the study groups in the number of LAD, RAD and GG positive ICMS sites in either left or right face-M1. LAD and RAD had a significant contralateral predominance (MMRM ANOVA, Bonferroni: $p<0.0001$). In the trim group, GG had a non-significant decreased number of sites. Only in the trim recovered group in comparison with the right face-M1, the left face-M1 had significantly more positive sites for GG (MMRM ANOVA, Bonferroni: $p=0.0027$), AD/GG overlapping sites (MMRM ANOVA, Bonferroni: $p<0.0001$), LAD/GG overlapping sites (* MMRM ANOVA, Bonferroni=0.0081) and only in the trim recovered and sham trim groups were there significantly more RAD/GG overlapping sites within the left than within the right face-M1 (*MMRM ANOVA, Bonferroni: $p=0.084$, $p<0.0001$, respectively).

Appendix 4-2

A. Number of positive ICMS (60μA) penetrations within face-M1



B. Number of ICMS (60μA) sites and penetrations and onset latency for GG within face-M1



For legend see Figs. 4-2 and 4-4 in chapter 4

Appendix 4-3

Number of positive ICMS sites within face-M1

Summary of Univariate Group Comparisons
(Mean ± SEM)

Stimulus Intensity	Cortical Side	Muscle	Naïve Group	sham trim Group	Trim recovered Group	Statistical significance
40	Left	AD and/ or GG	19.3 ± 4.8	17.0 ± 3.3	24.7 ± 3.7	p=0.29
		LAD	8.8 ± 3.4	7.3 ± 2.7	15.0 ± 3.7	p=0.22
		RAD	12.2 ± 4.5	13.0 ± 2.5	17.8 ± 2.9	p=0.46
		GG	12.2 ± 4.3	11.3 ± 3.5	15.3 ± 3.8	p=0.68
		AD + GG	6.0 ± 2.4	8.3 ± 2.7	10.8 ± 3.5	p=0.54
		AD	13.2 ± 4.3	14.0 ± 2.5	20.2 ± 3.7	p=0.30
		RAD + LAD	7.8 ± 3.6	6.3 ± 2.4	12.7 ± 2.8	p=0.30
		LAD + GG	4.8 ± 2.0	5.6 ± 2.4	8.7 ± 3.4	p=0.48
		RAD + GG	5.5 ± 2.4	7.7 ± 2.6	9.7 ± 2.9	p=0.61
	Right	AD and/ or GG	16.2 ± 3.1	17.1 ± 4.6	20.3 ± 3.1	p=0.56
		LAD	12.2 ± 3.5	12.3 ± 3.1	15.8 ± 2.5	p=0.59
		RAD	4.5 ± 1.9	5.1 ± 2.4	8.7 ± 2.8	p=0.56
		GG	8.5 ± 2.7	10.1 ± 4.4	6.8 ± 2.3	p=0.62
		AD + GG	5.0 ± 2.3	5.4 ± 2.5	4.0 ± 1.5	p=0.67
		AD	12.7 ± 3.7	12.4 ± 3.1	17.5 ± 2.8	p=0.47
		RAD + LAD	4.0 ± 1.6	5.0 ± 2.4	7.0 ± 1.5	p=0.68
		LAD + GG	5.0 ± 2.3	5.4 ± 2.5	3.7 ± 1.3	p=0.58
		RAD + GG	2.7 ± 1.5	3.6 ± 1.9	3.0 ± 1.3	p=0.80
60	Left	AD and/ or GG	32.5 ± 6.0	32.7 ± 5.0	40.7 ± 4.1	p=0.55
		LAD	18.3 ± 4.4	15.3 ± 4.3	24.0 ± 3.7	p=0.34
		RAD	23.3 ± 5.2	26.9 ± 4.0	32.0 ± 2.5	p=0.52
		GG	21.3 ± 6.2	20.7 ± 5.7	28.8 ± 4.6	p=0.43
		AD + GG	13.8 ± 4.2	16.4 ± 4.6	22.3 ± 3.5	p=0.33
		AD	25.0 ± 4.9	28.4 ± 3.9	34.2 ± 3.1	p=0.45
		RAD + LAD	16.7 ± 4.7	13.7 ± 3.9	21.8 ± 2.7	p=0.42
		LAD + GG	12.2 ± 3.5	11.4 ± 4.0	17.8 ± 3.3	p=0.35
		RAD + GG	13.5 ± 4.3	15.9 ± 4.5	21.3 ± 2.8	p=0.38
	Right	AD and/ or GG	31.0 ± 3.2	31.7 ± 6.9	33.2 ± 4.6	p=0.92
		LAD	25.5 ± 3.7	23.0 ± 4.8	29.3 ± 4.1	p=0.72
		RAD	9.8 ± 3.2	11.6 ± 3.3	14.5 ± 3.0	p=0.70
		GG	18.0 ± 3.9	21.3 ± 6.6	12.5 ± 3.3	p=0.41
		AD + GG	13.0 ± 2.6	14.3 ± 4.2	9.3 ± 2.3	p=0.50
		AD	26.0 ± 3.8	24.7 ± 4.6	30.0 ± 4.2	p=0.82
		RAD + LAD	9.3 ± 3.0	9.9 ± 3.5	13.8 ± 2.6	p=0.67
		LAD + GG	13.0 ± 2.6	14.3 ± 4.2	9.3 ± 2.3	p=0.50
		RAD + GG	7.2 ± 2.5	8.1 ± 3.5	6.7 ± 2.0	p=0.97

Summary of the total numbers (Mean± SEM) of positive ICMS sites for each muscle or group of muscles within left and right face-M1, at stimulation intensities of 40 and 60 μA as compared across the study groups through a series of ANOVA followed by *post hoc* Bonferroni-adjusted pairwise comparisons where applicable. There were no significant differences across the study groups.

Appendix 4-4
Number of positive ICMS sites within face-M1
(Naive and sham trim groups pooled)
Summary of Univariate Group Comparisons
(Mean ± SEM)

Stimulus Intensity	Cortical Side	Muscle	Control Group	Trim Group	Trim recovered Group	Statistical Significance
40	Left	AD and/or GG	18.1 ± 2.7	14.7 ± 2.5	24.7 ± 3.7	p=0.1631
		LAD	8.0 ± 2.1	6.5 ± 1.8	15.0 ± 3.7	p=0.1099
		RAD	12.6 ± 2.4	11.2 ± 1.9	17.8 ± 2.9	p=0.2739
		GG	11.7 ± 2.6	8.5 ± 3.8	15.3 ± 3.8	p=0.4643
		AD + GG	7.2 ± 1.8	5.5 ± 2.4	10.8 ± 3.5	p=0.3978
		AD	13.6 ± 2.3	11.7 ± 2.0	20.2 ± 3.7	p=0.1551
		RAD + LAD	7.0 ± 2.0	6.0 ± 1.6	12.7 ± 2.8	p=0.1700
		LAD + GG	5.2 ± 1.5	3.3 ± 1.3	8.7 ± 3.4	p=0.2934
		RAD + GG	6.7 ± 1.8	5.3 ± 2.3	9.7 ± 2.9	p=0.4854
	Right	AD and/or GG	16.7 ± 2.8	13.0 ± 2.2	20.3 ± 3.1	p=0.3541
		LAD	12.2 ± 2.2	10.0 ± 2.3	15.8 ± 2.5	p=0.3753
		RAD	4.8 ± 1.5	5.3 ± 1.4	8.7 ± 2.8	p=0.3602
		GG	9.4 ± 2.6	4.3 ± 2.2	6.8 ± 2.3	p=0.4299
		AD + GG	5.2 ± 1.6	2.2 ± 1.1	4.0 ± 1.5	p=0.4560
		AD	12.5 ± 2.3	10.8 ± 2.2	17.5 ± 2.8	p=0.2728
		RAD + LAD	4.5 ± 1.4	4.5 ± 1.2	7.0 ± 1.5	p=0.5059
		LAD + GG	5.2 ± 1.6	1.8 ± 1.1	3.7 ± 1.3	p=0.3713
		RAD + GG	3.2 ± 1.2	1.5 ± 1.0	3.0 ± 1.3	p=0.6560
60	Left	AD and/or GG	32.6 ± 3.7	31.7 ± 3.8	40.7 ± 4.1	p=0.3359
		LAD	16.7 ± 3.0	14.3 ± 3.1	24.0 ± 3.7	p=0.2104
		RAD	25.2 ± 3.1	28.0 ± 3.5	32.0 ± 2.5	p=0.3848
		GG	21.0 ± 4.0	15.7 ± 5.1	28.8 ± 4.6	p=0.2463
		AD + GG	15.2 ± 3.0	12.0 ± 3.4	22.3 ± 3.5	p=0.1967
		AD	26.8 ± 3.0	28.0 ± 3.5	34.2 ± 3.1	p=0.3191
		RAD + LAD	15.1 ± 2.9	14.3 ± 3.1	21.8 ± 2.7	p=0.2767
		LAD + GG	11.8 ± 2.6	9.0 ± 2.4	17.8 ± 3.3	p=0.1902
		RAD + GG	14.8 ± 3.0	12.0 ± 3.4	21.3 ± 2.8	p=0.2335
	Right	AD and/or GG	31.4 ± 3.8	28.2 ± 3.9	33.2 ± 4.6	p=0.7776
		LAD	24.2 ± 3.0	24.0 ± 3.6	29.3 ± 4.1	p=0.5576
		RAD	10.8 ± 2.3	14.3 ± 3.2	14.5 ± 3.0	p=0.5230
		GG	19.8 ± 3.9	11.0 ± 3.9	12.5 ± 3.3	p=0.2577
		AD + GG	13.7 ± 2.4	8.5 ± 2.7	9.3 ± 2.3	p=0.3159
		AD	25.3 ± 2.9	25.7 ± 4.0	30.0 ± 4.2	p=0.6427
		RAD + LAD	9.6 ± 2.2	12.7 ± 2.5	13.8 ± 2.6	p=0.4566
		LAD + GG	13.7 ± 2.4	8.5 ± 2.7	9.3 ± 2.3	p=0.3159
		RAD + GG	7.7 ± 2.1	6.5 ± 2.4	6.7 ± 2.0	p=0.9166

Summary of the total numbers (Mean± SEM) of positive ICMS sites for each muscle or group of muscles within left and right face-M1, at stimulation intensities of 40 and 60 μA as compared across the study groups through a series of ANOVA followed by *post-hoc* Bonferroni -adjusted pairwise comparisons where applicable. There were no significant differences across the study groups.

Appendix 4-5

Face-M1 Positive ICMS sites
Repeated-measures ANOVA results
(Naive and sham trim groups pooled)

Muscle	Predictor	F-statistic, DF, Statistical significance
AD and/or GG	**Overall Model**	**chi-sq=25.94, df=1, p<0.0001**
	Study group	F=1.48, df=2,22, p=0.2503
	Cortical side	**F=5.59, df=1,22, p=0.0273 ✶**
	Intensity	**F=51.32, df=1,22, p<0.0001 ✶**
	Study group * Cortical side	F=1.04, df=2,22, p=0.3696
	Study group * Intensity	F=0.06, df=2,22, p=0.9456
	Cortical side * Intensity	F=0.15, df=1,24, p=0.7055
LAD	**Overall Model**	**chi-sq=49.44, df=1, p<0.0001**
	Study group	F=1.80, df=2,22, p=0.1893
	Cortical side	**F=31.13, df=1,22, p<0.0001**
	Intensity	**F=52.17, df=1,22, p<0.0001**
	Study group * Cortical side	F=1.15, df=2,22, p=0.3365
	Study group * Intensity	F=0.04, df=2,22, p=0.0=9594
	Cortical side * Intensity	**F=4.89, df=1,24, p=0.0368**
RAD	**Overall Model**	**chi-sq=36.16, df=1, p<0.0001**
	Study group	F=1.25, df=2,22, p=0.3056
	Cortical side	**F=150.02, df=1,22, p<0.0001**
	Intensity	**F=54.23, df=1,22, p<0.0001**
	Study group * Cortical side	F=1.04, df=2,22, p=0.3690
	Study group * Intensity	F=0.60, df=2,22, p=0.5601
	Cortical side * Intensity	**F=14.14, df=1,24, p=0.0010**
GG	**Overall Model**	**chi-sq=20.77, df=1, p<0.0001**
	Study group	F=1.22, df=2,22, p=0.3157
	Cortical side	**F=17.02, df=1,22, p=0.0004**
	Intensity	**F=15.71, df=1,22, p=0.0007**
	Study group * Cortical side	**F=4.72, df=2,22, p=0.0197**
	Study group * Intensity	F=0.17, df=2,22, p=0.8424
	Cortical side * Intensity	F=0.20, df=1,24, p=0.6560
AD and GG	**Overall Model**	**chi-sq=23.78, df=1, p<0.0001**
	Study group	F=1.02, df=2,22, p=0.3787
	Cortical side	**F=24.31, df=1,22, p<0.0001**
	Intensity	**F=25.47, df=1,22, p<0.0001**
	Study group * Cortical side	**F=6.04, df=2,22, p=0.0081**
	Study group * Intensity	F=0.16, df=2,22, p=0.8544
	Cortical side * Intensity	F=0.35, df=1,24, p=0.5604
AD	**Overall Model**	**chi-sq=35.51, df=1, p<0.0001**
	Study group	F=1.68, df=2,22, p=0.2093
	Cortical side	F=3.93, df=1,22, p=0.0601
	Intensity	**F=70.02, df=1,22, p<0.0001**
	Study group * Cortical side	F=0.38, df=2,22, p=0.6856
	Study group * Intensity	F=0.24, df=2,22, p=0.7911
	Cortical side * Intensity	F=0.19, df=1,24, p=0.6703
RAD and LAD	**Overall Model**	**chi-sq=30.03, df=1, p<0.0001**
	Study group	F=1.80, df=2,22, p=0.1895
	Cortical side	**F=21.42, df=1,22, p=0.0001**
	Intensity	**F=30.93, df=1,22, p<0.0001**
	Study group * Cortical side	F=2.38, df=2,22, p=0.1157
	Study group * Intensity	F=0.18, df=2,22, p=0.8329
	Cortical side * Intensity	F=1.31, df=1,24, p=0.2631
LAD and GG	**Overall Model**	**chi-sq=23.88, df=1, p<0.0001**
	Study group	F=1.15, df=2,22, p=0.3348
	Cortical side	**F=6.08, df=1,22, p=0.0219**
	Intensity	**F=26.06, df=1,22, p<0.0001**
	Study group * Cortical side	**F=6.63, df=2,22, p=0.0056**
	Study group * Intensity	F=0.09, df=2,22, p=0.9106
	Cortical side * Intensity	F=0.04, df=1,24, p=0.8390
RAD and GG	**Overall Model**	**chi-sq=32.65, df=1, p<0.0001**
	Study group	F=0.56, df=2,22, p=0.5794
	Cortical side	**F=64.48, df=1,22, p<0.0001**
	Intensity	**F=25.03, df=1,22, p<0.0001**
	Study group * Cortical side	**F=4.43, df=2,22, p=0.0242**
	Study group * Intensity	F=0.15, df=2,22, p=0.8634
	Cortical side * Intensity	**F=5.28, df=1,24, p=0.0306**

Mixed model repeated-measures ANOVA, followed by *post-hoc* Bonferroni-adjusted pairwise comparisons where applicable, was performed in order to determine whether study group, cortical side, stimulation intensity (40 vs 60μA), or any combination of these effects significantly affected the number of positive ICMS-sites. These tests were performed separately for each muscle and each combination of muscles. (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus).

Appendix 4-6

A. Onset latencies of ICMS (60μA)-evoked EMG activities in LAD, RAD and GG within face-M1

(Mean ± SEM)

Cortical Side	Muscle	Naïve Group	Sham trim Group	Trim Group	Trim recovered Group	Statistical Significance
Left	LAD	22.6 ± 2.1	22.7 ± 2.0	19.6 ± 1.3	21.5 ± 1.8	p=0.6274
	RAD	14.2 ± 1.0	14.3 ± 0.8	13.1 ± 0.6	14.7 ± 0.9	p=0.6329
	GG	19.9 ± 1.9	18.4 ± 1.2	22.0 ± 2.3	18.4 ± 0.7	p=0.3734
Right	LAD	14.2 ± 0.6	15.4 ± 1.3	15.2 ± 0.9	16.0 ± 1.2	p=0.6983
	RAD	21.2 ± 1.6	21.9 ± 1.9	24.8 ± 1.1	23.1 ± 2.3	p=0.5471
	GG	23.1 ± 2.9	22.1 ± 1.7	28.8 ± 1.4	20.2 ± 1.1	p=0.5557

B. Onset latencies of ICMS (60μA)-evoked EMG activities in LAD, RAD and GG within face-M1 (Naïve and sham trim groups pooled)

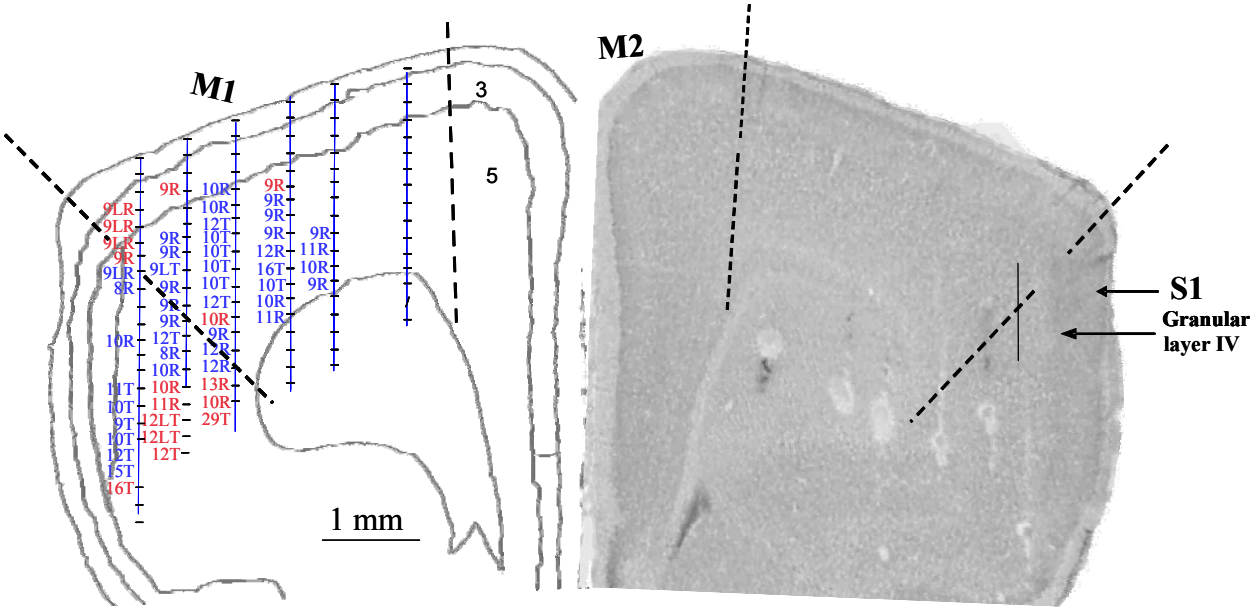
(Mean ± SEM)

Cortical Side	Muscle	Control	Trim Group	Trim Recovered	ANOVA
Left	LAD	22.6 ± 1.4	19.6 ± 1.3	21.5 ± 1.8	p=0.4105
	RAD	14.2 ± 0.6	13.1 ± 0.6	14.7 ± 0.9	p=0.4155
	GG	19.1 ± 1.1	22.0 ± 2.3*	18.4 ± 0.7	p=0.2520
Right	LAD	14.9 ± 0.8	15.2 ± 0.9	16.0 ± 1.2	p=0.6852
	RAD	21.6 ± 1.2	24.8 ± 1.1	23.1 ± 2.3	p=0.3484
	GG	**22.6 ± 1.6**	**28.8 ± 1.4**	**20.2 ± 1.1**	**p=0.0219**

A. Onset latencies of ICMS-evoked EMG responses in LAD, RAD and GG. There were no significant differences across the study groups. In all groups, LAD had a significantly shorter onset latency within the right face-M1 and RAD had a significantly shorter onset latency within the left face-M1 (paired *t-test*, $p<0.05$). Only in the trim group did GG have a significantly longer onset latency within the right face-M1 than within the left face-M1 (paired *t-test*, $p<0.001$). **B.** Similar results were obtained following pooling the naïve and sham groups into one control group, except that after pooling, within the right face-M1, GG onset latency was significantly longer in the trim group than in the trim recovered group (ANOVA: p=0.022; Bonferroni: p=0.027).

Appendix 5-1

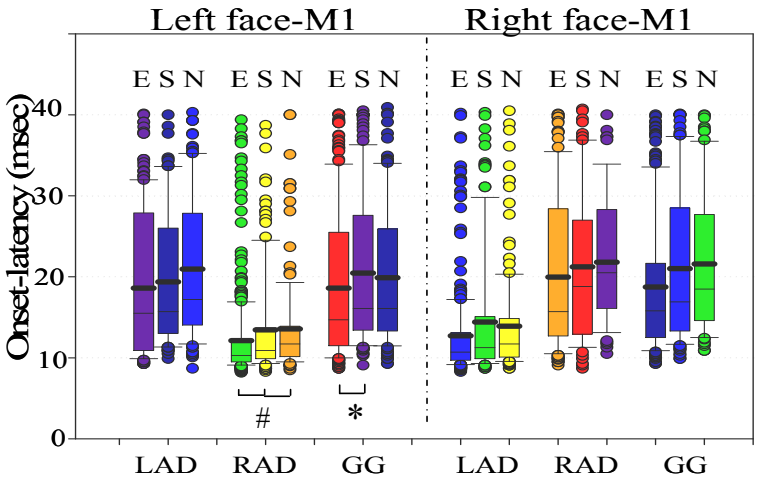
Cortical cytoarchitecture and motor representation map within face-M1 and face-S1



Muscle representation superimposed on histological coronal cross section. Each positive response site represents the muscle with the smallest value of onset latency at different stimulation intensities: Red - 60μA; Blue - 40μA. T-Tongue, L-left anterior digastric, R-right anterior digastric. S1 – primary somatosensory cortex; M1 – primary motor cortex; M2 – secondary motor cortex.

Appendix 5-2

Onset latencies of ICMS (60μA)-evoked EMG activities in LAD, RAD and GG within face-M1



Box plot showing the distribution of all onset latencies (0-40 msec) for each muscle within the left and right face-M1 of the extraction (E), sham (S) and naïve (N) groups at 60 μA ICMS intensity. In comparison to the sham group, in the extraction group, GG had a significantly shorter mean median of onset latency and the shortest value of mean onset latency (*ANOVA, Bonferroni, $p<0.05$). For RAD, the mean median of the onset latency had a trend toward a shorter onset latency in the extraction group than in the sham and naïve groups (# ANOVA $p<0.1$). (LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus; E-extraction; S-sham; N-naïve; Thick line – median; Thin line- mean; Boxes - 25–75% quartiles; Whiskers – 10th and 90th percentile).

Appendix 5-3
Number of positive ICMS sites within face-M1
Summary of univariate group comparisons
(Means ± SEM)

The number of positive ICMS sites obtained for each muscle or group of muscles within left and right face-M1 of each of the study groups at ICMS intensities of 40 and 60μA. In the left face-M1, in comparison with the sham and naïve groups, the extraction group had significantly more RAD and RAD-only site (*ANOVA: Bonferroni, $p<0.05$). (AD-anterior digastric; LAD-left anterior digastric; RAD-right anterior digastric; GG-genioglossus; LAD and RAD are for the overall representation of each muscle including the overlapping representations of LAD, RAD and/or GG. RAD only and LAD only are for site representing just one muscle, either LAD or RAD muscle).

Stimulus Intensity	Cortical Side	Muscle	Extraction Group	Sham Group	Naive Group	ANOVA
40 μA	Left	**AD**	**27.4 ± 2.6**	**10.3 ± 3.8**	**13.2 ± 4.3**	**p=0.0051**
		LAD	10.0 ± 2.3	6.3 ± 2.2	8.8 ± 3.4	p=0.62
		RAD	**26.5 ± 2.5**	**9.3 ± 3.7**	**12.2 ± 4.5**	**p=0.005***
		LAD Only	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0.2	p=0.99
		RAD Only	**10.4 ± 2.2**	**1.7 ± 0.5**	**3.7 ± 1.3**	**p=0.0036***
		GG	18.0 ± 3.5	16.3 ± 9.4	12.2 ± 4.3	p=0.77
		RAD + GG	13.5 ± 2.5	5.8 ± 3.6	5.5 ± 2.4	p=0.096
	Right	AD	20.0 ± 3.0	12.3 ± 2.2	12.7 ± 3.7	p=0.14
		LAD	19.5 ± 2.8	11.2 ± 2.5	12.2 ± 3.5	p=0.11
		RAD	8.1 ± 2.0	7.5 ± 1.1	4.5 ± 1.9	p=0.34
		LAD Only	6.0 ± 5.0	4.2 ± 3.5	5.7 ± 6.0	p=0.78
		RAD Only	0.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	p=0.26
		GG	16.9 ± 3.8	12.3 ± 3.9	8.5 ± 2.7	p=0.28
		LAD + GG	11.8 ± 2.5	5.7 ± 1.7	5.0 ± 2.3	p=0.089
60 μA	Left	**AD**	**52.4 ± 4.7**	**23.5 ± 4.2**	**25.0 ± 4.9**	**p=0.0004 ***
		LAD	21.5 ± 3.2	13.5 ± 3.5	18.3 ± 4.4	p=0.32
		RAD	**51.3 ± 4.6**	**23.2 ± 4.2**	**23.3 ± 5.2**	**p=0.0004 ***
		LAD Only	0.6 ± 0.4	0.2 ± 0.2	0.8 ± 0.5	p=0.48
		RAD Only	**20.8 ± 3.9**	**6.0 ± 1.7**	**5.2 ± 1.1**	**p=0.0016 ***
		GG	30.6 ± 4.4	26.3 ± 12.6	21.3 ± 6.2	p=0.70
		RAD + GG	25.3 ± 2.9	14.5 ± 5.0	13.5 ± 4.3	p=0.081
	Right	**AD**	**38.9 ± 5.5**	**23.8 ± 2.5**	**26.0 ± 3.8**	**p=0.054**
		LAD	**38.9 ± 5.5**	**23.8 ± 2.5**	**26.0 ± 3.8**	**p=0.051**
		RAD	38.9 ± 5.5	23.8 ± 2.5	26.0 ± 3.8	p=0.13
		LAD Only	12.6 ± 3.0	8.0 ± 2.1	10.0 ± 4.6	p=0.61
		RAD Only	0.9 ± 0.5	0.2 ± 0.2	0.5 ± 0.5	p=0.54
		GG	24.9 ± 4.9	20.3 ± 7.4	18.0 ± 3.9	p=0.66
		LAD + GG	20.0 ± 4.0	11.8 ± 2.8	13.0 ± 2.6	p=0.19

Appendix 5-4
Number of positive ICMS (60μA) **sites within face-S1**
Summary of univariate group comparisons
(Means ± SEM)

Cortical Side	Muscle	Extraction Group	Sham Group	Naïve Group	ANOVA
Left	**AD**	21.4 ± 3.0	6.7 ± 1.7	6.2 ± 1.4	**p=0.0003 ***
	LAD	3.6 ± 1.3	2.3 ± 0.8	3.0 ± 1.7	p=0.78
	RAD	21.3 ± 3.0	6.7 ± 1.7	4.8 ± 1.3	**p=0.0002 ***
	GG	13.9 ± 4.7	5.2 ± 2.0	10.0 ± 4.7	p=0.36
	RAD + GG	7.8 ± 2.4	3.0 ± 1.5	1.2 ± 0.8	p=0.055
Right	AD	20.5 ± 4.0	9.8 ± 5.5	9.3 ± 2.9	p=0.12
	LAD	20.5 ± 4.0	9.7 ± 5.5	9.3 ± 2.9	p=0.12
	RAD	3.6 ± 1.0	3.7 ± 1.6	3.0 ± 1.6	p=0.93
	GG	13.8 ± 5.8	7.5 ± 3.1	9.3 ± 3.1	p=0.61
	LAD + GG	6.1 ± 2.7	3.7 ± 2.0	3.7 ± 1.6	p=0.66

Summary of the total numbers (Mean± SEM) of positive ICMS sites for each muscle or group of muscles within left and right face-S1, at stimulation intensities of 60 μA as compared across the extraction and control groups (i.e., sham-extraction and naïve groups) through a series of ANOVA followed by *post-hoc* Bonferroni -adjusted pairwise comparisons, * $p < 0.05$.