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Title

Role of cutaneous and proprioceptive inputs in sensorimotor integration and plasticity occurring in the facial primary motor cortex.

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Key Points summary

• Previous studies investigated the effect of somatosensory afferent input on cortical excitability and neural plasticity often using TMS of hand motor cortex (M1) as a model. However, it is difficult to separate out the relative contribution of cutaneous and muscle afferent input to each effect.

• In the face, cutaneous and muscle afferents are segregated in the trigeminal and facial nerves respectively. We studied their relative contribution to corticobulbar excitability and neural plasticity in the depressor anguli oris M1.

• Stimulation of trigeminal afferents induced short-latency (SAI) but not long latency afferent inhibition (LAI) of face M1. In contrast, facial nerve stimulation evoked LAI but not SAI. Plasticity induction was observed only after a paired associative stimulation protocol using the facial nerve.

• Physiological differences in effects of cutaneous and muscle afferent inputs on M1 excitability suggest they play separate functional roles in behavior.

Abstract:

We examined the physiological mechanisms of sensorimotor integration and plasticity in face motor cortex (M1), with specific regard to the role of cutaneous and proprioceptive inputs activated by trigeminal and facial nerve stimulation, respectively.

In 16 healthy volunteers, the short-afferent inhibition (SAI), long-afferent inhibition (LAI) and the LTP-like plasticity following paired associative stimulation (PAS) were investigated in the depressor anguli oris muscle (DAO). Trigeminal nerve stimulation induced a significant inhibition ($p<0.05$) of DAO motor evoked potentials (MEP) at SAI inter-stimulus interval (ISI, 15, 20 and 30 ms), while no significant effects were observed at LAI ISIs (100-200 ms) and after PAS intervention. On the contrary, facial nerve stimulation induced a significant MEP inhibition in the LAI paradigm ($p<0.05$) as well as a significant facilitation at 10-30 minutes after PAS ($p<0.05$). The trigeminal-induced SAI and the facial-induced LAI showed a cranio-facial specificity. The facial F-wave was unaffected by both nerve stimulations. The present findings provide evidence that in face M1 cutaneous and proprioceptive afferents play a different functional role on sensorimotor integration and plasticity phenomena. Cutaneous inputs may exert a paucisynaptic inhibitory effect, while proprioceptive information is likely to target inhibitory and excitatory polysynaptic circuits involved in LAI and LTP-like plasticity.

The understanding of the physiology of face M1 may pave the way to further investigations on the physiopathology of several disorders involving the cranio-facial system.

Abbreviations.

1, area 1 of SI; 2, area 2 of SI; 3a, area 3a of SI; 3b, area 3b of SI; a, accessory nerve; ANOVA, analysis of variance; BS, brainstem; CMAP, compound muscle action potential; DAO, depressor anguli oris muscle; ES, electrical stimulation; f, facial nerve; FDI, first dorsal interosseus muscle; ISI, interstimulus time interval; LAI, long-afferent inhibition; LTD, long-term depression; LTP, long-term potentiation; M1, primary motor cortex; MEP, motor evoked potential; MSO, maximal stimulator output; PAS, paired associative stimulation; PMN, paramedian nuclei; PPC, posterior parietal cortex; PT, perceptual threshold; RMT, resting motor threshold; SI, primary sensory cortex; SII, secondary sensory cortex; SAI, short-afferent inhibition; SKIN, facial skin; t, trigeminal nerve; TH, thalamus; TMS, transcranial magnetic stimulation; TS, test stimulus; VII, facial motor nucleus; Vcn, fifth cranial nerve; VIIcn, seventh cranial nerve; VPM, ventroposteromedial nuclei.

Introduction

The influence of sensory inputs on motor cortex can be explored by examining their effect on the motor response to transcranial magnetic stimulation (TMS). The simplest method is to apply a sensory conditioning stimulus in the periphery and, at a variety of interstimulus intervals (ISIs), to measure how it affects the size of the TMS-evoked motor potential (MEP). In the upper limb, an electric stimulus to peripheral nerve suppresses MEPs at both short (20-25ms) and long (>100 ms) ISIs. These phenomena are termed short- (SAI) and long-latency (LAI) afferent inhibition, respectively (Chen et al., 1999; Classen et al., 2000; Tokimura et al., 2000; Kobayashi et al., 2003; Bikmullina et al., 2009; Devanne et al., 2009). Trans-cortical sensorimotor loops can be used experimentally to manipulate motor cortical excitability. For example, repetitive pairing of peripheral and cortical stimulation at ISIs around 20-25ms (paired associative stimulation, PAS) leads to long lasting increases in MEP amplitude that are thought to be due to early processes of synaptic long-term potentiation (Stefan et al., 2000; Wolters et al., 2003; Kujirai et al., 2006; Quartarone et al., 2006).

Most experiments have measured responses in intrinsic hand muscles after stimulation of the mixed nerves at the wrist or after stimulation of predominantly cutaneous fibres in the digital nerves. Although muscle afferents might have been expected to have a predominant input to motor cortex, the effects of pure cutaneous inputs are similar to those of mixed inputs, although the former are often weaker (Chen et al., 1999; Classen et al., 2000; Stefan et al., 2000; Tokimura et al., 2000), perhaps because fewer total afferent fibres are recruited (Bailey et al., 2016, Turco et al., 2017).

Pilurzi and colleagues studied these phenomena in the facial motor system using facial nerve stimulation paired with TMS of lower facial muscles M1. Interestingly, they showed significant LAI but not SAI after facial nerve stimulation and LTP-like facilitation in the PAS paradigm (Pilurzi et al, 2013). They hypothesized that stimulation of the mandibular branch of facial nerve was insufficient in generating a synchronous afferent volley to inhibit facial motor cortex at short latencies. Facial system provides a unique model to address the question of the relative roles of cutaneous and muscle afferent input. Cutaneous afferent inputs from the skin travel in the trigeminal nerve whereas proprioceptive afferents, i.e muscle spindles and tendon receptors, are generally thought to be absent (Connor & Abbs, 1998; Cattaneo & Pavesi, 2014). Human and animal works suggested that proprioceptive function is mediated by mechanoreceptors, present in high density in the overlying skin (Edin et al., 1995; Johansson et al., 1988; Connor & Abbs, 1998; Cattaneo & Pavesi, 2014) and probably within the facial muscles (Cobo et al., 2017a). These anatomo-functional features allow to activate separately cutaneous inputs in trigeminal nerve and mechanosensitive afferents excited by the contraction of the facial muscles following facial nerve stimulation. In addition, at the stylomastoid foramen the facial nerve is a pure motor nerve with a unimodal distribution of fiber diameter (Nordin et al., 1986) and previous study using microneurography of facial nerve demonstrated that no somatosensory signal were recorded in the facial nerve. However, in the peripheral branches, proper facial motor fibers are adjoined by trigeminal anastomoses (Hwang et al. 2007, Cobo et al., 2017b) that terminate in the facial tissue and supposedly carry putative proprioceptive information.

The present study extends the work of Pilurzi and co-workers (2013) by comparing the effects of facial and trigeminal stimulation on SAI, LAI and PAS in the depressor anguli oris muscle (DAO). The results revealed that, differently from the arm, in the face muscle SAI can be evoked by cutaneous inputs in trigeminal nerve but is absent after stimulation of mechanosensitive afferents in the facial nerve. In contrast, LAI and PAS are absent after trigeminal nerve stimulation and only observed after facial nerve stimulation. This may mean that activity in mechanosensitive muscle afferents is necessary for LAI and PAS. Alternatively, because stimulation of facial nerve evokes a muscle twitch, it may be that LAI and PAS in the face require natural patterns of repetitive sensory activity in stretch sensitive and other skin receptors that overlie facial muscles (Johansson et al., 1988; Edin et al., 1995; Edin & Johansson, 1995; Cattaneo & Pavesi, 2014) rather than the single synchronous volley evoked by trigeminal electrical stimulation, sufficient to induce SAI.

Methods

Ethical Approval

Experiments were conducted in sixteen healthy volunteers (10 females and 6 males; mean age 28.69 (4.84 SD: standard deviation) years), all right handed according to the Oldfield inventory scale. All subjects gave their informed written consent to participate in the study, which was approved by the local ethical committee (Bioethics Committee of ASL. n. 1 – Sassari, ID 2075/CE/2014) and conducted in accordance with the Helsinki declaration, except for registration in a database. None of the subjects had a history of neurological diseases. Subjects sat in a comfortable chair and were asked to stay relaxed but alert during the experiments.

EMG

EMG was recorded, in different experimental sessions, from the right DAO, from the right first dorsal interosseous (FDI), from the right masseter (MM) and from the right trapezius muscles, using 9 mm diameter Ag-AgCl surface electrodes. For the DAO EMG recordings, the active electrode was placed at the midpoint between the angle of the mouth and the lower border of the mandible, with the reference electrode over the mandible border, 1 cm below the active electrode and the ground electrode over the right forehead. For the FDI EMG recordings, the active electrode was placed over the muscle belly, the reference electrode at the second finger metacarpo-phalangeal joint and the ground electrode over the forearm. For the MM EMG recording active electrode was positioned in the lower third of the masseter muscle with reference electrode placed in the middle part of the zygomatic arch. For the trapezius muscle recording electrode was placed in the upper trapezius over the muscle belly and the reference electrode over the acromion-clavicular joint. Unrectified EMG signals were recorded (D360 amplifier, Digitimer Ltd, Welwyn Garden City, UK), amplified (x1000), filtered (bandpass 3-3000 Hz for MEP and 50-5000 Hz for F-waves recordings), and sampled (5 kHz per channel; window frame length: 500 ms for MEPs; window 250 ms for F-waves) using a 1401 power analog-to-digital converter (Cambridge Electronic Design, Cambridge, UK) and Signal 5 software on a computer and stored for off-line analysis.

TMS

TMS of the left hemisphere was performed using a figure-of-eight shaped coil with external loop diameter of 7 cm connected to a Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK). The optimal stimulation site, for the contralateral DAO or FDI, was carefully searched and then marked with a soft tip pen over the scalp, to maintain the same coil position throughout the experiments. The handle of the coil pointed posteriorly and laterally, at approximately 30-45 deg to the interhemispheric line (Kujirai et al., 2006; Pilurzi et al., 2013). The resting motor threshold (RMT) was taken as the lowest TMS intensity, expressed as percentage of the maximum stimulator output (MSO), that elicited, in the relaxed muscle, MEPs of 0.05 mV in at least 5 out of 10 consecutive trials. The intensity of the test stimulus (TS) for TMS of face M1 was 120% of RMT. In experiment 3, TS was set at 110% of RMT, adjusted to evoke in the FDI MEPs of nearly 1mV.

Electrical stimulation

Electrical stimulation (square-wave pulses of 0.2 ms duration) was applied through a pair of cup electrodes (cathode distal), connected to a constant current stimulator (model DS7; Digitimer, Welwyn-Garden City, Herts, UK), to the mentalis branch of the right trigeminal nerve, to the marginal branch of the right facial nerve and to the right accessory nerve as a conditioning stimulus (ES) in different sessions (Figure 1). Due to great individual anatomical variability of mandibular branch, electrodes position was adjusted in each subject to obtain supramaximal DAO excitation using the lowest stimulus intensity. In order to avoid MM activation by facial nerve stimulation, due to a conducted volume, MM EMG was recorded (Figure2).

The intensity of the electrical stimulus was set at an intensity of three times the Perceptual threshold (PT) of the subject for the trigeminal nerve; while for both facial and accessory nerve stimulations ES was set at a value able to evoke a small stable compound muscle action potential (CMAP) in the right DAO and the right- trapezius muscle respectively.

Facial F-waves, were evoked through ES of the right marginal branch of the facial nerve at supramaximal intensity (TS).

Experimental design

Main experiments

Experiment 1. Effects of trigeminal versus facial nerve stimulation on DAO MEP in the SAI and LAI protocols.

In all sixteen subjects, the effects of trigeminal and facial nerve stimulation on DAO MEPs were compared in the SAI and LAI paradigms. Single pulse TMS of the left face M1 was preceded by ES of the right trigeminal or facial nerves at various ISIs. The experiment was divided up into four blocks: trigeminal-SAI (tSAI), facial-SAI (fSAI), trigeminal-LAI (tLAI) and facial-LAI (fLAI). In tSAI and fSAI blocks, TS alone and 10, 15, 20, 25, 30 ms ISIs were tested. Each tLAI and fLAI block consisted of TS, 100, 150, 180 and 200 ms ISIs. The four blocks and all states (TS alone and ISIs) were randomized in each subject using a semi-randomized protocol. Ten unconditioned MEPs and 10 conditioned responses for each ISI were recorded from the right DAO at rest.

Experiment 2. After-effects of trigeminal versus facial nerve stimulation on DAO MEP in the PAS protocol.

Fifteen out of the 16 subjects enrolled in experiment 1 participated in experiment 2. Eight subjects (5 females and 3 males; mean age 29.25(4.74) years) underwent facial-PAS (fPAS), seven subjects (4 females and 3 males; mean age 28.22(4.87) years) underwent trigeminal PAS (tPAS). The PAS intervention was administered by pairing ES of the right facial or trigeminal nerves (fPAS and tPAS group, respectively) with TMS of the left face M1 using a ES-TMS ISI of 20 ms. Two hundred pairs of stimuli were given at 0.25 Hz. Subjects were instructed to keep facial muscles relaxed and stay alert. Twenty MEPs were collected from the resting DAO before and immediately (T0), 10 (T10), 20 (T20) and 30 (T30) minutes after PAS delivery.

Control experiments

Control experiments took place at least two weeks apart from the main experiments. SAI and LAI were tested using the same experimental and data collection procedure as experiment 1.

Experiment 3. Effects of trigeminal versus facial nerve electrical stimulation on facial F-Wave

To test the origin of the tSAI and fLAI, the effects of trigeminal and facial nerve stimulation on facial F-waves were investigated in 8 of the subjects who had participated in experiment 1 (5 females and 3 males; mean age 31.86(3.80) years). F-waves were obtained from the right DAO following TS of the marginal branch of the facial nerve for each subject. The same ES used in experiment 1 were given to the mental (ISIs of 10-15-20-25-30 ms ISIs) and marginal (ISIs of 100-150-180-200 ms) nerves before the TS. Twenty unconditioned and twenty conditioned recordings were collected for each ISI, in randomized order. Then, the persistence of the facial F waves, expressed as the number of F-waves clearly detectable (amplitude >20 μV) divided by number of recordings, was compared between the two conditions.

Experiment 4. Effects of accessory nerve stimulation on DAO MEP in SAI and LAI protocols

To compare the effects of homotopic and heterotopic cranial nerve stimulations (close and far from the target muscle, respectively), in 11 out of 16 subjects (8 females and 3 males; mean age 29.54(4.55) years), the effects of heterotopic accessory nerve stimulation on DAO MEPs were tested using SAI (aSAI) and LAI (aLAI) paradigms, where the stimulation of the accessory nerve was paired with TMS of face M1, and results compared with SAI and LAI induced by stimulation of homotopic cranial nerves. The accessory nerve was chosen due to the fact that it is the only one of the cranial nerves, except trigeminal and facial nerves, that can be easily stimulated by surface electrodes and it is thought to be purely motor thus not contain the sensory supply to the innervated muscles.

Experiment 5. Effects of trigeminal and facial nerve stimulation on FDI MEP in SAI and LAI protocols

Topographic muscle specificity of trigeminal and facial effects was tested in a distant muscle. FDI was chosen because of its accessibility and well standardized use in SAI and LAI protocols. All sixteen subjects underwent trigeminal and facial nerve stimulation (same stimulation procedure described in experiment 1) paired with TMS of hand M1. Results obtained in the FDI were then compared with significant effects obtained in the DAO muscle.

Statistical Analysis

Statistical analysis was performed with SPSS 18 software (SPSS Inc, Chicago, IL, USA).

Differences in PT, ES, RMT, TS intensities and test MEP amplitudes were assessed using Student's paired *t* test in experiment 1, 3, 4 and 5 with Student's unpaired *t* test in experiment 2. Values are expressed as a means ± standard deviation (SD).

Data processing

After processing of the EMG signal, each trial was characterized by a single number, i.e. the MEP amplitude. For each subject, each experimental condition contained a series of 10 repeated trials. Given the small number of repetitions we adopted, as a measure of central tendency, the median value. We therefore extracted the median of each pool of MEP amplitudes within each experimental condition. The data from conditioned conditions were then expressed as a ratio of the conditioned MEP over the unconditioned MEP. In this way values between 0 and 1 indicate an inhibitory effect of the conditioning stimulus and values larger than 1 indicate an excitatory effect of the conditioning stimulus. To ensure normality of the distribution, instead of the raw ratio (distributed between 0 and + infinity) we calculated the log of the ratio (distributed between -infinity and +infinity). The log-transformed data indicate inhibition of the conditioning stimulus whenever negative and facilitation whenever positive.

At this point two parallel analyses were performed. One was aimed at finding different distributions of the data according to the factorial designs of each experiment. This was done by feeding the individual data in ANOVAs with different structures according to each experiment. This approach is informative of the different distribution of data between experimental conditions (for example trigeminal stimulation vs facial stimulation) but is not informative of the absolute polarity (inhibition or excitation) of the effects of the conditioning stimulus on the test stimulus. We performed therefore a second, independent analysis consisting of t-tests for single samples applied to the data from each experimental condition against the null hypothesis of mean value = 0 (corresponding to the absence of modulation from the conditioning stimulus on the test stimulus).

Distribution analysis

Experiment 1: Independently for SAI and LAI a two way repeated measure (RM) ANOVA was performed with NERVE (facial or trigeminal) and ISI (SAI: 10, 15, 20, 25 or 30 ms; LAI: 100, 150, 180, or 200 ms) as a within factors.

Experiment 2: A mixed ANOVA was performed with NERVE (facial or trigeminal) as between-subjects factor, and TIME (baseline, 0, 10, 20 or 30 ms) as within-subjects factor.

Experiment 3: A two way RM-ANOVA was performed separately for both SAI and LAI protocols, with NERVE (facial or trigeminal) and ISI (SAI: 10, 15, 20, 25,30 ms; LAI: 100, 150, 180, 200 ms) as within factors.

Experiments 4 and 5: data from these experiments were merged with those from Experiment 1. Being the subjects participants in both the main and control experiments, this made it possible to perform a within-subjects analysis. In Experiment 4, a RM-ANOVA was performed separately for SAI and LAI, with NERVE (accessory, facial or trigeminal) and ISI (SAI: 10, 15, 20, 25 or 30; LAI: 100, 150, 180, 200ms) as within-subjects factors. In Experiment 5, tSAI and fLAI, were analyzed independently using RM ANOVA with MUSCLE (DAO or FDI) and ISI (SAI:10, 15, 20, 25 or 30; LAI: 100, 150, 180, 200 ms) as within-subjects factors.

Data distributions highlighted by significant ANOVA results were explored systematically by Tukey's Honestly Significant Difference Test.

Analysis of the effect of the conditioning stimulus

In each experiment we compared every set of data within each cell of the experimental design to the null hypothesis of mean=0. The significance threshold was adjusted for the number of comparisons using the Bonferroni-Holme method.

Results

Experiment 1. Effects of trigeminal versus facial nerve stimulation on DAO MEP in the SAI and LAI protocols.

SAI: Data indicated a clear difference between facial and trigeminal conditioning stimuli (Figure 3), which was specific for the 15 ms, 20 ms and 30 ms ISIs. ANOVA showed a significant main effect of NERVE ($F(1,15)=6.84$; $p=0.019$), ISIs ($F(4,15)=6.44$; $p=0.0002$) and a significant interaction NERVE*ISI ($F(4,15)=2.77$;$p=0.03$). Post-hoc analysis indicated a significant difference between trigeminal and facial stimulation at 15 ($p=0.014$), 20 ($p=0.014$) and 30 ms ($p=0.003$) ISIs. The one-sample t-tests indicated absolute inhibitory effects only for trigeminal nerve stimulation at 15 ($p=0.007$), 20 ($p=0.003$) and 30 ms ($p=0.005$) ISIs.

LAI: The results indicated that overall facial stimulation had a different effect comparing to trigeminal stimulation, at all ISIs. ANOVA showed a main effect of NERVE ($F(1,15)=8.06$; $p=0.012$) but a non-significant effect of ISI and interaction among the factors (all $p>0.26$). The one-sample t-tests indicated absolute inhibitory effects for facial nerve stimulation at 200 ms ISI ($p=0.003$).

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Data obtained from experiment 1 are shown in Figure 3 and recordings from a representative subject are reported in Figure 4.

Experiment 2. After effects of trigeminal versus facial nerve stimulation on DAO MEP in the PAS protocol.

Statistical analysis showed a significant effect of NERVE ($F(1,13)=18.43$; $p=0.0009$) but a non-significant effect of ISI or interaction among the factors (all $p>0.52$). Compared to trigeminal nerve stimulation, facial stimulation showed a clear PAS effect at all intervals measured. Polarity analysis indicated absolute facilitatory effects, compared with baseline only for facial stimulation, at T10 ($p=0.002$) and T30 ($p=0.005$) time points after PAS (Figure 5).

Experiment 3. Effects of trigeminal versus facial nerve electrical stimulation on facial F-Wave

F-waves were recorded from the right DAO, following supramaximal stimulation (mean intensity: 24(3.9) mA) of the ipsilateral marginal branch of the facial nerve. Each TS evoked a stable CMAP at 2.3(0.5) ms. In regard to conditioning stimuli, the mental nerve was stimulated at the SAI ISIs at a mean intensity of 3.8(0.6) mA and the facial nerve at the LAI ISIs at 4.4(1.4) mA. Mean F-wave latency was 14.7(1) ms. The mean F-waves persistence value (number of F-waves/number of stimuli), measured at baseline was 0.56(0.12) and 0.59(0.1) ($p>0.05$) in the trigeminal and facial conditioning trials, respectively. One-way ANOVA with ISI as within-subjects factor, showed no significant effect of both the trigeminal and facial CS at SAI and LAI ISIs, respectively (Figure 6).

Experiment 4. Effects of accessory nerve stimulation on DAO MEP in SAI and LAI protocols.

SAI: ANOVA showed a no significant main effect of NERVE ($F(2, 20)=2.93$, $p=0.077$) but a significant effect of ISI ($F(4, 40)=6.54$; $p=0.0004$) and interaction NERVE*ISI ($F(8, 80)=2.12$, $p=0.044$). Post-hoc analysis showed that at 15 ms ISI the effects induced by accessory nerve stimulation were significantly different from those of trigeminal nerve stimulation ($p=0.04$) but not from those induced by facial nerve stimulation ($p=1.00$). On the contrary, at 20 ms ISI, the effects of accessory nerve stimulation on DAO MEP were significantly different from those induced by facial nerve stimulation ($p=0.02$) but not from those induced by trigeminal nerve stimulation ($p=0.56$). No significant difference between effects of accessory and trigeminal or facial nerve stimulation was found at any other ISIs.

LAI: The effects of accessory nerve stimulation resulted non different from those induced by facial nerve stimulation; a trend of difference was instead detected when compared with the effects of trigeminal nerve stimulation. ANOVA showed a main effect of NERVE ($F(2, 20)=3.47$, $p=0.05$) and ISI ($F(3, 30)=4.99$, $p=0.006$) but no significant interaction among the factors ($p=0.35$). Post-hoc analysis using Tukey's HSD to investigate the main effect of NERVE, indicated that the trigeminal stimulation was significantly different form the facial stimulation ($p=0.02$).

Polarity analysis showed that accessory nerve stimulation was ineffective at SAI ISIs, but induced a clear inhibitory effect at 100 ms ISI ($p=0.002$) in the LAI protocol. Data obtained from experiment 4 are shown in Figure 7.

Experiment 5. Effects of trigeminal and facial nerve stimulation on FDI MEP in SAI and LAI protocols

SAI: ANOVA showed a significant main effect of ISI ($F(4,60)=3.78$; $p=0.008$) and an interaction MUSCLE*ISIs ($F(4,60)=3.52$; $p=0.012$). Post-hoc analysis detected a different effect exerted by trigeminal stimulation on the FDI MEPs compared to DAO MEPs at 15 ms ($p=0.022$) and 20 ms ($p=0.009$) ISIs.

LAI: ANOVA did not show a significant effect of MUSCLE, ISI or interaction among the factors (all p values >0.17).

No absolute inhibitory effect for both tSAI and fLAI on FDI MEPs were found (all p's < 0.05). These results are shown in Figure 8.

Discussion

The main finding of the present study is that SAI could be evoked by stimulation of cutaneous afferents in the trigeminal nerve but was absent after stimulation of distal facial nerve branches. In contrast, LAI and PAS required stimulation of facial nerve (see also Pilurzi et al., 2013), but were absent after trigeminal stimulation.

Sensorimotor integration and LTP-like plasticity in the facial motor cortex

Since facial muscles are devoid of muscle spindle and joint receptors (Connor & Abbs, 1998; Cattaneo & Pavesi, 2014; Cobo et al., 2017a), we hypothesized that stimulation of the marginal branch of the facial nerve can excite cutaneous mechanoreceptors activated by the muscle twitch (Edin & Johansson, 1995) and/or nerve fibers directed to "Ruffini-like" mechanoreceptors within facial muscles (Cobo et al., 2017a), possibly travelling in distal trigemino-facial anastomoses (Cattaneo & Pavesi, 2014; Hwang et al., 2007). Our results suggest that these receptors do not contribute to SAI or that there are too few of them to generate a measureable effect.

It could be that both LTP-like plasticity and LAI depend solely on input from the mechanoreceptors in facial muscles since they are not seen after trigeminal stimulation. Furthermore, facial nerve stimulation additionally excites motor fibres, and the resulting muscle contraction will be sensed by "proprioceptive" mechanoreceptors contained in the skin overlying the DAO belly (Edin & Johansson, 1995). Although a single volley in cutaneous afferents, such as that after stimulation of the trigeminal nerve, may not be sufficient to evoke LAI and PAS, it could be that the more natural sustained pattern of activation produced during an evoked muscle contraction can contribute to LAI and PAS. Note though that if this is the case, then the same pattern of natural input does not contribute to the later phases of SAI (ISIs 25-30 ms), even though these intervals would leave adequate time for the delayed afferent input produced by muscle contraction to reach M1.

Future experiments could test the "natural stimulation" hypothesis in more detail. For example, stimulation of pure cutaneous receptors with stimuli such as light brush or skin stretch (Edin et al., 1995; Ito & Ostry, 2010), which are likely to produce a more dispersed afferent volley from slow-adapting receptors, might also produce trigeminal LAI and even PAS. Whatever the explanation, the difference in effects on SAI and LAI provides further evidence that the mechanisms underlying these phenomena are different (Chen et al., 1999; Sailer et al., 2002, 2003; Paulus et al., 2008; Bailey et al., 2016, Turco et al., 2017). It complements observations in the hand, that show GABAa and cholinergic systems (Di Lazzaro et al., 2000, 2007; Paulus et al., 2008) underlie SAI, while GABAb pathways may mediate LAI (Sailer et al., 2002, 2003; Paulus et al., 2008). Recently, it was shown that SAI could be modulated by a directed stimulation, using TMS protocols, to SI but not M1 (Kojima et al., 2015; Tsang et al., 2014, 2015). Furthermore, PAS does not alter the expression of SAI but may decrease LAI (Russmann et al., 2009; Meunier et al., 2012).

Our conclusion is that in face M1, SAI depends on cutaneous input only with no role from mechanosensitive receptors in muscle. In fact this may be similar to the situation in the hand since there is no evidence there that activation of muscle afferents is necessary (Tokimura et al., 2000). All we know is that stimulation of cutaneous fibres, whether in digital nerves or in mixed nerves, can produce SAI. Moreover, Bailey and colleagues showed that SAI is influenced by the volume of the sensory afferent volley in fact, it showed the largest effect was obtained when the sensory fibers are fully recruited (Bailey et al., 2016). On the other hand there is a lack of experiment at investigating the role of pure muscle receptor input in SAI evocation.

The source of afferent input responsible for LAI and PAS is less clear. A recent study by Turco and co-workers (2017) showed that LAI in the arm was strongest using mixed nerve stimulation but it was not influenced by further recruitment of sensory afferents. These data indicate that LAI is less reliant on the sensory afferent volley once a minimum afferent volley to activate the circuit is achieved (Turco et al., 2017.).

In view of the uncertain status of the "natural stimulation" hypothesis, it may well be that they depend more strongly on muscle afferent input. If so there may be a resemblance to the situation in the hand where muscle afferent input appears necessary to evoke PAS with anterio-posterior TMS (Kujirai et al., 2006). However, it may also be that co-activation of mechanoceptive information is crucial to induce a PAS-dependent LTP-like plasticity in face M1, while pure cutaneous afferent information is not. This might explain why, in the hand, digital nerve stimulation leads to smaller effects compared with those obtained with mixed nerve stimulation at an intensity sufficient to generate a muscle twitch (Stefan et al., 2000; Wolters et al., 2003; Kujirai et al., 2006; Quartarone et al., 2006).

Origin of tSAI and fLAI in facial muscles

Besides hand muscles, F-waves have been characterized in the upper and lower facial muscles (Zappia et al., 1993; Wedekind et al, 2001). Their amplitude and persistence are considered as an expression of facial motoneuron activity and are currently used to test brainstem excitability (Öge et al., 2005; Ishikawa et al., 1996). In our study, we analyzed the persistence of the facial F-waves rather than the amplitude, since this parameter is highly variable in the general population (Fisher, 1992; Wedekind et al., 2001) and is considered an index related to the pool of motoneurons excited rather than to motoneuronal excitability, the latter being represented more appropriately by the F-wave persistence (Rivner, 2008).

The lack in modulation of H reflex and F-waves were used to prove the cortical origin of SAI and LAI in hand muscles (Chen et al., 1999; Tokimura et al., 2000). Likewise, in the DAO the same conditioning trigeminal and facial nerve inputs, which were able to produce a significant SAI and LAI, respectively, did not alter the persistence of the facial F-waves, suggesting a cortical origin for these phenomena in face M1.

Possible sensorimotor interactions at subcortical level

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The origin of SAI evoked by trigeminal stimulation is less clear. A cutaneo-muscular silent period has been previously described in the DAO at a variable latency. An early, ipsilateral component appears around 15 ms from trigeminal stimulation, followed by a longer and bilateral silent period appearing after 40 ms (Pavesi *et al.*, 2000, 2003; Cattaneo *et al.*, 2007; Cattaneo & Pavesi, 2010). The corticobulbar volley evoked by TMS in the current work could interact with the inhibitory afferent information mediating the trigemino-facial silent period, especially at the 15 and 20 ms ISIs, while the descending volley at the 30 ms ISI would fall in the reprise of voluntary activity between the early and late components. If any interaction occurs between the peripheral inhibition and the descending cortico-bulbar volley, it does so at pre-motoneuronal level, because in the current experiment we showed that facial F-waves were unaffected by trigeminal stimulation at the ISIs of interest. Another consideration regards the intensity of trigeminal stimulation. The recruitment of the cutaneous silent period in the DAO muscles is maximal only at 7xPT, therefore the intensity of cutaneous stimulation used in the present experiment (3xPT) is not optimal to elicit the cutaneous silent period, though this phenomenon is known to occur already at stimulation intensities of 2xPT). In conclusion, we cannot exclude with the present findings that the trigeminal inhibitory effects can be due at least in part to a brainstem reflex circuitry rather than being transcortical in origin. This is particularly true for the short ISIs of 15 and 20 ms, but it is unlikely for the 30 ms ISI.

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Homo- and heterotopic stimulations on SAI and LAI in face M1

We observed no differences in the amount of DAO MEP inhibition induced by homotopic (trigeminal and facial nerves, respectively) and heterotopic (accessory nerve) stimulations, given to separate but contiguous districts (cranial versus cervical). This finding seems in contrast with hand data demonstrating a topographic MEP inhibition depending on homo- and heterotopic stimulation (Classen et al., 2000; Tamburin et al., 2001; Helmich et al., 2005). However, this specificity was not apparent at higher stimulus intensities (Tamburin et al., 2001; Helmich et al., 2005). Thus, the apparent discrepancy between face and hand data, could be accounted for by the intensity required for the heterotopic accessory nerve stimulation to induce SAI and LAI in the DAO muscle, which was significantly higher than that used for the homotopic stimulation of the trigeminal and facial nerves. In addition, the aLAI showed a shorter duration than the fLAI, which might be attributed to a possible facilitatory long-interval effect described on distant muscles (Bikmullina et al., 2009).

However, the similar effect obtained for homotopic and heterotopic stimulations might simple reflect anatomical and functional interactions in the cranio-cervical district (Danziger et al., 1995; Watson & Drummond, 2014; Boehm & Kondrashov, 2016). Recent studies in humans suggested that the accessory nerve carries not only motor fibers, but also sensory inputs. In particular, visible ganglia or clustered cells were detected in the accessory nucleus, mainly at C1 spinal level (Boehm and Kondrashov, 2016). Furthermore, evidence supported the existence of a functional connectivity between the trigeminal and cervical systems at the level of the cervical-brainstem junction. In fact, functional connections between cutaneous trigeminal afferents and the spinal root of the accessory nerve were suggested to occur in patients with reinnervation of the VII-XI nerves (Danziger et al., 1995).

Cranio-facial topographic specificity of tSAI and fLAI

While the heterotopic stimulation of the accessory nerve did not reveal a clear topographic effect in the SAI and LAI paradigms, the absence of any effect on the FDI exerted by the activation of trigeminal and facial nerves suggests that "cranio-facial" selectivity for these inhibitory effects exist.

The FDI was chosen for its easy access and standardized responses in comparison with other upper limb muscles closer to DAO, such as shoulder muscles. Other cranial muscles non-pertinent to the trigeminal and facial systems, such as sternocleidomastoid or trapezius muscles, were instead excluded since they are technically difficult to stimulate with TMS, SAI and LAI are not standardized in these muscles and the sternocleidomastoid muscle has been reported to be innervated by an ipsilateral cortico-bulbar projection (Odergren & Rimpiläinen, 1996).

A trigeminal-induced MEP inhibition in the relaxed FDI has been previously described (Siebner et al., 1999), but this effect required longer ISIs (30-60 ms versus 10-30 ms) and higher stimulation intensities (10xPT versus 3xPT) than those used in our experiments. Here, trigeminal stimulation at 3xPT was sufficient to produce a consistent inhibition of the DAO MEPs at 20 ms ISI, but ineffective on the FDI up to 30 ms ISIs.

Possible circuits involved in sensorimotor integration and paired associative stimulation protocols

It can be hypothesized that cutaneous trigeminal inputs activate oligosynaptic circuits which might primarily involve inhibitory connections between areas 3b and 1 of the contralateral primary somatosensory cortex (SI), (Allison et al., 1991; Forss et al., 1994) and layers 5/6 of M1 (Kaneko et al., 1994a, 1994b; Porter, 1996; Classen et al., 2000; Tokimura et al., 2000; Aronoff et al., 2010; Mao et al., 2011; Cash et al., 2015) and that by these connections they mediate the SAI (Porter et al., 1996; Cash et al., 2015; Kojima et al., 2015; Tsang et al., 2014, 2015; Bailey et al., 2016). Proprioceptive facial inputs activate inhibitory circuits involving areas 3a and 2 of contralateral SI (Friedman & Jones, 1981; Allison et al., 1991) and bilaterally the secondary somatosensory cortex (SII) and the posterior parietal cortex (PPC) (Allison et al., 1991; Forss et al., 1994; Karhu & Tesche, 1999; Chen et al. 1999; Boakye et al., 2000; Sailer et al. 2002), at LAI intervals (Chen et al. 1999; Classen et al., 2000). In line with the idea that LAI and PAS share their underpinning circuits (Russmann et al., 2009; Meunier et al., 2012), it seems reasonable to suppose that the same LAI-inducing proprioceptive input, at short intervals might engage excitatory interneurons in SI and M1 (layers 2/3) mediating

PAS-induced LTP-like plasticity (Kaneko et al., 1994b; Cash et al., 2015), but also SII and PPC. The crucial role of SII for sensory processing and sensorimotor integration in face M1 has been confirmed recently by a fMRI study where the Bell's palsy condition induced significant changes in connectivity in SII (Klingner et al., 2014).

Taken all together, this information may allow the drawing of a generic model (Fig. 9) that attempts to illustrate the possible pathways underlying sensorimotor integration processes and PAS-induced LTP-like plasticity in face M1.

Conclusions

The present findings provide evidence that cutaneous and proprioceptive afferents could play a different functional role in sensorimotor integration and plasticity of face M1. Cutaneous inputs seem to have a paucisinaptic inhibitory access to face M1. Proprioceptive information is likely to target a more complex higher order network, via excitatory and inhibitory polysynaptic circuits involved in sensorimotor integration and motor learning.

The understanding of the physiology of sensorimotor integration processes at the level of face M1 may pave the way to future studies aimed at clarifying the physiopathology of several motor disorders involving the cranio-facial system.

Author contributions

The experiments were performed at the laboratories of neurophysiology of the Department of Biomedical Sciences, University of Sassari, Sassari (Italy).

Conception and design of the experiments: G.P., J.C.R and F.D.; acquisition, analysis and interpretation of data: G.P., F.G., B.M., L.C., G.P., J.C.R and F.D. drafting the article or revising it critically for important intellectual content: G.P., L.C., G.P., J.C.R and F.D. All authors approved the final version for publication, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Competing interests.

The authors do not have any competing interest in and did not receive any funding for this research.

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Figure legends

Figure 1. Position of the electrodes for the recording and electrical stimulation of the facial, trigeminal and accessory nerves.

For both trigeminal and facial nerves stimulation EMG of DAO muscle was recorded, the active electrode is placed at the midpoint between the angle of the mouth and the lower border of the mandible (-), the reference electrode over the mandible border, 1 cm below the active electrode (+). (A) For the electrical stimulation of the trigeminal nerve, the cathode electrode is positioned in the chin border (+) and the anode electrode in the right mental foramen (-). (B) For the electrical stimulation of the facial nerve, electrodes are placed over the marginal branch of the right facial nerve with cathode distal (+) and anode proximal (-), nearly 2 cm far from the mandibular angle. The correct position was carefully searched for each subject moving 1 cm up and down over the mandible border in order to have a stable CMAP in the DAO muscle with the lowest intensity, but not conduction volume in the masseter muscle .

(C) For the accessory nerve stimulation EMG of the upper trapezius was recorded. The electrical stimulation electrodes are placed in the cervical triangle, 1-2 cm posteriorly to the lateral border of sternocleidomastoid and anteriorly to the trapezius muscle with cathode distal (+) and anode proximal (-).

Electrical stimulation electrodes are shown as white circle while EMG electrodes as black circle.

Figure 2. Effects of trigeminal and facial nerve stimulation on depressor anguli oris muscle (DAO) and in the masseter muscle (MM).

EMG recordings from the DAO and MM muscles of a representative subject are reported for each stimulation condition. The electrical stimuli (duration 0.2 ms, intensity 3xT, frequency 0.25 Hz) were applied over the right facial and trigeminal nerves.

Figure 3. Effects of trigeminal and facial nerve stimulation on motor evoked potentials (MEP) of the depressor anguli oris muscle (DAO) in the short afferent inhibition (SAI) and long afferent inhibition (LAI) paradigms.

A – In the SAI protocol (10-30 ms interstimulus intervals, ISI), the amplitude of DAO MEPs was significantly reduced by trigeminal stimulation (tSAI, black line) at 15, 20 and 30 ms ISIs while it appeared unaffected by facial nerve stimulation (fSAI, grey line).

B- In the LAI protocol (100-200 ms ISI), DAO MEPs showed a significant inhibition at each ISI tested after facial nerve stimulation (fLAI, while trigeminal stimulation was ineffective at any ISI tested.

Ordinates report MEP amplitude expressed as a ratio of the unconditioned MEP. * $p<0.05$. The graphs report the group means (N = 16 subjects). Error bars represent 95% confidence interval of the mean.

Figure 4. Effects of trigeminal and facial nerve stimulation on motor evoked potentials (MEP) of the depressor anguli oris muscle (DAO) with a paired stimulation in short afferent inhibition (SAI) and long afferent inhibition (LAI) paradigms.

Recordings of MEPs from the DAO of a representative subject are reported for each condition (unconditioned MEP, induced by the test stimulus (TS), and conditioned MEPs at interstimulus intervals (ISIs) of 20 and 200 ms). Conditioning stimulus was applied over the right facial and trigeminal nerves.

Figure 5. Effects of facial and trigeminal paired associative stimulation (fPAS and tPAS, respectively) on the magnitude of motor evoked potentials (MEP) recorded from the depressor anguli oris muscle (DAO).

The graphs show the time course of effects on DAO MEP amplitudes after 0 (T0), 10 (T10), 20 (T20), 30 (T30) minutes from fPAS (white boxes) and tPAS (grey boxes) interventions.

Compared with each other, MEP ratio after fPAS and tPAS were significantly different at all time points, being significantly increased following the fPAS intervention. *$p<0.05$. The graphs report the group means (N = 15 subjects). Error bars represent 95% confidence interval of the mean.

Figure 6. F-waves in the depressor anguli oris muscle (DAO) after stimulation of the trigeminal and facial nerves at SAI and LAI intervals, respectively.

The graphs report the F wave persistence expressed as percentage number of trials eliciting an F-wave following 20 facial nerve stimuli. We report data from unconditioned stimuli (baseline) and stimuli preceded by trigeminal stimulation at SAI intervals (A- left panel) and facial stimulation at LAI intervals (B- right panel). F-waves persistence was not altered by either of the two conditioning stimuli, at any ISI tested. The graphs report the group means (N = 8 subjects) Error bars represent 95% confidence interval of the mean. The dashed line indicates the mean baseline value.

Figure 7. Effects of homotopic and heterotopic nerve stimulation on motor evoked potentials (MEP) of the depressor anguli oris muscle (DAO).

A- In the short afferent inhibition (SAI) protocol, the amplitude of DAO MEPs was significantly reduced at 20 ms interstimulus interval (ISI) by stimulation of both homotopic trigeminal (tSAI, grey boxes) and heterotopic accessory (aSAI, black boxes) nerve stimulation

B- In the long afferent inhibition (LAI) protocol DAO MEPs were significantly inhibited by both homotopic facial (fLAI, white boxes) and heterotopic accessory (aLAI, black boxes) nerve stimulations.

Ordinates report MEP amplitude expressed as a ratio of the unconditioned MEP. *$p<0.05$. The graphs report the group means (N = 11 subjects). Error bars represent 95% confidence interval of the mean.

Figure 8. Muscular somatotopy of trigeminal short afferent inhibition (tSAI) and of facial long afferent inhibition (fLAI) in the cortical representation of the depressor anguli oris muscle (DAO) and first dorsal interosseous muscle (FDI)

A- Effects of trigeminal nerve stimulation on motor evoked potentials (MEP) recorded from the DAO (white boxes) and from the FDI (grey boxes) at SAI inter-stimulus intervals (ISI). The DAO exhibited a significant SAI at 15 and 20 ms ISIs, while the FDI was unaffected at any ISI tested.

B- Effects of facial nerve stimulation on DAO and FDI MEPs in the LAI protocol. The box plot shows no significant difference between the two muscles.

Ordinates report MEP amplitude expressed as a ratio of the unconditioned MEP. *$p<0.05$. The graphs report the group means (N = 16 subjects). Error bars represent

95% confidence interval of the mean.

Figure 9. Schematic model of circuits in the facial motor system engaged by SAI, LAI and PAS paradigms.

Cutaneous inputs from the facial skin, carried by the Vth cranial nerve (Vcn) join areas 3b and 1 of the primary somatosensory cortex (SI), via the ventral postero-medial nucleus (VPM) of the thalamus (TH). From SI-3b and SI-1, oligosynaptic pathways project to layers 5/6 of the facial primary motor cortex (M1) exerting a short afferent inhibition (SAI) on pyramidal cells innervating the facial motor nucleus (VII) in the brainstem (BS). The same inputs, may produce a SAI phenomenon in the depressor angulis oris muscle (DAO), via sensory-motor integration processes occurring at brainstem (BS) level or mediated by the paramedian nuclei (PMN) of the TH.

Single pulse stimulation of the VIIth cranial nerve (VIIcn) excites proprioceptive afferents that project to neurons in the SI areas 3a and 2. These neurons modulate the activity of cortical interneurons in layers 2/3 of M1 producing a short-latency cortical facilitation (SICF) and also send connections to the secondary somatosensory cortex (SII). From SI-3a, SI-2 and SII polysynaptic projections to layers 5/6 of M1 produce a long afferent inhibition (LAI) on the DAO. Paired associative stimulation (PAS) of M1 and of the VIIcn acts via polysynaptic excitatory circuits on both M1 layers 2/3 and SII inducing a long-term potentiation (LTP)-like plasticity in M1.