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Titolo: Emotion processing and brain plasticity in patients with congenital facial palsy: the case of Moebius Syndrome

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Riassunto

Numerose indagini neuroscientifiche degli ultimi trent'anni hanno ampliato la conoscenza del funzionamento del Sistema dei Neuroni Specchio (SS), le cui rappresentazioni neuronali sono attive sia durante l'esecuzione che durante l'osservazione di un'azione. In particolare, la componente facciale del SS è stata proposta come uno dei network principali per i processi socio-emotivi, data la presenza di regioni coinvolte sia nelle rappresentazioni sensorimotorie delle espressioni facciali che nella modulazione delle risposte autonomiche legate alle emozioni. È stato inoltre suggerito come l'organizzazione del SS facciale sia probabilmente predisposta già prima della nascita e poi definita sulla base dell'esperienza durante periodi sensibili dello sviluppo cerebrale.

A tal proposito, è stato dimostrato come bambini con paralisi facciale congenita, affetti da Sindrome di Moebius (SM), riportino difficoltà nel riconoscimento delle espressioni facciali e ridotta attività autonomica in risposta a stimoli emotivi, probabilmente a causa di un ipo-funzionamento del SS.

Per verificare questa ipotesi, scopo di questa tesi di dottorato è valutare l'organizzazione anatomo-funzionale sensorimotoria del volto in relazione alla capacità di elaborare le emozioni in pazienti con SM. Un secondo obiettivo è studiare la plasticità corticale dei pazienti con SM a seguito di chirurgia facciale e interventi neuroriabilitativi.

Il primo studio ha mostrato, tramite un'indagine elettrocardiografica, la presenza di una ridotta reattività autonomica in risposta ad espressioni facciali in termini di bassa modulazione dell'aritmia sinusale respiratoria (RSA) in bambini con SM. Ciò suggerisce come pazienti con SM possano avere minore capacità di rispondere adeguatamente a stimoli sociali.

Il secondo studio consisteva in un'indagine cinematica longitudinale sulla produzione del sorriso in pazienti affetti da SM che hanno seguito un protocollo di neuroriabilitazione FIT-SAT (Facial-Imitation and Synergistic hand-mouth Activity) dopo un intervento chirurgico facciale, tramite procedura di *free muscle transfer*. I risultati, sebbene siano stati condotti su un numero limitato di pazienti, hanno mostrato che il protocollo FIT-SAT 1) facilita l'attivazione dei muscoli trapiantati sul viso e 2) promuove una migliore modulazione del sorriso rispetto ai trattamenti tradizionali, probabilmente favorendo un più efficace reclutamento delle strutture neurali responsabili dell'esecuzione del sorriso.

Il terzo studio ha mostrato come pazienti con SM che hanno svolto un intervento chirurgico per aumentare la motilità del viso in età avanzata abbiano, rispetto ai soggetti di controllo, una minore consapevolezza sensoriale del volto durante la stimolazione tattile simultanea

faccia-mano (la mano estingue la faccia). Al contrario, i pazienti operati durante l'infanzia non mostrano tale difficoltà, riportano prestazioni simili a soggetti di controllo. Questi risultati suggeriscono che 1) la paralisi facciale congenita potrebbe causare un restringimento della rappresentazione somatotopica del volto a favore di un'aumentata estensione della regione della mano, e 2) che gli interventi in età precoce potrebbero essere più efficaci nell'indurre riorganizzazione sensorimotoria facciale simile a persone normali.
Nel complesso, questi risultati confermano come l'impossibilità congenita di produrre gesti facciali complessi possa influenzare l'organizzazione sensorimotoria facciale, supportando anche l'ipotesi che un'ipo-funzionamento del SS in pazienti con SM sia alla base di alcune delle difficoltà nell'elaborazione delle emozioni. Infine, questi risultati suggeriscono come interventi chirurgici e di riabilitazione possano indurre riorganizzazione cerebrale e comportamentale anche nel caso di malattie congenite, soprattutto durante l'infanzia, grazie a processi di plasticità attività-dipendenti più efficaci.

PAROLE CHIAVE:
Paralisi Facciali; Neuroni Specchio; Emozioni; Neuroriabilitazone; Plasticità Cerebrale;

Abstract

Several neuroscientific investigations over the last thirty years have extended our knowledge about the anatomo-functional organization of the sensorimotor "Mirror Neurons System" (MNS), whose neuronal representation are active during both action execution and observation. In particular, the facial component of the MNS has been proposed as a core network for socio-emotional processes, with regions involved both in sensorimotor representations of facial expressions and in the modulation of autonomic responses related to emotion. Moreover, the organization of the facial MNS is thought as already predisposed even before birth and then refined based on the actual experience of an individual, especially during highly sensitive periods of brain development.

To this regard, some studies have reported emotion processing difficulties in children with congenital facial paralysis, such as Moebius Syndrome (MBS). In particular, it has been demonstrated that MBS children reported difficulties in facial expressions recognition and reduced autonomic activity in response to emotional stimuli, probably because of an abnormal functioning of their facial MNS.

To test this hypothesis, the aim of this doctoral thesis is to evaluate MBS patient facial sensorimotor anatomo-functional organization in relation to the capacity of processing emotions. A second objective is to investigate MBS patients' cortical plasticity following facial surgery and MNS-based neurorehabilitative interventions.

The first study investigated MBS children autonomic response to facial expressions, by means of Electrocardiographic measurements. Results showed significant lower autonomic modulation in MBS children in terms of Respiratory Sinus Arrythmia (RSA) reactivity in response to faces. This confirm already demonstrated MBS children difficulties in emotion processing, probably even in terms of lower predisposition to adaptively react to social stimuli.

The second study consisted in a post-surgery longitudinal kinematics investigation of smile production in MBS patients who followed a Facial-Imitation and Synergistic hand-mouth Activity (FIT-SAT) neurorehabilitation protocol after facial surgery, namely a free muscle transfer procedure. Results based on a limited number of patients showed that the FIT-SAT protocol 1) facilitates the activation of the transplanted muscles on the face and 2) promotes better modulation of the smile compared to traditional treatments, probably fostering a more effective recruitment of neural structures responsible for smile execution.

The third study showed that MBS patients who underwent surgery to increase face motility late in life reported lower sensory facial awareness during simultaneous face-hand

stimulation, compared to age-matched control subjects. In contrast, patients operated during childhood reported control-like performances. These results suggest that 1) congenital facial palsy could cause a shrinkage of the facial somatotopic field in favour of an over-represented hand region and 2) interventions in early age could be more effective in inducing facial sensorimotor reorganization.
As a whole, these findings confirm that the impossibility to produce complex facial gesture since gestational development could affect the actual facial sensorimotor organization, supporting also the hypothesis of a hypo functioning facial MNS in patients with congenital facial palsy. This supports the idea that emotion processing difficulties in MBS patients could be linked to deficits in motor simulation. Finally, these results suggest that brain and behavioral reorganization following interventions could occur also in congenital disease, especially if performed during childhood, probably because of more effective activity-dependent plasticity process.

KEY WORDS:
Facial Palsy; Mirror Neurons; Neurorehabilitation; Brain Plasticity; Emotion Processing;

1. INTRODUCTION

1.1 *ESSE EST PERCIPI*: FACIAL SENSORIMOTOR REPRESENTATIONS AND EMOTIONAL PROCESSING NETWORK

Since Darwin seminal work "The Expression of emotion in animals and man"(Darwin 1872), several ethological and psychological researchers have stressed the role of the body for social, communicative and emotional processes. On this propose, the famous sentence "[…] we feel sorry because we cry, angry because we strike, afraid because we tremble […]" by William James (James 1884), it has been taken as a representative concept about peripheral body feedback theories for emotion processing. Interestingly, James pointed out another crucial and pioneering aspect for emotion processing theories: it is not the body *per se* but its brain representation that allows emotion experience (James 1884). In particular, he posits the existence of a sensory feedback following body expressions and movement that constitutes a basic circuit for emotion processing sited in the sensory and motor regions of the brain. After James' first theorizations, the role of sensorimotor networks for emotion processing has been debated through many theoretical accounts and it has been more recently supported and further elaborated by several neuroscientific researches.

Moreover, this hypothesis, further proposed by several neuroscientific scholars in the field of emotion, implicitly entails the presence of a mechanism that allows an individual to become conscious of his own sensation related to emotions, and that even consciousness, one of the most complex and still partially unknown brain phenomena, relies on these sensorimotor processes. In the words of Berkeley, the famous Irish philosopher "*esse est percipi*": the existence of objects and of individuals is based on the fact that the perception of the world is made possible thanks to an individual conscious interpretation of what he is sensing about the word. To this end, as the phenomenologist philosopher Merleau-Ponty has already suggested, self-consciousness as well as perceiving the surrounding world and understanding other individuals are processes related to a strict self-other interconnection mediated by individuals motor bodily experience rather than symbolic thoughts and cognitive interpretations: "the body is our general medium for having a world" (Merleau-Ponty, 1945).

From a neuroscientific point of view, for several years these two processes, sensing and *processing* of the sensing allowing perception, consciousness and the production of appropriate behaviours in relation to context, have been considered as related to segregated brain moduli. Although, many researches of the last decades have contributed to establish a different and more complex view, proposing a bottom-up approach in which the sensorimotor system, thanks to its anatomo-functional organisation, plays a crucial role for sensorimotor integration, emotion processing, social interaction and other cognitive processes (such as language and symbolic thought)(Corballis 2010; P.F. Ferrari et al. 2017; Fogassi and Ferrari 2004, 2007, 2007; Garbarini and Adenzato 2004; Glenberg et al. 2008; Rizzolatti and Arbib 1998; Rizzolatti and Craighero 2004).

In this chapter I will summarize and discuss the main outcomes of the large amount of studies which have contributed to define the new organisation of the sensorimotor system, both form an anatomical and functional point of view.

1.1.1 *TO SEE A WORLD IN A GRAIN OF SAND*: FROM SINGLE CELL RECORDINGS TO THE DEFINITION OF A NEW ANTATOMO-FUNCTIONAL ORGANISATION OF THE SENSORIMOTOR SYSTEM AND ITS MIRROR NEURON COMPONENT

Based on the pioneering researches of Penfield, Jackson, Sherrington and Woosley, the classical neuroscientific view about the motor and sensory regions posited that the first is involved mainly in action planning and execution, while sensory processing and higher perceptual and cognitive elaboration was related to other specific and segregated associative brain regions. The somtatosensory region was considered to be organised in a primary sensory area sited in the post-central gyrus, while the Motor regions, located in the pre-central gyrus in the rostral part of the brain, were thought to be composed by two main areas: the primary motor area (M1), devoted to the output and kinematic control of movement execution; the premotor and supplementary motor regions, involved in motor planning and preparation. Since the first investigation through intracranical microelectrical recordings and stimulation, both the motor and sensory cortex have been described as topographically and somatotopically organized (Penfield and Rasmussen 1950). Thus, it has been shown that each body part is cortically represented by nearby neurons, whose activity is related to the same peripheral effector, leading to the formation of different brain body maps. One of the first crucial aspect pointed out by these seminal researches is that the localisation and the width of the body maps are not directly related to the peripheral body parts but are rather based on the amount of peripheral innervation and, in particular, to the numerosity of neurons devoted to the sensory and motor processing of a specific effector (Figure 1).

Although some crucial aspects have been confirmed, such as the general somatotopic organisation of the primary cortices (Harding-Forrester and Feldman 2018), several subsequent researches have significantly changed the traditional view about sensory and motor region organisation, showing that these areas are rather characterized by a more complex anatomo-functional network as well as by a large amount of cortico-cortical and cortico-subcortical connection.

Since the seminal researches of Matelli and collogues of the University of Parma on the monkey motor cortex organization, it has been shown that, apart from the primary motor cortex (F1), the premotor cortex (Broadmann area 6) is composed by at least six different sub-regions (F2-F7), each one with its own somatotopic, functional, anatomical and connectivity specificity (Rizzolatti and Luppino 2001). In particular, while areas F6 and F7 are mainly connected with prefrontal regions, F1-F5 regions are characterized by a large number of interconnections with the sensory regions of the parietal lobule. Thanks to this connectivity pattern, different parieto-premotor circuits allow specific sensory-motor transformations and elaboration. In other words, somatosensory or visual sensory information are directly elaborated thanks to motor representation and used for supporting action production, modulation and coordination.

Among all the premotor sub-areas, of particular relevance for the purpose of the current Ph.D. work is the anatomo-functional organization of area F5. Several neurophysiological researches have demonstrated that area F5, sited in the rostral part of the ventral premotor cortex in the macaque monkey (Di Pellegrino et al. 1992; Rizzolatti et al. 1996; Rizzolatti and Luppino 2001), encodes sensorimotor representation of hand (medially) and mouth (laterally) motor acts, with some degree of overlapping between neuronal pool for both hand and mouth (Di Pellegrino et al., 1992; Ferrari et al., 2017; Ferrari et al., 2003; Gallese et al., 1996; Maranesi et al., 2012; Rizzolatti et al., 1988, 1996).

Besides purely motor neurons encoding for specific motor acts, it has been also demonstrated that several neurons of this region encodes the goal of a movement (i.e. a motor act) rather than specific movements around a joint (Rizzolatti and Luppino 2001). Moreover, many neurophysiological investigations have reported visuomotor properties in F5, thanks to the parieto-frontal connections previously described. Thus, it has been shown that the so called "canonical" visuomotor neurons in F5 are active both when a grasping motor act is executed and when a graspable object is passively observed (Murata et al. 1997).

Furthermore, in the early 1990's the team led by Giacomo Rizzolatti and colleagues of the University of Parma discovered a particular subset of visuomotor neurons of F5 called "mirror neurons" (MNs)(Di Pellegrino et al. 1992; Vittorio Gallese et al. 1996; Rizzolatti et al. 1996). The peculiarity of these neurons, first identified for simple goal-directed hand or mouth actions (such as grasping an object or a piece of food), was to be active not only during the execution of an action but also during the observation of the same act performed by another individual (Di Pellegrino et al. 1992; Vittorio Gallese et al. 1996; Rizzolatti et al. 1996).Thereafter, it has also been demonstrated the existence of "broadly congruent" mirror neurons, being active not only for one specific type of action during execution and observation ("strictly congruent MNs" (Vittorio Gallese et al. 1996)), but also for similar one, like grasping and holding (Di Pellegrino et al. 1992; Vittorio Gallese et al. 1996; Rizzolatti et al. 1996). Further investigations revealed also audio-visual mirror neurons, MNs responding not only for visual inputs related to an action but also to the auditory correlates of an action listened by the subject (such as. the breaking of a nutshell or tearing a paper) (Kohler et al. 2002). Interestingly, among MNs population some of them are active even if the action is partially occluded: after showing the object to which the action is directed, these MNs fired even if the final part of the action observed was occluded, demonstrating their sensitivity not only to the motor act but to the action goal, inferred through different sensory modalities (Umiltà et al. 2001). All these data contributed in defining F5 organization not only in terms of action and related somatotopic effectors, but also in terms of goal representations besides the specific act required to achieve them (Umiltà et al. 2008). Moreover, thanks to its MNs component, F5 is thought to represent a core region for action recognition and others' intention understanding (Fogassi et al. 2005).

Besides F5, MNs have been described in the inferior parietal lobule (IPL), a critical region for visual and sensory processing and sensorimotor integration and transformation (Fogassi et al. 2005). In particular, motor neurons with mirror properties are present in specific regions of the convexity of the IPL widely interconnected with F5 (Nelissen et al. 2011): the prefrontal gyrus area (PFG) and the anterior intraparietal area (AIP). Thus, the parieto-frontal circuit connecting F5 to IPL is thought to represent a core network for controlling hand-object interactions and also for understanding others' actions. To this regard, recent researches have shown that perspective or effector identity (e.g. hand) do not dramatically modulate mirror responses of the fronto-parietal mirror network during action-observation (Fiave and Nelissen 2020), suggesting the presence of a more general rather than specific functioning of a mirror mechanism suitable for action and intention understanding.

Notably, such specificity in mirror network activation seems to be modulated by higher-order features such as the final goal of an action or the object value in relation to which the observed action is directed. To this end, neurophysiological investigations have demonstrated that F5-IPL circuits are part of a wider parieto-prefrontal network involved in the organization of chain of sequential motor acts on the basis of a final goal and, given its mirror component, in the understanding of others' intention (Fogassi et al. 2005). In particular, this study revealed that, although the kinematics of the first part of the action (grasping) was identical, there was a specific and differential parieto-frontal neuronal activity during the first motor act observation based on the final goal of the action (placing an object or eating a piece of food): specific grasping-related neurons were more active during the placing condition while others responded more during the eating condition, thus suggesting that the MNS allow not only action anticipation and understanding but also the comprehension of final goal and intentions of action performed by others (Fogassi et al. 2005).

Furhter researches have also shown that object value (e.g. food or non-food) (Caggiano et al., 2012) as well as a predicted value (in terms of positive or negative reward administered to another individual following object grasping) (Pomper et al. 2020) seems to modulate different sub-set of F5 mirror neurons population activation during action observation. These data on the one hand further confirm the goal hierarchical organization of the mirror network, with neuronal population coding of "simple" goal (e.g. grasping/holding) rather than specific motor act, and neuronal network coding for the final objective (intention) of an action (Fogassi et al. 2005). On the other hand, they also expand our knowledge about mirror network prediction properties not only in terms of action/intention but also of motivational/value of an action.

Following non-human primate studies, several researches with noninvasive techniques have demonstrated the existence of a similar anatomic and functional organization of the sensorimotor system and of the mirror network also in humans (Rizzolatti and Craighero 2004).

One of the first study showing the existence of "mirror" properties of the human sensorimotor system was conducted by Fadiga and collegues (Fadiga et al. 1995). Following Transcranial Magnetic Stimulation (TMS) of hand premotor regions and the simultaneous Motor Evoked Potential (MEP) recordings of specific hand muscles, these authors showed that observing grasping actions performed by others increased the subjects' hand MEP responses induced by the TMS.

Subsequently, several non-invasive techniques (such as functional Magnetic Resonance Imaging - fMRI, electroencephalogram - EEG) have revealed sensorimotor activity with mirror properties during action execution and observation also in humans (Fabbri-Destro & Rizzolatti, 2008; Rayson et al., 2016; Giacomo Rizzolatti & Craighero, 2004; Giacomo Rizzolatti & Fogassi, 2014). Hence, these studies showed the existence of an homologue MNS in humans (Giacomo Rizzolatti & Craighero, 2004; Giacomo Rizzolatti & Fogassi, 2014), including IPL, PMv and Inferior frontal Gyrus (IFG), with the superior temporal sulcus providing the visual inputs to the system (Fabbri-Destro & Rizzolatti, 2008; Iacoboni & Dapretto, 2006). In a brain imaging study conducted by Buccino and colleagues (Buccino et al., 2001) it has been shown that when a subject observed both intransitive or object-related actions performed with different effectors (hand, mouth, foot), different region of the pre-motor cortex of the observer were activated, revealing a somatotopic pattern of mirror activity coherent with the classical human sensorimotor homunculus (Wilder Penfield & Rasmussen, 1950).

Another fMRI study carried out by Buccino and collogues (Buccino et al., 2004) investigated brain activity of human subjects while they were observing mouth actions performed by humans (silent speech), monkeys (lipsmacking) and dogs (barking). Coherently with previous results, the observation of human silent speech activated the *pars opercularis* of the left inferior frontal gyrus (IFG), the premotor section of Broca's region. The observation of monkey lipsmacking activated the same regions but to a smaller extent. Interestingly, the observation of the barking dog activated only the extrastriate visual areas. These results suggest that the observation of actions belonging to the motor repertoire of an observer triggers a motor resonance process, mapped on the observer's motor representation. In contrast, actions that do not belong to the observer's repertoire are elaborated mainly through visual neural mechanisms.

As I will further discuss, these results show that the sensorimotor organization of an individual is based on his own experience and this, in turn, modulates the MNS activity during action observation (Calvo-Merino et al., 2005). On this regard, interestingly MNS experience-driven modulation has been described even in infants (Rayson et al., 2017; van Elk et al., 2008), in a period of development in which brain structures are highly sensitive to experience (Knudsen, 2004).

Another peculiarity of the human MNS, unlike non-human primates MNS, is being sensitive not only to goal-directed actions but even to intransitive gestures (Maeda et al., 2002). Moreover, it has also been demonstrated that MNS plays a crucial role in learning novel motor acts through imitation (Giacomo Rizzolatti & Craighero, 2004). In particular, in a fMRI investigation naive human subjects were asked to observe and then imitate specific combination of finger movements performed by expert guitarists (Buccino, Vogt, et al., 2004). Results showed that during the observation of an unknown motor act the MNS areas (IFG, PMv and IPL) were active. Furthermore, it has been shown that during the preparation delay period before imitating the already observed action, prefrontal regions, like the middle frontal gyrus (are 46), plus motor regions involved in motor preparation were active. The authors suggest that prefrontal regions may recruit the already activated MNS motor pattern in order to recreate a motor performance that fit at best with the already observed action (Buccino, Vogt, et al., 2004)

Thus, although individual motor experience could affect the intensity of individual sensorimotor activity during action observation, these results suggest that, MNs system could be considered a crucial network also in motor learning of novel behaviors through imitations (Buccino, et al., 2004). As I will further discuss, these mechanisms have been exploited in order to facilitate neuronal rehabilitation after brain strokes and peripheral body deficits, through Action-Observation therapy approaches (Buccino et al., 2006).

Summing up, according to these studies, MNs system activity crucially contribute to complex process, such as action understanding and motor learning through a mechanism of "direct matching" of the observed action with the internal sensorimotor representation of the observer. In other words, action-observation allows the understanding of the goal of an ongoing action by internally simulating it (Vittorio Gallese et al., 1996; Vittorio Gallese & Goldman, 1998), thus also constituting a core network for social process. In fact, MNS activity could subserve interactions dynamics between individuals allowing the understanding of others' intention and subsequently planning appropriate responses and behavior in relation to the context by correctly predicting actions performed by others (Fogassi et al., 2005; Pomper et al., 2020). Coherently, a countercheck of the critical role of Action-Observation mechanism in action recognition derived from some investigations aimed at perturbing sensorimotor activity. In particular, some Transcranial Magnetic Stimulation investigations have revealed that the perturbation of specific knob of the MNS (Inferior Frontal cortex; Anterior intraparietal regions; Somatosensory cortices) during action observation decrease individual's performance in correctly recognizing specific features of the observed actions (such as the kind of grip used by the performer or the final goal of the observed action) (Jacquet & Avenanti, 2015). In the field of social interaction, another proof about the crucial MNS involvement in social processes came from a bulk of studies aimed at investigating mirror neurons activity in patients with Autism Spectrum Disorders (ASD), whose main clinical characteristic are motor deficits and social impairments (Iacoboni et al., 2006; for a recent review see Oberman & Ramachandran in Ferrari and Rizzolatti, 2015). For example, an fMRi investigation carried out by Dapretto and coworkers (Dapretto et al, 2005) have shown that high-functioning children with ASD reported strongly lower activation of a typical region of the MNS, namely the Inferior Frontal Gyrus (IFG), during emotional facial expression observation and imitation. Interestingly the intensity of activity in this region was highly corelated with the severity of symptoms in the social domain: the more the activity in IFG during imitation, the higher the social competence of the patients (Dapretto et al., 2005).

To this end, as I will accurately discuss in the next paragraph, several investigations have deepened the role of MNS networks about emotion processing and, in particular, of facial expressions of emotion, given their critical relevance for social interactions.

1.1.2 THE FACIAL MIRROR NEURON SYSTEM: AN INTEGRATED MULTI-HUB CORTICO-SUBCORTICAL NETWORK FOR EMOTION PROCESSING

The relevance for social interactions of the MNs system has been further demonstrated by several studies investigating mirror properties not only for goal-directed actions, but also for communicative facial expressions with affective content. The first study investigating premotor activity related to communicative facial gestures at the neuronal level was conducted by Ferrari and collogues of the University of Parma (Ferrari et al., 2003). In particular, this neurophysiological investigation showed that specific neuronal pool of monkey area F5 were active during both the production and the observation of different mouth movement, that is, sucking (ingestive actions) and, more interestingly, lipsmacking, an affiliative social gesture consisting in rapid repetitive cycle of opening-closing of the lips (Ferrari et al., 2003). Several subsequent non-invasive investigations showed parieto-frontal activity related to the production and observation of facial expressions of emotion also in humans (Carr et al., 2003; Sato et al., 2015; Van der Gaag et al., 2007; Wicker et al., 2003). Besides the classical parieto-frontal MN network (IFG, IPL) all these studies investigating sensorimotor activity in relation to facial expression of emotions found mirror properties also in brain regions involved both in sensorimotor integration and emotional processing such as other mesial, temporal and parietal regions like superior temporal sulcus (STS), middle temporal gyrus (MTG), insula, amygdala, supplementary motor area (SMA), and the somatosensory cortex (Carr et al., 2003; Iacoboni & Dapretto, 2006; Van der Gaag et al., 2007; Wicker et al., 2003).
The existence of an “extended” mirror neurons system involved in emotion processing has been confirmed also by neurophysiological investigation of both the macaque and human brain activity. On this regard, Caruana and colleagues conducted a study showing that the electrical stimulation of different section of the insula induced specific pattern of facial gestures (disgust or lipsmaking) and autonomic responses (Caruana et al., 2011). Due to its connectivity pattern with several regions involved in somatosensory, interoceptive inputs

and autonomic activity, this region has been considered crucial for integrating and coordinating sensorimotor and vegetative responses related to emotion. Moreover, rare micro-electrical brain recordings and stimulation investigations on patients who underwent brain surgery, confirmed the involvement of the anterior cingulate cortex in both the production and the observation of facial expressions, in particular of smiling and laughing (Caruana et al., 2017). Based on this previous data and on recent studies combining connectivity, anatomical and microelectrical investigation of macaque brain, a recent work of Ferrari et al. (Ferrari et al., 2017) points out the existence of a specific mirror networks for facial/communicative processing, distinguished from the hand-mirror network, mainly involved in sensorimotor transformation for reaching/grasping/manipulating actions. In particular, these authors showed that the mouth regions, laterally represented in the F5/opercular regions, are mainly connected with the parietal areas involved in somatosensory and motor representation of the face, as well as with regions related to gustatory and somatosensory intraoral inputs. Moreover, while hand premotor regions receive their visual inputs from IPL, premotor facial representation could receive visual information from ventrolateral prefrontal cortex. Moreover, the authors described the presence of strong connections between facial/communicative premotor representations and limbic structures dedicated to emotions, facial expressions and motivational processing, such as the orbitofrtonal cortex, the anterior cingulate cortex, the insula, and the amygdala, which, in turn, have downstream connection with subcortical structures controlling autonomic and vegetative activity. Direct F5 premotor-subcortical connections have been described with the putamen area hosting face/mouth representation and dedicated to movement execution (Alexander & DeLong, 1985), with thalamic regions involved both sensory-motor functions and in wider thalamic-prefrontal circuits contributing to hand-mouth synergies and coordination of motor sequences. Moreover, it has been described the presence of facial F5/operculum downstream projections to mesencephalic and pontine regions like the solitary tract, the trigeminal nuclei, the facial nuclei and the gray matter, which encode and integrates sensory-motor information related to the control of facial muscles movements in order to produce different pattern of facial expression and mouth movements. Considering this wide connectivity pattern of F5 hosting facial representations, mirror system for facial expressions could be considered as a core brain network for emotion processing, composed by several interconnected regions, each one involved in important functions related to the sensorimotor integration and the coordination of viscero-motor pattern related to emotion.

A core tenet of the mirror mechanism is that perceiving and understanding an action, or an emotion expressed by an individual must rely on the observer's neuronal representation responsible for producing the observed movement/gesture. Considering also the limbic and subcortical regions involved in the facial mirror network, the anatomical and functional overlap between the regions devoted to experiencing/producing and observing an emotion allows individuals to immediately share their affective state even at a visceral and pre-verbal level. Indeed, the function of mirror systems has been considered of particular relevance since the first mother-neonate social interactions (Ferrari et al., 2012; Salo, 2018), where the affective attunement of the dyad appears to be crucial for the cognitive and social development of the child himself (Ainsworth, 1993; Lyons-Ruth, 1996). In relation to this, it has been hypothesized that the facial component of the mirror system is probably already predisposed and highly canalized since birth, allowing the infant to actively interact with the caregiver thanks to face-to-face exchanges since the first days of life (Ferrari et al. 2012, 2013; Tramacere, Pievani, and Ferrari 2017). Although the presence of a MNS at birth is very likely, it has also been stressed out the importance of individual experience in refining its basic organization (Ferrari et al., 2013). In the next chapter I will discuss the main aspect related to the role of social interactions for the development of the mirror neuron system, considering in particular the interaction between genetically-guided maturation defined over phylogenesis and experience-dependent developmental process related to the ontogenesis of an individual.

1.2 SENSORIMOTOR SYSTEM DEVELOPMENT AND NEUROPLASTICITY

Brain development begins during the first embryonic maturation period and continues after birth with processes of maturation and refinement which reshape its organization through the complex interconnection between gene expressions and individual's interactions with the surrounding world.

Following the first cell divisional processes, which give rise to the blastula (first cell agglomeration), during the *neurulation* process the cells of the ectoderm, part of the blastula from which the NS will derive, begin to differentiate into the various cells that will constitute the different brain structures. This first phase of neuronal development takes place through predetermined sequences of neuronal formation: neurulation, differentiation and *migration,* a process by which neuronal cells reach their final sites, constituting brain structures (Jessell & Sanes, 2000; Rice D & Barone S, 2000). Finally, when the architecture of the nervous system has reached structural organization similar to the adult one, different processes of circuits and synaptic differentiation and specification take place. In fact, the effective brain organization in neuro-functional circuits depends on processes of programmed cell death (apoptosis), as well as on the elimination (pruning) of unused synapses on the basis of an individual's experience and the consequent consolidation of the most useful brain connections (Jessell & Sanes, 2000; Knudsen, 2004; Rice D & Barone S, 2000)

On this regard, the extracellular environment, as well as the physical and social one to which the infant interact with, play a fundamental role in this process of neurophysiological development (Jessell & Sanes, 2000; Rice D & Barone S, 2000). Therefore, starting from the perinatal period, the NS refines its anatomo-functional organization through brain experience-dependent plasticity processes, which can be explained in terms of structural and/or functionl brain changes on the basis of individual experience during the interaction with his physical and social environment (experience-dependent neuroplasticity). To this regard, the complex interaction between biological predisposition and brain plasticity process support the development of the different sensorimotor, cognitive, and socio-emotional process. In particular, several researches have shown that these interactive developmental processes play a fundamental role for the brain formation especially within specific time windows, namely *sensitive periods* (Knudsen, 2004), in which brain plasticity is particularly high. As I will further discuss, it has been demonstrated that, depending on the process or brain structure involved, such experience-dependent brain plasticity are active even in late period of life (such as adolescence) and partially preserved also in adulthood, even with some important limitations (Hensch & Bilimoria, 2012; Nelson, 2000).

Therefore, based on this considerations, early experience has an important long-term consequence in brain functioning. To this regard, as suggested from the first researches focused on the infant psychological development, it has been proposed that sensory-motor process support subsequent individual cognitive and social competence. From a neuroscientific perspective, further evidences demonstrated that sensorimotor system on the one hand supports the primary development of the child's cognitive and emotional skills during the early period of life (Ferrari et al. 2013; Nelson, Zeanah, and Fox 2019), on the other hand it contributes to higher-level social and cognitive skills even in adulthood (e.g. language and symbolic thought, attentional processes, emotional processes, intersubjectivity, skills relational and empathic) (Engel et al., 2013; P.F. Ferrari et al., 2017; Rizzolatti et al., 1987; Rizzolatti & Arbib, 1998; Rizzolatti & Craighero, 2004). Vice-versa, early social interaction influences the actual individual sensorimotor organization thanks to the experience-dependent neuroplasticity process previously mentioned (Ferrari et al., 2013). In the next paragraph I will discuss in depth the main plasticity processes related to early social interactions, outlining their role in sensorimotor development and, in particular, of its mirror network component.

1.2.1 EARLY SOCIAL INTERACTION AND DEVELOPMENT OF FACIAL SENSORIMOTOR REPRESENTATIONS

Even in the context of social interaction, biological brain maturation is related to the interaction between genetical instructions, environmental inputs and experience. In particular, the exposure to environmental stimuli that the child's brain expects to receive on the basis of phylogenetical predisposition (experience-expectant process) is crucial for the brain structures to develop. In other words, during evolution certain brain neural system has evolved in order to expect interactions with specific stimuli during likewise specific early windows of time in which the exposure to these stimuli is fundamental for inducing brain development (Fandakova & Hartley, 2020).

Moreover, as already mentioned before, environmental inputs modulate the effective neuro-functional organization of brain networks through experience-dependent plasticity processes. Thus, a partial or total lack of exposure to social relevant inputs can produce deficits in the child's development and in the correct maturation of brain structures and functions.

To this regard, several researches have shown that, besides physical stimulation, exposure to caregiver nurturing through multisensory modalities is a crucial aspect for the appropriate development of brain network related to socio-emotional processes.

The exposure to face and, in particular, to facial expressions of emotions represents a core aspect for the infant social development. In a series of interesting researches based on the rhesus macaque model, it has been shown that exposure to faces is fundamental in order to develop brain structures involved in face processing and recognition (Livingstone et al., 2017).

In fact, a neonate macaque not exposed to conspecific or human faces during the first month of development reported weaker performances in recognizing conspecific face later in life and no formation of the typical dedicated face-regions of the inferior temporal sulcus (Arcaro et al., 2017; Sugita, 2008).

To this regard, several psychological, ethological and neuroscientific studies have shown infant sensitivity to face and, in particular to facial expressions, since birth (Farah et al., 2000; Ferrari et al., 2006; Ferrari et al., 2012; Meltzoff & Moore, 1977; Morton & Johnson, 1991). Interestingly, studies in the field of developmental psychology have highlighted the infant ability to communicate with the caregiver through rhythmic behavioral modules made of complex pattern of vocal and facial expressions (Murray et al., 2016). In particular, since the seminal work of Meltzoff and Moore (Meltzoff & Moore, 1977), many researches have shown neonates ability to actively imitate the caregivers facial gestures since the first hours of life (Meltzoff & Moore, 1989). In addition, it has been shown that 36 hours after birth human infants can discriminate and reproduce different facial expressions of emotion (happiness, sadness and surprise) (Field et al., 1982).

These neonatal abilities entail two important aspects. First, that infants at birth are able to produce specific pattern of facial gestures, also in order to actively communicate their own needs and internal states. On this regard, interestingly, it has been shown that even during gestational development, starting from the 24-35 weeks, the fetus initially produces spontaneous non-specific facial movements, which are subsequently organized in specific pattern of facial expressions already before birth (Reissland et al., 2011).

A second crucial aspect about neonatal sensitivity to face, is that infants are able to perceive facial expressions of others and to actively imitate them, showing their precocious capacity of intentional communication and emotional reciprocity expressed through face-to-face interactions.

Considering the action-perception mechanism properties of the *mirror* component of the sensorimotor system previously discussed, it has been proposed that the infant precocious communicative skills mainly depends on the MNS network (Ferrari et al., 2009, 2012; Murray et al., 2016).

More specifically, the first evidence of MNS activity related to facial expressions in newborns derives from two seminal works of Ferrari and colleagues at the National Institute of Health.

In a first behavioral study these authors demonstrated that newborn macaques are capable of facial gesture imitation since the first days of life, such as opening of the mouth, tongue protrusion and lipsmacking (Ferrari et al., 2006). Moreover, further EEG investigation on newborn macaques revealed the involvement of sensorimotor system during facial expressions observation and imitation (Ferrari et al., 2012).

Subsequent EEG studies on human subjects showed sensorimotor activity in relation to facial expressions of emotion observation and imitation even in infants of 30 months of age (Rayson et al., 2016). As a whole, these results suggest that sensorimotor system for facial expression is already sufficiently structured at birth, allowing infants to express their own intentions and emotions and to actively interact with their caregivers.

Although these results show that sensorimotor system is already settled at birth, several researches have stressed the role of experience in refining and shaping the actual development trajectories of the system itself. In particular the kind of social stimulation can make sensorimotor system more or less sensitive to emotional-social stimuli (Ferrari et al., 2013; Rayson et al., 2017; Vanderwert et al., 2015).

To this regard, electroencephalographic recording studies carried out on both infant macaques and humans have shown that the greater or lesser exposure to early social interactions correlates with a relative greater or lesser responsiveness of the sensorimotor system to the observation of facial expressions. In particular, in a study of Vanderwert et al. (Vanderwert et al., 2015) on newborns macaques, it has been shown that the sensorimotor activity in relation to the observation of affiliative facial gestures (lipsmacking) of a group of nursery reared was less intense than the one of a same-age group of mother-reared. Interestingly, other studies have shown that during the first month of life macaques mother-infant interaction are characterized by intense communicative exchanges trough facial gestures, in particular of lipsamcking (Ferrari et al., 2009). Thus, even within few days of life, the lack of crucial social stimulation could impact the sensorimotor functional activity of the nursery-reared baby macaques, thus leading to their less effective MNS responses during facial gesture observation.

A subsequent EEG longitudinal investigation on human subjects revealed that babies who had been highly exposed to maternal mirroring (i.e. when the mother reproduces and imitates responsively and synchronously facial expressions produced by the infant) within the first two months of life reported greater cerebral response of the sensorimotor systems (and therefore of the mirror systems) during the observation of facial expressions at 9 month of age, compared with age-matched babies who received less early maternal mirroring (Rayson et al., 2017).

These studies underline how social experience during particularly sensitive periods of brain plasticity can play a crucial role for the effective functional organization of the mirror system (Ferrari et al., 2013). To this end, recent models posit a crucial role of epigenetic mechanisms for brain and socioemotional development (Ferrari et al., 2013), that is the role of experience in modulating genes expression as well as of the eventual generational transmission of experience leading to genetical predisposition at birth. Therefore, behavioral and social experiences could influence brain development by triggering neuronal changes even at a molecular level on the basis of genetical predisposition inherited over the evolution. In turn, these predispositions allow infants to be particularly sensitive and receptive to the exposure at specific stimuli and experiences, even before birth. To this regard, infant's facial sensorimotor activity and also their sensitivity to face represent a *sine qua non* condition on which social experience could exert its influence in canalizing brain formation, with an important impact on the following development of cognitive and socio-emotional processes. The caregiver's visual input could therefore play an important role in facilitating the characteristic sensory-motor coupling of the mirror system previously described. The infant, in fact, being unable to see his own face, must have an internal representation which allows him to map the vision of a facial gesture on a corresponding internal motor program. On this perspective, it’s interesting to remind the studies briefly mentioned before reporting the presence of spontaneous and then organized facial expressions already before birth (Reissland et al., 2011). Interestingly, another study revealed that fetus modulates their mouth gestures (such as opening/closing of the mouth) in response to the characteristics of mother vocalization (G. A. Ferrari et al., 2016).

Taken together, these results support the hypothesis about the crucial role of the sensorimotor system in subserving early intersubjective competence of the child, also testified by its ability to actively stimulate a maternal response (Trevarthen & Aitken, 2001) and in scaffolding further complex cognitive and social process. Moreover, they are in line with the importance of interaction between biological predisposition and experience-dependent plasticity process for the actual brain development. For the same reason, atypical brain formation may belong not only to genetical structural disease or to adverse environmental condition, but rather to a dysfunctional and vicious cycle in individual-environment interactions. Thus, a structural malformity, especially during sensitive period of development, may affect brain organization, which in turn will influence individual sensitivity to social environment, which in turn will affect brain organization because of experience-dependent neuroplasticity process.

Conversely, adverse early social environment could affect brain maturation by limiting the kind of experience to which biological predisposition are exposed. The consequent atypical anatomo-functional organization may in turn tune brain sensitivity to specific kind of inputs, thus affecting the final brain functioning and the relative behavioral, cognitive and socio-emotional individual organization.

1.2.2 HOW ADVERSE SOCIAL EXPERIENCE AND PERIPHERAL BODY IMPAIRMENTS COULD AFFECT SENSORIMOTOR CORTICAL STRUCTURAL AND FUNCTIONAL ORGANIZATION

Since Hubel and Wiesel seminal studies on kittens visual cortex reorganisation (Wiesel & Hubel, 1965) due to peripheral eyes impairments, several researches have demonstrated the influence of peripheral body impairments on cortical organization. In fact, it has been widely documented that in patients suffering from birth from congenital cataracts, even with some differences depending on the specific aspects of vision, individuals who underwent eye surgery later in life the congenital disorder leads to a less effective visual recovery and to an abnormal organization of the visual cortex, caused by the lack of correct exposure to visual stimuli during sensitive periods of brain development (Lewis & Maurer, 2005; Wiesel, 1982).

Several subsequent researches on human and animal model (Pascual-Leone et al., 2005; Rice D & Barone S, 2000) have shown that, especially during critical developing periods, the loss of a primary peripheral inputs could cause an augmented anatomo-functional cortical representation of an alternative one, mainly coming from cortically adjacent territory, due to horizontal cortico-cortical synaptic connections (Buonomano & Merzenich, 1998).

As previously mentioned, such plasticity process has been documented even in adult individuals. In particular, considering the somatotopic sensorimotor cortical representation with face and hand closely represented, several studies on patients who underwent limb amputation showed that the cortical hand regions were invaded by the face one, reporting also "phantom" tactile sensation of the absent arm or referred sensation of the hand while stimulated on the face (V. S. Ramachandran & Hirstein, 1998; Vilayanur S. Ramachandran & Rogers-Ramachandran, 2000). Conversely, some studies on patients with peripheral acquired facial injuries or face transplants reported an inverse pattern with referred face sensation while touched on the hand (Clarke S et al., 1996) and with cortical regions typically dedicated to the face responding to hand stimulation and hand movements (Rijntjes et al., 1997; Uysal et al., 2016). Furthermore, some studies have investigated cortical re-organization following arm transplant in a former amputee, reporting a reverse effect in which the hand territory, previously invaded by face representation, recovered its typical organization responding again to hand (Farnè et al., 2002; Giraux et al., 2001). Interestingly, these results have demonstrated that dramatic cortical reorganization could occur even in adulthood. As I will further discuss on this thesis, these results show that somatosensory maps are not static but rather dynamic and modulated by competing processes. Although, as

I will extensively discuss, some studies have reported opposite results, showing important limitation in plasticity process during adulthood (Hensch, 2005; Hensch & Bilimoria, 2012; Makin & Bensmaia, 2017). In fact, experience-dependent plasticity has been shown to be higher during early development, where the lack of proper sensory inputs might cause permanent loss of a function (Wiesel & Hubel, 1965). Thus, even if the cortical somatotopy seems to be approximately already settled precociously after birth (M. Desmurget et al., 2014; Graziano & Aflalo, 2007; Saby et al., 2015), several researches on congenital peripheral deficits have proposed that the actual sensorimotor organisation, far from being stable, mainly depends on behaviourally relevant experiences, thanks to use-dependent neuroplasticity process (Ferrari et al., 2013; Stoeckel et al., 2009). As already briefly discussed before, even in the field of social process early experiences have a crucial role in refining the actual brain anatomo-functional organization. Several researches have investigated how the perturbation of early social interactions during the first period of development could affect both the development of the nervous system and of process of higher-order domains such as cognitive, emotional and socio-adaptive ones. On this regard, an important contribution has been given by studies conducted on non-human primates. Following the pioneering research of Harlow and collaborators (Harlow & Zimmermann, 1959), it has been highlighted how babies separated from birth by their mothers have serious consequences on development. For example, it has been reported the presence of morphological and/or functional brain deficits in structures related to emotional and cognitive processes (amygdala, hippocampus, and prefrontal cortex), as well as behavioral problems (e.g. motor stereotypies, stress-related behaviors, increase in anxious behaviors and aggressive), less social interactions, and impaired cognitive performance (for a review see French & Carp, 2016; Kundakovic & Champagne, 2015; Nelson, 2000; Nelson et al., 2019).

About sensorimotor development, it has also been shown how young macaque monkeys separated from mothers at birth tend to be less responsive when observing facial expressions with affiliate communicative content, reporting less neonatal imitating behaviors less the observed expressions and reporting less cortical activation in the sensorimotor regions inherent the mirror systems during the observation of affiliative facial gestures (Vanderwert et al., 2015).

Similarly to what has been found on animal models, some important longitudinal studies on children institutionalized and thus separated from their own mother during the first months of life have shown how early social deprivation from the primary care could cause brain anatomo-functional deficits, such as a decrease in the volume of gray and white matter and functional dysregulations of structures involved in emotional processing (amygdala and amygdala-prefrontal cortex connectivity pattern), as well as cognitive deficits, socio-emotional difficulties, and also high incidence of psychiatric disorders and "quasi-autistic" psychopathological conditions (for a review see Nelson et al., 2019). Furthermore, studies in the field of mother-infant interaction, have demonstrated that the presence of physical deficits that interfere with the interaction dynamics of the dyad have an impact on the child developmental trajectories. In this regard, some studies have shown how congenital facial malformations (such as the presence of cleft lip) can disrupt the typical early mother-child face-to-face interaction patterns, with negative consequences on the emotional and cognitive development of the child himself (Murray et al., 2008). In fact, the mothers of these children tend to avoid looking their child's mouth and also show less activation of brain areas, such as the orbitofrontal cortex, involved in coding the value of the sensory stimuli related to the face. Other studies have shown that children with rare forms of congenital blindness, for example, have a more limited facial expressive repertoire at 9 and 12 months compared to sight-impaired children (Tröster & Brambring, 1992).

Considering the previously mentioned results about the importance of early maternal mirroring for facial sensorimotor network development, these results are likely to be attributable to alterations in early caregiver-child communication dynamics that, in turn, could affect long-term brain functional organization.

In other cases, the communicative disturbances between mother and newborn in the first months of life can be mainly linked to parental vulnerabilities as in the case of maternal depressive disorders. In fact, these mothers are generally less responsive and sensitive to the infant's signals, tend to mimic their behaviors less and also tend to have an altered vocal tone compared to non-depressed mothers (a descending and monotonous contour of the intonation of the voice). These maternal characteristics have been seen to have long-term effects on the emotional state of the baby, orienting it in a depressive sense, as it could emerge in the adolescent period (Murray et al., 2011).

Summing up, these results are in line with the models focused on the predisposition-experience interaction that characterize brain and socio-cognitive function development.

As already proposed (Ferrari et al., 2013), biological predisposition phylogenetically inherited provide pivotal sensorimotor functions thanks to which infants could actively interact with the surrounding physical and social environment. Afterwards, the kind of experience made throughout infancy and, within certain constraints, the entire life, will narrow the actual brain and cognitive organization thanks to experience-dependent plasticity. Usually, these brain plasticity processes are thought to allow the individuals to better adapt his own behavioral, cognitive and social organization to the actual environment to which they have to deal with. However, in presence of genetical or physical impairments, as well as adverse social conditions, "negative" counterproductive plasticity outcomes could occur, thus affecting brain anatomo-functional organization and limiting the individual cognitive and socio-emotional functioning.

In the next chapter I will discuss recent researches aimed at investigating emotion processing and brain sensorimotor organization in individuals affected by Moebius Syndrome (MBS), an extremely rare congenital disease characterized by the lack of mimic facial movements because of impaired cranial nerves formation.

In particular, after a brief introduction about clinical and etiological features of the Moebius syndrome, I will present previous studies about emotion processes in MBS children. Afterwards I will discuss three studies carried out during my Ph.D. course and aimed at further investigating MBS children sensorimotor anatomo-functional organization and neuroplasticity in relation to emotion processing.

1.3 EMOTION PROCESSING AND CONGENITAL FACIAL PARALYSIS: THE CASE OF MOEBIUS SYNDROME

1.3.1 MOEBIUS SYNDROME AETIOLOGY AND CLINICAL FEATURES

Moebius syndrome (MBS) has been originally described in the 1880s by Moebius(Möbius, 1888) and von Graefe (von Graefe, 1880). To date, the it his known as an extremely rare (estimated prevalence 1 in 50.000 to 1 in 500.000 live births) congenital disorder characterized by altered development of facial (VII) and abducens (VI) cranial nerves and nuclei, resulting in "uni- or bilateral facial paralysis and deficient lateral movement of the eye(s)"(Abramson et al., 1998; Bianchi et al., 2009; W. Briegel, 2006; Carta et al., 2011; Cattaneo et al., 2006; Patel et al., 2017; Sjögreen et al., 2001; Terzis & Anesti, 2011; Terzis & Noah, 2003a; Webb et al., 2012). It can also be associated with other cranial nerves weakness (affecting XII, X, IX, III, VIII, V, IV and XI CN in order of decreasing frequency)(Kulkarni et al., 2012; Shashikiran et al., 2004; Singham et al., 2004), orofacial malformations (epicanthic folds, micrognathia), limb defects (such as club feet and missing or underdeveloped fingers or hands), musculoskeletal abnormalities and hypoglossia (weakness or malformation of the tongue)(W. Briegel, 2006; Rogers et al., 1977; Singham et al., 2004; Terzis & Noah, 2002; H. T. F. M. Verzijl et al., 2003, 2005). The main functional deficits are the lack of facial expressions, as well as difficulties in speaking, eating, sucking and swallowing(Singham et al., 2004). Some MBS individuals retain residual lower facial muscle activity, possibly due to aberrant innervation from other cranial nerves(Verzijl et al., 2005).

Even the aetiology of the syndrome is still debated, the most accredited explanations posits that MBS is caused by a cascade of secondary events after an initial insult at the brainstem level, as well as a possible genetic aetiology (Kulkarni et al. 2012), during a specific window of time of the embryonic development in which the formation of the VII and of VI cranial nerves nuclei take place (Patel et al., 2017).

In particular, cranial nerve nuclei are being formed during the 4th and 5th week of development, when cephalic neural crest cells begin to differentiate (Abramson et al., 1998; Raroque et al., 1988). Moreover, during the 6th week of gestation the blood supply to the developing hindbrain changes from the basilar to the vertebral artery, allowing adequate blood supply to the cranial nerve nuclei(Bavinck & Weaver, 1986; Terzis & Anesti, 2011) formation. Therefore, non-genetic causes of the syndrome are thought to involve vascular events with interruption of blood supply during the initial development of the embryo, causing deficient cranial nerves centres formation (Bavinck & Weaver, 1986; Morales-Chávez et al., 2013; Terzis & Anesti, 2011). To this regard, the proximity between facial and the abducens nucleus at the cerebellopontine angle is considered as a probable reason of the concurrent nerves palsies in MBS (Terzis & Anesti, 2011). In line with this hypothesis, radiological imaging, ultrasonography, computed tomography, and magnetic resonance imaging investigations in individuals with MBS showed calcifications and hypoplasia of the brainstem (Dooley et al., 2004; Singham et al., 2004), as well as, even in a few cases, cerebellum abnormalities (Benbir et al., 2011; Harbord et al., 1989; Pedraza et al., 2000).

According to the current literature, the vascular insult could be caused by infection, hyperthermia, hypoxia, and vasculitis (Terzis & Anesti, 2011), as well as by drug abuse, like benzodiazepines (Courtens et al., 1992) and misoprostol (an abortifacient drug) (Pachajoa & Isaza, 2011; Puvabanditsin et al., 2005), thalidomide (Elsahy, 1973) and also alcohol (Martínez-Frías et al., 2001) or cocaine (Puvabanditsin et al., 2005) exposure during pregnancy (Elsahy, 1973; Pastuszak et al., 1998; Sánchez & Guerra, 2003).

However, given that not all MBS patients show signs of cranial nerve nuclei hypoplasia(Ali et al., 2018), it is reasonable to assume other con-causes besides vascular insult resulting in the MBS clinical condition.

In fact, drugs such as ethanol, thalidomide and misoprostol are able to modulate the expression of many genes (through epigenetic mechanisms) involved in brain developing processes like neuronal proliferation, apoptosis, differentiation and migration, as well as synaptogenesis and synaptic activity(Dufour-Rainfray et al., 2011). For example, fetal exposure to teratogens has been shown to be associated with an increased incidence of autism (Tordjman et al., 2014). Presumably, similar epigenetic mechanisms could have a role also in causing MBS clinical configuration.

Another hypothesis, as already mentioned, rely on genetical causes. Although genetical aspects underlying MBS aetiology remains partially unclear, some familial trends have been revealed with both autosomal dominant and recessive patterns (Becker-Christensen & Lund, 1974; Legum et al., 1981; Rojas-Martínez et al., 1991; Schröder et al., 2013; H. T. Verzijl et al., 1999). In particular, it has been shown that considering MBS musculoskeletal anomalies the risk of hereditariness is about 2%, while it increases to 25-30% suggesting a specific genetic aetiology for other clinical features such as isolated facial palsy, deafness, ophthalmoplegia, and digital contractures (MacDermot et al., 1991).

To this regard, despite genetic investigations are particularly difficult due to the syndrome's rarity and genetic heterogeneity, some studies have been able to select candidate genes and chromosomal loci as well as their relative mutation by analysing the genomes of MBS individuals.

To date, the most implicated loci are linked to particular homeobox genes (including HOXA1, HOXB1, and SOX14), necessary for spatial and temporal development of the brain. These loci include 1p22, 3q21-q22, 10q21.3-q22.1, and 13q12.2-1345. Some of them have been defined as MBS1 at 13q12.2-q13 identified by Slee and colleagues(Slee et al., 1991), MBS2 or HCFP1 (hereditary congenital facial paresis 1) at 3q21-q22 as reported by Kremer et al.(Kremer et al., 1996), and MBS3 or HCFP2 (hereditary congenital facial paresis 2) at 10q21.3-q22.1 from a report by Verzijl et al.(H. T. Verzijl et al., 1999) of dominant MBS in a Dutch family. Mutations in homeobox genes may also influence other genes, therefore determining the varying phenotypes characterizing the syndrome. Other selected genes are PLEXIN-A1, GATA2, EGR2, BASP1 and TUBB3(Patel et al., 2017; van der Zwaag et al., 2002), which are crucial for neuronal development. Of particular interest is another gene probably implicated in the syndrome that is FLT1/VEGFR1: being linked to an aberrant vascular growth, its involvement support both genetic as well as the previously mentioned intrauterine vascular event (Kadakia et al., 2015) hypothesis.

Recently, a study of Tomas-Roca and colleagues (Tomas-Roca et al., 2015) showed that neuropathological deficiencies found in MBS individuals correlated with those found in mutant mice model. In particular, this study reported de novo mutations affecting two genes, PLXND1 and REV3L, in six MBS individuals. In this regard, analysis of PLXND1 and REV3L in knock-out mice(Tomas-Roca et al., 2015) demonstrated that mutations in these genes were responsible for deficient facial branchiomotor neuron migration and craniofacial bone abnormalities/vertebral defects both in knock-out mice and in MBS individuals. These results strongly support a crucial causative role of these genes in inducing a proportion of MBS cases (Tomas-Roca et al., 2015).

1.3.2 TREATMENT: SURGICAL AND REAHBILITATION PROCEDURES

As already mentioned above, the main characteristic of MBS is the lack of mimic facial movements, dramatically affecting their speaking abilities and their possibility to communicate emotions through facial expressions.

Considering how important face is for social interactions (Darwin, 1872; U. Dimberg & Thunberg, 1998; Ulf Dimberg et al., 2002; Ekman & Friesen, 1986; Pier F. Ferrari et al., 2013a; Korb et al., 2010; Murray et al., 2016; Niedenthal, 2007; Niedenthal et al., 2001), not surprisingly many socioemotional difficulties have been reported in MBS patients, especially during developmental age, thus probably becoming a risk factor leading to individuals' social and psychological difficulties later in adulthood (Bogart & Matsumoto, 2010; W. Briegel, 2007; Briegel et al., 2010).

For this reason, many intervention programs have been proposed to enhance speech and oral competence and to improve their social skills and quality of life, such as respiration control, neuromuscular training, massage, meditation-relaxation(Devriese, 1994; Lindsay et al., 2010), as well as psychological and communication strategies interventions (Bogart, 2015; Michael et al., 2015). It has been also developed a series of surgical strategies in order to increase their facial motility, thus overcoming one of the most important and psychologically dramatic deficits for MBS subjects, namely the lack of facial expressions (R. M. Zuker et al., 2000). In particular, the *smile surgery*, recommended for individuals of at least 6 years old, consists in a micro-neurovascular transfer of a muscle (usually the Gracilis) that is grafted from the leg to the corners of the mouth and innervated by the masseteric nerve (B. Bianchi et al., 2010, 2013; Bianchi et al., 2009; Terzis & Noah, 2002, 2003; R. M. Zuker et al., 2000). Thus, *smile surgery* can allow MBS individuals to voluntary smile, as well as to improve their general oral competence related to mouth movements (e.g. speaking, eating) (Bianchi et al. 2010; 2013). To this regard, post-surgery rehabilitative treatments are fundamental for promoting the achievement of a more spontaneous, naturalistic and symmetrical smile (Bianchi et al., 2013; Ferrari et al. 2017). In particular, as I will further discuss, recently has been implemented a new neurorehabilitation protocol based on the actual knowledge of the sensorimotor system properties in order to foster the recruitment of brain network responsible of smile production following facial animation surgery in MBS patients. From a functional point of view, improving MBS patient's possibility to communicate and express emotions through their face could positively affect their socioemotional abilities and competence, thus inducing relevant enhancement in the patient's quality of life.

As previously discussed, neuroscientific studies of the last 20 years have deeply demonstrated the crucial role of facial expressions and relative brain representation for social and emotional process (Ferrari et al. 2017; Ferrari et al. 2003; Van der Gaag, Minderaa, e Keysers 2007), both in terms of facial expressions recognition and autonomic activity related to emotion. Thus, it is possible to assume that the congenital lack of mimic facial expressions could affect social interactions not only limiting their communicative modalities but also compromising MBS patient's ability to adequately process others' emotion expressed through the face. To further discuss these aspects, in the next paragraph I will summarize previous researches that have investigated emotion processing in patients with facial palsy and, especially of MBS patients.

1.3.3 *FACE EVOLUTION*: EMOTION PROCESSING IN CONGENITAL FACIAL PALSY

As already mentioned, Charles Darwin was the first scholar scientifically investigating and discussing the crucial role of face in the expression of emotions (Darwin, 1872). Since Darwin first theorization, facial expressions of emotion have been considered crucially relevant for their evolutionary value. In particular, they are thought to be preserved and evolved across phylogenesis, fostering communication between individuals and emotion regulation during social interaction and thus allowing an individual to better adapt to his social environment (Parr et al., 2005). To this end, several ethological investigations have demonstrated the communicative value of facial expressions in several non-human primates' species (for a review see Preschoff, 2000 and Parr et al., 2005). For example, it has been demonstrated that in several non-human primates species the silent exhibition of the teeth (silent bared-teeth), considered homologous to human smile, usually occur after a fight or during affiliative interactions, probably promoting reconciliation and/or tension relief (Van Hooff, 1972; Preschoff, 2000). Moreover, cross-cultural investigations have shown that humans of different ethnic groups exhibit and recognize facial gestures with same typical patterns related to emotions (Ekman, Sorenson and Friesen, 1969; Ekman & Friesen, 1971) thus confirming the pioneering hypothesis suggested by Charles Darwin about a common phylogenetic origin with universal communicative meaning of facial expressions in humans and animals.

From a functional point of view, subsequent studies have described a mechanism that allows humans and non-human primates to rapidly communicate and share inner emotional state through facial expressions, that is *facial mimicry* (Holland et al., 2020; Wood et al., 2016), described as the automatic rapid imitation of an observed facial expression. Facial mimicry has been studied through several behavioural and electromyographic (EMG) investigations (U. Dimberg et al., 2000; U. Dimberg & Thunberg, 1998; Ulf Dimberg et al., 2002; Korb et al., 2010). These studies have shown that when viewing emotional expressions, people tend to react automatically and quite rapidly (after 300–400ms (U. Dimberg, 1982; U. Dimberg & Thunberg, 1998)) with congruent facial expressions and thus activating the corresponding facial muscles (U. Dimberg et al., 2000). Similar facial responses during social interactions have been described also in nonhuman primates within the context of friendly and playful interactions (Palagi & Mancini, 2011), as well as during early mother-infant interactions (Mancini et al., 2013), further suggesting its common evolutionary origin and shared

adaptive functions between different species. In particular, facial mimicry is thought to play a fundamental role for regulating social interactions and even fostering social bounding.
To date, several simulation models have proposed different neuronal substrates subserving facial mimicry as a critical mechanism for emotion processing. While the *reverse simulation models* emphasize facial peripheral proprioceptive feedback information elaborated from facial representation of the observer following the automatic reproduction of the observed facial expression and thus generating the corresponding emotional experience within the observer itself (Mancini, Ferrari, e Palagi 2013), the *unmediated resonance model* suggests that the observation of a facial expression directly triggers a sub-threshold activation of the same neural substrate associated with the observed emotion (Goldman & Sripada, 2005), namely the previously described "mirror neuron system" (MNS)(Blakemore & Frith, 2005; Iacoboni & Dapretto, 2006; Niedenthal, 2007).
Although some important specific differences, both simulation models posit that individual facial brain representations (facial somatosensory and/or sensorimotor regions) are crucial in supporting facial mimicry and consequently emotion processing. However, the actual mechanism (e.g. if concerning central nervous system rather than peripheral activity) subserving facial mimicry, as well as its critical role for socioemotional process, are still debated.
In order to clarify and disentangle some of these questions, several studies have investigated emotion processing and, in particular, elaboration of facial expressions of emotion when facial movements are experimentally blocked or impaired due to clinical conditions.
Studies that have artificially inhibited participants' facial movements, like requesting them to hold a pen between the lips (Oberman et al., 2007), have found a reduced ability to recognize emotions (Oberman et al., 2007; Ponari et al., 2012) supporting the hypothesis that being able to freely express facial mimicry facilitates the emotion recognition process (Neal & Chartrand, 2011; Sato et al., 2013).
Apparently, these results clearly support the facial feedback hypothesis, where the recognition of emotional facial expressions would be critically supported by the activation of the facial musculature.
However they cannot conclusively exclude the role of inner simulation process, because, for example, actively blocking facial muscles by holding a pen with the lips would generate an incongruent proprioceptive feedback, or even a sensorimotor activity that could interfere with the internal simulation of the observed facial expression (Wood et al., 2016).

To further deepen these aspects, other studies have been focused in investigating emotion recognition in clinical conditions characterized by impaired facial expression production.
For example, clinical investigations have found lower facial expressions recognition performance in Parkinson's disease patients (Gray & Tickle-Degnen, 2010; Ricciardi et al., 2017). However, this neurological diseases is usually associated with facial amimia but also with other symptoms such as impaired movement initiation, rigidity, tremors, reduced expressivity of the whole body and voice (Gray & Tickle-Degnen, 2010), as well as comorbid disorders like depression, that may concur in affecting their decoding of facial expressions ability(Gray & Tickle-Degnen, 2010), and thus not allowing a clear identification of the actual role of the lack of facial mimicry on emotional processing.
To this regard, a series of studies have investigated emotion elaboration in individuals mainly characterized by peripheral facial palsy.
In a recent longitudinal research, for example, Storbeck and colleagues (Storbeck et al., 2019) reported longer reaction time in facial expression recognition on patients affected by acute acquired facial palsy (Bell's palsy) with respect to control subjects. Interestingly, after clinical remission, Bell's patients improved their performances in facial expression recognition, although still reporting significantly prolonged response time. No significative differences emerged in recognition accuracy scores.
These authors suggest that these results on one hand support embodied simulation theories, considering that acquired paralysis could have limited the typical functioning of the facial sensorimotor mirror networks, on the other hand, given the normal accuracy of emotion recognition, suggest the presence of compensatory strategies allowing adult individuals with facial palsy to correctly recognize facial expressions of emotions.
Among facial palsy, probably the most intriguing model for studying the interaction between impaired facial mimicry and emotion processing are individuals with MBS, being mainly characterized by a congenital form of peripheral mimic facial block, usually with normal intelligence and cognitive development.
Considering the rarity of the syndrome, only few studies have investigated MBS individuals' emotions recognition ability, and not rarely with difference in procedures and methodologies thus reporting results that are ambiguous and not yet conclusive.
Giannini et al.'s study (Giannini et al., 1984), for example, reported difficulties in facial expressions processing in a single 36 years old MBS woman compared to normative data of 300 control participants. In particular, the participant was unable to correctly interpret facial expressions of slot machine players in relation to prizes. Similar but not completely

overlapping results have been reported by a study of Calder and colleagues (Calder et al., 2000). These authors provided evidence that three adult people with bilateral MBS (mean age: 28,7 years) were able to recognize basic facial expressions. However, MBS participants showed difficulties in a more complex task, consisting in evaluating morphed facial expressions.

In a study of Bogart and Matsumoto (Bogart & Matsumoto, 2010) 37 adult individuals with MBS accuracy did not differ from the performance of a matched control group. This study consisted in a facial expression recognition task performed through an online assessment where participants had to correctly identify emotions among a set of set of 42 validated photos representing seven basic facial expressions of emotions (anger, contempt, disgust, fear, happiness, sadness, and surprise). The authors suggested that the absence of facial expressions recognition difficulties revealed by this study indicate that facial mimicry and in general sensorimotor simulation are not necessary for facial expression processing.

A recent investigation of Caramazza and collegues (Vannuscorps et al., 2020) tested 11 MBS individuals (mean age 27.7, SD=9.25) through several task of facial expressions of emotion and facial identity recognition. The authors used a single case analysis approach by comparing each individual performance with normative data. Results showed that only two out of eleven MBS participants reported control-like performances. Based on these findings, the authors posit that simulative process are not necessary to adequately process facial expressions.

As previously mentioned, the studies discussed so far on facial expression recognition in MBS are clearly not conclusive. For example, in all the studies MBS participants were adults who never produced or mimicked facial expressions for their entire lives. Thus, they could have developed alternative cognitive strategies for emotion recognition, probably involving visual rather than sensorimotor representations, like specific face configurations (eyebrow, retractions of the lips corners of the mouth, etc.). These alternative modalities of emotion processing could be thought to be particularly effective when the observed facial expressions represent a prototypical emotion, and especially if static rather than dynamic and with no time limit of exposure. Although facial mimicry could be thought as fundamental for social interactions, it is well known that individuals also use other non-verbal channels to communicate emotions such as gestures, posture, proximity (Calbi et al., 2017; de Gelder et al., 2015) or voice prosody (Liebenthal et al., 2016). MBS individuals could than integrates all these aspects with other cognitive (e.g. language and symbolic thoughts) and contextual ones in order to obtain adequate representation of emotional meanings and then associate them with visual aspects of facial expressions. From a neuroconstructivist point of view (Westermann et al., 2007), these strategies may have allowed MBS patients' to overcame physical constraints (that is the block of facial muscles) in order to adaptively interact with others and detecting others' emotions by integrating different elements related to both bodily and contextual aspects. This, for example, could allow MBS individuals to associate an observed specific facial configuration to its cognitive and social meaning (e.g. a face expressing happiness to a pleasant state/situation). However, as previous studies have suggested, presumably these alternative strategies might not be effective in recognizing and processing complex emotion as an immediate matching and inner sensorimotor simulation of the observed facial expression. Moreover, they could necessitate a long learning period and thus probably becoming effective later after childhood. Therefore, it would be more informative to investigate emotion processing and facial expressions of emotions recognition in MBS children rather and in adult individuals and using more ecologic and complex stimuli rather than static and basic facial expression of emotion.

Another important aspect to be taken into account is that previous studies on MBS patients didn't focused on implicit emotion processing (e.g. physiological correlates of emotions).

In fact, considering the large amount of research showing the modulatory function of facial sensorimotor representations network on the autonomic nervous system (ANS) activity previously discussed, it is reasonable to assume that if the congenital absence of facial mimicry could result in an inability to simulate others' facial expressions, this should affect not only explicit facial expression recognition but also autonomic response in relation to emotion.

To this regard, a recent study of Nicolini and colleagues led by Pier Francesco Ferrari at the University of Parma investigated the autonomic response to emotional stimuli in MBS children through a non-invasive and accurate technique to asses ANS activity(Ioannou et al., 2013; Kuraoka & Nakamura, 2011; Nakayama et al., 2005), namely the functional Infrared Thermal Imaging (fITI) (Nicolini et al., 2019). In particular, children face was video-recorded with a high-resolution thermal camera while participants were observing a series of cartoon video-clips with different emotional contents (happiness, sadness, fear). Results showed that MBS children reported significant weaker thermal response than an age-matched control group, as well as a parallel difficulty to recognize basic expression of emotions (assessed through an adapted version of Test of Emotion Comprehension (O. Albanese & Molina, 2008)). Considering that peripheral thermal variation is modulated by autonomic activity (Ioannou, Gallese, et al., 2014), these results do show the presence of atypical autonomic responses in relation to emotion in MBS individuals. To this end, in the next chapter I will present a study conducted during my Ph.D. course and aimed at further investigate MBS children autonomic responses in relation to dynamic facial expressions.

2. Ph.D. COURSE RESEARCH ACTIVITY

2.1 Objectives and hypothesis of the thesis

To date, although facial sensorimotor representations involvement in emotional facial expressions have been widely demonstrated, their crucial role for socio-emotional processes is still debated.

More recent investigations have tried to disentangle these issues by studying emotion processing in clinical conditions, in particular in patients with congenital facial paralysis, affected by Moebius Syndrome. However, it is still not clear if congenital mimic facial weakness could have led to a deficient organization of the sensorimotor system, with a consequent impaired simulation mechanism during facial expression observation.

As previously discussed, relevant behavioral experiences during early period of development, namely *sensitive periods*, are fundamental in order to narrow and define the actual brain organization. Indeed, experience-dependent neuroplasticity processes are already active during gestational brain formation and then continue during childhood and adolescence. Thus, the leading hypothesis of this doctoral thesis is that the MBS individual's limitations (or total absence in some patients) to produce facial expressions could have led to a hypo-functioning facial representation, with a subsequent impairment of the activity of the *mirror neuron system* (MNS) during emotional processing.

In particular, objective of this Ph.D thesis is to evaluate MBS patient facial sensorimotor anatomo-functional organization in relation to the capacity to process facial expressions of emotion. A second objective is to investigate MBS patient's cortical plasticity following facial animation surgery and MNS-based neurorehabilitative interventions.

In the following chapters I will present and discuss three studies carried out during my Ph.D course and aimed at further testing these hypotheses.

The first study investigated MBS children autonomic response to facial expressions, by means of thermal imaging and Electrocardiographic measurements. These results have been already published in the following article: "Children with facial paralysis due to moebius syndrome exhibit reduced autonomic modulation during emotion processing"(De Stefani, et al. 2019).

The second study consisted in a post-surgery longitudinal kinematics investigation of smile production in MBS patients who followed a Facial-Imitation and Synergistic hand-mouth

Activity (FIT-SAT) neurorehabilitation protocol after *smile surgery*, namely a free muscle transfer procedure.

Finally, the third study was focused in investigating facial sensitivity and sensorimotor somatotopic organization on MBS patients who underwent surgery to increase face motility at different ages.

2.2 STUDY 1 – CHILREN WITH FACIAL PARALYSIS DUE TO MOEBIUS SYNDROME EXHIBIT REDUCED AUTONOMIC MODULATION DURING EMOTION PROCESSING

Text and results of this study have been already presented and discussed in an article published on *Journal of neurodevelopmental disorders*, with the following reference:

De Stefani, E., Ardizzi, M., Nicolini, Y., Belluardo, M., Barbot, A., Bertolini, C., ... & Ferrari, P. F. (2019). Children with facial paralysis due to Moebius syndrome exhibit reduced autonomic modulation during emotion processing. *Journal of neurodevelopmental disorders*, *11*(1), 12.

2.2.1 Background

When individuals are exposed to emotional faces, they spontaneously react with distinct electromyography responses in emotion-relevant facial muscles, a mechanism termed "facial mimicry" (Ardizzi et al., 2013, 2014, 2016; Ulf Dimberg & Thunberg, 2012). Notably, artificially interfering with participants' spontaneous facial muscular activation during observation of facial expressions significantly reduces emotion recognition performance (Ipser & Cook, 2016; Oberman et al., 2007; Ponari et al., 2012). This evidence indicates a close relationship between the ability to express facial emotions and the ability to recognize facial expressions displayed by others. According to motor theories of perception, the observation of others' facial expression activates the sensorimotor representations involved in the execution of that expression, facilitating recognition processes (Niedenthal et al., 2010). In particular, information concerning one's own emotion is hypothesised to be retrieved through both somatovisceral and motor re-experiencing of an observed emotion (Vittorio Gallese, 2014).

The ''mirror neuron system" (MNS) is considered part of the neurobiological substrate supporting this shared representation (Ferrari et al., 2003, 2012; Rizzolatti & Sinigaglia, 2008). When we observe an individual performing an action, our motor cortices become active in the same way as if we were experiencing that action ourselves (Buccino, Binkofski, et al., 2004). This simulation mechanism is useful for understanding others' actions and goals within a motor framework (De Stefani et al., 2016; De Stefani, Innocenti, De Marco, et al., 2013; Giacomo Rizzolatti et al., 2014; Giacomo Rizzolatti & Craighero, 2004), and can be applied to the domain of language and emotional development (Corballis, 2010; De Marco et al., 2015, 2018; De Stefani, Innocenti, Secchi, et al., 2013; Fabbri-Destro et al., 2015). Although neuroimaging investigations have shown that a number of cortical and subcortical areas (involving the premotor cortex, the anterior cingulate cortex and the anterior insula) that support first person experience of a specific emotion, also become active during the observation of that emotion in others (Carr et al., 2003; Caruana et al., 2011; De Stefani, Nicolini, et al., 2019; Jezzini et al., 2012; van der Gaag et al., 2007; Wicker et al., 2003), the debate concerning the role of simulation processes in emotional recognition remains an open one in the literature.

The study of facial expression processing in patients with peripheral facial palsy could be a potentially powerful empirical strategy for assessing simulation processes in emotion recognition. Among facial palsies, Moebius syndrome (MBS) is the most interesting condition, because it is present from birth and is characterised by weakening or paralysis of the facial muscles. The cranial nerves that are predominantly involved in this extremely rare syndrome (1/250.000 live births (Picciolini et al., 2016) are the sixth and seventh; these directly control lateral eye movements and facial muscles, respectively (Picciolini et al., 2016). These nerves are either absent or underdeveloped, resulting in bilateral or unilateral facial palsy. MBS is sometimes associated with musculoskeletal abnormalities and other cranial nerve palsies: these include, most commonly, the hypoglossal nerve (Bianchi et al., 2010), which often leads to atrophy of the tongue and, accordingly, speech problems (Bianchi et al., 2010). Some patients with MBS may also present with additional deformities, such as orofacial, limb and musculoskeletal malformations, whereas the patient's intelligence is usually preserved (Bianchi et al., 2010; Bianchi et al., 2009; Briegel et al., 2009, 2010; De Stefani, Nicolini, et al., 2019; Terzis & Noah, 2003).

The diagnosis of MBS is based exclusively on clinical criteria. The classical diagnostic criteria are bilateral facial paralysis affecting both sides of the face (7th cranial nerve) and paralysis of sideways (lateral) movement of the eyes (6th cranial nerve) (Bianchi et al., 2010; De Stefani, Nicolini, et al., 2019; Picciolini et al., 2016; Terzis & Noah, 2003). Recently, cases with unilateral facial palsy have also been included in the spectrum of this disease (Bianchi et al., 2010; Bianchi et al., 2009; Picciolini et al., 2016). Because of their congenital lack of capacity for facial mimicry, the study of children with MBS is of great relevance for investigating the contribution to emotion recognition of facial simulation processes.

Here, we focus on an under-investigated topic: the contribution of the capacity for facial mimicry to autonomic regulation in response to others' emotions. In fact, the autonomic nervous system (ANS) regulates the physiological reactions of the entire body to environmental stimuli (J. F. Thayer & Lane, 2000), fostering either prosocial (e.g., a parasympathetic calm and relaxed state (Porges, 2009; Porges & Furman, 2011)), or defensive (e.g., sympathetic fight of flight responses), behavioral strategies. The centrality of the ANS in emotion has been demonstrated in a large body of research aimed at assessing different aspects of the ANS–emotion relationship (for a review see (Kreibig, 2010), and the link between motor simulation and ANS reactivity is supported by several neuroimaging studies demonstrating how observation of others' emotional facial expressions activates not only motor pathways (Carr et al., 2003), but also brain structures (e.g. amygdala, insula) (Likowski et al., 2012; van der Gaag et al., 2007; Wicker et al., 2003) regarded as part of the extended MNS (Bonini, 2016; P. F. Ferrari et al., 2017), and thought to be responsible for emotional information processing. Despite the volume of studies, investigations of the effects of deficits in facial mimicry on autonomic regulation are still lacking. We hypothesized that MBS patients might present an alteration in autonomic responses to emotional stimuli as a consequence of the inability to express emotions from birth.

Among several techniques commonly adopted for ANS recording, functional infrared thermal imaging (fITI) and electrocardiography (ECG) were implemented in the present study. fITI is a technology that offers the advantage of a non-contact approach, which is suitable for human psychological and physiological studies (Ioannou, Gallese, et al., 2014). fITI records the body's naturally emitted thermal irradiation, which depends on cutaneous blood perfusion controlled by the ANS innervating the vessels that irrigate the skin (Ioannou, Gallese, et al., 2014). Recently, it has been demonstrated that many emotional states are associated with variations in facial temperature (Ioannou et al., 2013; Merla & Romani, 2007; Nakanishi & Imai-Matsumura, 2008). Specifically, measuring thermal effects of emotional arousal may provide useful information about the sympathetic branch of the ANS, since skin temperature depends on cutaneous blood perfusion and local tissue metabolism, and sudomotor responses, all of which are controlled by the sympathetic system.

Using ECG, we estimated respiratory sinus arrhythmia (RSA) reactivity. RSA is a metric of heart rate variability associated with spontaneous breathing. RSA measures the parasympathetic branch of the ANS via cholinergic vagus nerve projections to the heart. During situations where active coping, or emotional regulation, is required, the vagal input increases RSA, supporting a flexible coping response. According to polyvagal theory, this response is a physiological indicator of the individual's ability to engage in appropriate regulatory behavior and provides a physiological substrate for affect regulation, which presumably underlies adaptive interpersonal functioning (Porges, 2001; Porges, 2003). Specifically, the vagal tone at rest is considered a stable neurophysiological mechanism reflecting potential autonomic reactivity in the absence of environmental challenge. In the literature, high resting RSA has been associated with appropriate emotional reactivity and indexes of the functional ability to engage and disengage with the environment (Thayer et al., 2012).

We conducted two experiments testing for emotional processing in children with MBS. In the first experiment, we tested whether, compared to a non-affected control group of the same age, children with MBS were able to recognize stimuli representing facial expressions. In fact, in the literature, there are no studies on facial recognition of emotions in children with MBS, but only in adult patients, and results are inconclusive (Bate et al., 2013; Calder et al., 2000; Bogart & Matsumoto, 2010). We used dynamic stimuli that, in the literature, have proved more effective than static images in inducing an emotional response (Arsalidou et al., 2011; Kessler et al., 2011; Sato et al., 2002). The stimuli were facial expressions representing emotions of disgust, surprise, anger, happiness. These emotions were selected based on the developmental stage of the participants. Thus, although even newborns are able to produce facial expressions (Meltzoff & Moore, 1989), the ability to recognize specific emotions from facial expressions increases with age (Lawrence et al., 2015; Rodger et al., 2018). Previous studies reported that, among the basic facial expressions, the emotions that are best recognized (from an actor's full face display) are happiness, anger, and disgust, followed by fear, with sadness being more difficult to recognize (Guarnera et al., 2015, 2017; Herba et al., 2006). More specifically, research has shown that, by 5 years of age, children are as sensitive as adults to displays of happiness, (Gao & Maurer, 2009); and from 8 to 11 years, they recognize happy, angry and disgust expressions more easily than those showing fear and sadness (Gao & Maurer, 2009; Mancini et al., 2013). For these reasons, among the basic facial expressions, we included two positive emotions (happiness and surprise) and two negative emotions (anger and disgust), whereas facial expressions of fear and sadness were discarded.

Once the ability of children with MBS to recognize facial expressions was ascertained, we determined whether the emotional processing and the responses of the ANS (physiological experiment) were less efficient in these children than in those in the control group.

2.2.2 Experiment 1: emotion detection probe

The first study tested participants' ability to recognize facial expressions with a high percentage of accuracy. (Note, establishing that children of this age group could recognize the expressions accurately was an important prerequisite for the valid assessment of ANS responses in experiment 2, in which we used the same set of stimuli. Thus, use of facial expressions that were not easily recognizable by children of this age would render uninterpretable the results obtained in the second experiment.)

Materials and methods

Participants

The study involved 26 subjects. Eight children with MBS (MBS group, MBS: 3 females, M_{age}=9 years; SD=2.3) were recruited at the Operative Unit of Maxillofacial Surgery, Head and Neck Department.

In table 1, demographic data and clinical information concerning all participants with MBS are reported. The children's medical history was confirmed with the treating physician prior to testing. The inclusion criteria for children with MBS were: 1. a certified diagnosis of unilateral or bilateral facial paralysis (B. Bianchi et al., 2010b; Bernardo Bianchi et al., 2009b; Picciolini et al., 2016) (We included unilateral paralysis based on previous studies demonstrating that patients with hemiparesis also show impairment in emotion recognition (Korb et al., 2016)); 2. a score >70 percentile on the Colored Progressive Matrices Test, CPM (*CPM*, n.d.). Exclusion criteria were 1. the presence of congenital limb malformations; 2. The presence of any psychiatric or physical illness at the time of participation.

The control group consisted of 18 children (control group, CG: 3 females, M_{age}=9 years; SD=1.4) who did not meet criteria for a clinical diagnosis of MBS, or present with any psychiatric or physical illness, or any other neurological disorder.

Table 1 Demographic and clinical characteristics of participants with Moebius syndrome in experiment 1

Group	Age	Sex	Paralysis	Cranial nerves involved	Dysfunction	IQ
MBS01	11	Female	Bilateral	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS02	11	Female	Unilateral left	Abducens nerve (VI)	No lateral eye movements	110
				Facial nerve (VII)	Facial palsy	
MBS03	11	Male	Bilateral	Facial nerve (VII)	Facial palsy	105
				Accessory nerve (XI)	Ipsilateral weakness in the trapezius muscle	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS04	8	Male	Bilateral	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS05	6	Male	Bilateral	Facial nerve (VII)	Facial palsy	100
MBS06	8	Male	Bilateral	Abducens nerve (VI)	No lateral eye movements	110
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS07	11	Male	Unilateral left	Abducens nerve (VI)	No lateral eye movements	120
				Facial nerve (VII)	Facial palsy	
MBS08	6	Female	Unilateral right	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	

Participants’ legal guardians gave written informed consent for the experimental procedure, which was approved by the Ethics committee of Parma (prot. 32074). Participation in the study was voluntary and the participants were not paid. The study was conducted in line with the Declaration of Helsinki 2013.

Stimuli

Stimuli were short video-clips lasting 4sec created using computer-morphing software (Abrosoft FantaMorph software package). Pictures (*800×560 pixels)* of four actors’ faces expressing five different emotions were selected from a set of validated pictures from the Nim Stim Face Stimulus Set (Tottenham et al., 2009). Pictures consisted of four Caucasian actors’ faces (two males and two females) expressing four emotional facial expressions (i.e., disgust, surprise, anger, happiness) or a neutral facial expression (figure 1A).

Each video-clip showed the transition from a neutral facial expression to an emotional one within the same actor (emotional video-clips) or from a neutral face to another (neutral face, non-emotional video-clips). In total, we created 60 stimuli (12 disgust, 12 surprise, 12 anger, 12 happiness and 12 neutral stimuli). E-Prime 2.0 software (Psychology Software Tools, Inc.) was used for stimuli presentation.

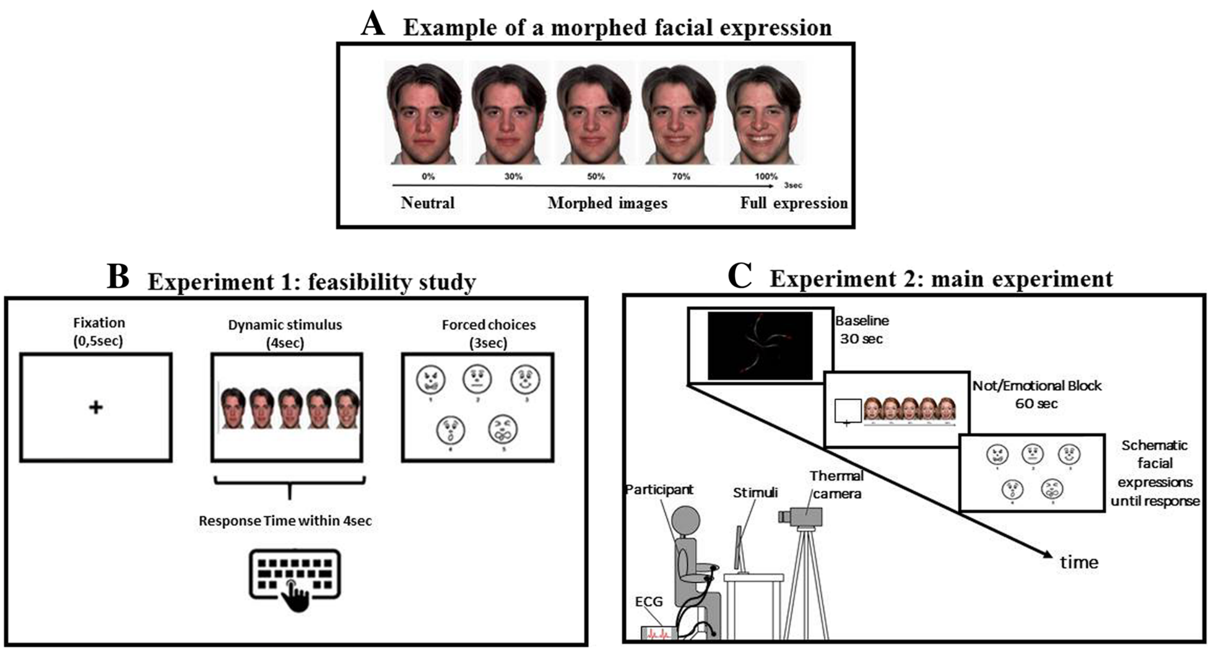

Fig. 1 **a**An example of the morph steps. Each video clip showed an actor's face morphing from the neutral expression to one of the five prototypical expressions (disgust, surprise, anger, happiness, and neutral). **b** Procedure of the experiment 1. Participants were presented with dynamic facial expressions one at a time. Each stimulus lasted for 4 s. When participants recognized the facial expression they pressed the space bar (stop button) and the stimulus disappeared (response time, RTs). RTs were considered an indicator of the time necessary to recognize the facial expression. Then participants were instructed to categorize each stimulus in a forced-choice procedure identifying the facial expression from a list of five stylized emotional faces (accuracy rate, RACC). **c** Procedure of the experiment 2. Participants were presented with 5 different blocks of morphed emotional faces (i.e., disgust, surprise, anger, happiness, and neutral). Each block lasted 60 s and was composed of different video clips representing the same emotion (12 facial expressions in total). Each video clip was preceded by 30-s baseline (i.e., a dynamic screensaver). At the end of each block participants underwent a control task (a forced-choice procedure identifying among five alternative pictures the emotion corresponding to the block previously seen)

Procedure

Once informed consent was obtained, participants were seated in a comfortable chair after being introduced to the experiment. Stimuli were presented centrally and the viewing distance was set at 60 cm from a 17inch computer-monitor (1024X768@75Hz). Written instructions were presented on the screen before the beginning of each task and were read aloud to the participant by the experimenter.

Video-clips were randomly presented one at a time. Each trial started with a fixation cross, presented for 0.5sec in the centre of the screen. Each video-clip lasted for 4sec (3 seconds of dynamic morph and 1 second of full emotion expression, figure 1B). Each stimulus was presented on a white background, with a dynamic morph starting from neutral and going to full facial expression.

Participants were told that the facial expressions appearing on the screen would look neutral at the beginning of the video-clip, and would gradually change to reveal one of five expressions (disgust, surprise, anger, happiness and neutral expressions). They were asked to watch the facial displays change and to press the space bar to stop the video as soon as they thought they knew which expression the face was displaying. Participants were also instructed to maximize speed and recognition accuracy. When participants pressed the stop button, the stimulus disappeared, and the response time was recorded as an index of the time necessary to recognize the facial expression. (The disappearance of the stimulus ensured that the response time reflected the actual recognition of the facial expression). If participants did not press the space bar, the stimulus disappeared after 4sec.

After the stimulus disappeared, participants were instructed to categorize each stimulus in a forced-choice procedure identifying the facial expression from five options (stylized emotional faces). One practice trial was run, prior to 10 test trials (two trials for each emotion).

Statistical data analyses

We analysed two dependent variables: response time (RTs) and accuracy rate (RACC). RTs were calculated as the time elapsed between the onset of the stimulus and the participants' button press (recognition of a single facial expression). RACC rate was computed as the proportion of correct responses out of the total answers given (discrimination of facial expressions).

We excluded RTs less than 920ms (less than 30% of morphing) in order avoid anticipatory responses. RACC data were arcsine transformed prior to the analysis; values ranged from a minimum of zero to a perfect score of 1.57 (which is the arcsine of 1 (*On Measuring Performance in Category Judgment Studies of Nonverbal Behavior | SpringerLink*, n.d.)).

RTs were included as dependent variables into two mixed-design analysis of variance (ANOVA) in which "emotion" (five levels: disgust, neutral, surprise, anger and happiness) was used as the within-subjects factor and "group" (two levels: MBS, CG) as the between-subjects factor. When the sphericity assumption was violated, Greenhouse–Geisser degrees of freedom corrections were applied. The probability value was set at $p < 0.05$ for all analyses. Partial eta square (ηp^2) was calculated as the effect size measure. Bonferroni post-hoc tests were conducted following the two-way ANOVA.

Since many participants were 100% correct in recognising some emotions, we considered only the total number of correct answers given by each group. The Kruskal-Wallis test was used as a non-parametric statistical procedure for comparing the RACC values of the two samples. The Statistical Package for the Social Sciences, version 25 (SPSS, Chicago, IL, United States) was used to perform the analyses.

Results

Table 2 contains means and standard deviations of participants' RTs during emotional expression recognition for the MBS group (MBS) and control group (CG), respectively. Overall, disgust was the emotion that required the longest RTs (2349ms), while happiness was the most rapidly recognized (1931ms).

Table 2 Experiment 1: Mean and standard deviation (SD) of response times (in milliseconds) for neutral, disgust, surprise, anger, and happiness stimuli for the Moebius syndrome group (MBS) and control group (CG)

	Response time (ms)									
	Neutral		Disgust		Surprise		Anger		Happiness	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MBS	2282	437	2382	409	2208	492	2027	373	1920	232
CG	2089	413	2335	416	2170	339	2108	416	1936	396

Mixed ANOVA on RTs revealed a main effect of emotion ($F(4,96) = 9.9$; $p = 0.001$; $\eta p^2 = 0.29$). Bonferroni post-hoc t-tests revealed that participants recognized happy video-clips significantly faster (1931ms) than disgust (2349ms, p=0.001), neutral (2149ms, p=0.014) and surprise (2182ms, p=0.003) video-clips. Conversely, disgust was the emotion that took the longest RTs (disgust vs. neutral, p=0.032; disgust vs. anger, p=0.001). No significant group or interaction (group x emotion) effects were observed (p>0.05).

Table 3 contains means and standard deviations of participants' RACC rates for the recognition of emotional expressions. In general, the judgments of facial stimuli were highly accurate (mean RACC= 96% ±4.7).

Table 3 Means and standard deviations (SD) of response accuracy rate for the recognition of each emotional expression in experiment 1 and at the end of each block in experiment 2 showed by Moebius Syndrome group (MBS) and control group (CG)

	Neutral		Disgust		Surprise		Anger		Happiness	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Experiment 1_Accuracy (%)										
MBS	96	8.9	79	24.3	96	6.4	96	9	95	14.7
CG	100	2	91	8.5	100	0	99	2.7	98	3.8
Experiment 2_Accuracy (%)										
MBS	69	0.5	85	0.4	100	0	100	0	92	0.3
CG	100	0	94	0.3	100	0	100	0	94	0.3

The Kruskal Wallis test comparing RACC values between the groups showed that RACC scores were significantly lower for MBS than CG (Chi-square=5.096; p =0.024, MBS=92.2%, CG=97.4% respectively; figure 2), indicating that, although they were very accurate, MBS participants' performance in discriminating facial expressions was poorer than that of the control group.

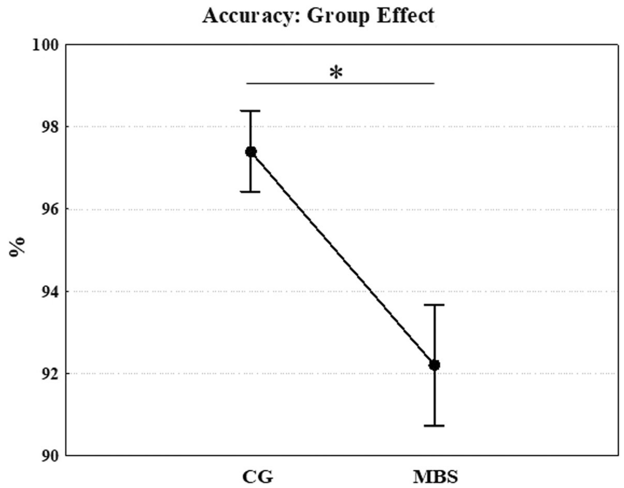

Fig. 2 Experiment 1: Kruskal–Wallis test was used for comparing the RACC values of the two samples. Mean accuracy scores (%) for control (CG) and Moebius syndrome group (MBS) groups on recognition of five facial expressions are reported. *$p < 0.05$. Error bars represent SE (standard errors of the means)

Discussion

Results of this first study revealed an absence of group difference in RTs, and this finding supports the hypothesis that children with MBS had a comparable level of emotion recognition to that of the control group. These results are in line with previous studies (Calder et al., 2000; Bogart & Matsumoto, 2010) in which face stimuli were presented singly, and exclude the possibility that children with MBS may have difficulty inspecting stimuli, because their response times were similar to those of the control group .

However, the RACC analysis showed that the control group was more accurate than children with MBS, suggesting some difficulties in the latter group in discriminating the facial expressions displayed. Although the high percentage of accuracy and the small number of participants preclude us from concluding that children with MBS have deficits in emotional recognition, the results nevertheless highlight some difficulty when required to identify facial expressions from an array of stimuli with emotional content (complex facial recognition task). We hypothesize that participants with MBS, not being able to benefit fully from motor simulation mechanisms, probably use alternative cognitive strategies, which may not be as effective as simulation (at least during early development) (De Stefani, Nicolini, et al., 2019). One of these cognitive mechanisms could involve strict, rule-based, strategies, in which memorized lists of characteristics defining emotional expressions, are employed. Such strategies may lead children to look for the presence of these specific features when performing emotional perception tasks. For example, a "rule" for disgust could be "corners of the actor's mouth turned down". This characteristic, present both in disgust and anger, was efficient for recognizing the facial expression when present, but was ineffective when choosing among multiple options when the stimulus was no longer present. Our findings are also in line with the conclusion of Calder et al. (Calder et al., 2000) and Bate et al. (Bate et al., 2013) that, while difficulties in emotional face recognition are prevalent in individuals with MBS, they are not invariable. It is also possible that the higher number of errors we found in children with MBS might be related to the young age of participants. Thus, previous studies have focused on adults, while here we included children who have probably not yet fully developed other cognitive strategies for recognizing others' emotions.

This study had a number of limitations. First, the small sample of patients, and the limited number of facial expressions used, limit the generalizability of our results. Second, the high level of accuracy in facial expression recognition observed in both groups may have masked group differences in recognizing specific emotions. In future, more refined measurements of emotion recognition and the use of more complex stimuli (e.g. morphed facial expressions of two or more emotions) could be useful in identifying subtler difficulties in children with Moebius syndrome. Further, these preliminary results will need to be further investigated using more complex experimental designs and a greater number of stimuli. Moreover, follow-up assessments should be carried out across development, from childhood to adolescence, in order to assess the improvement of facial expression recognition in these patients.

2.2.3 Experiment 2: physiological experiment

In this study, we tested whether the responses of the ANS during emotional processing were altered in children with MBS compared to a control group. Specifically, we monitored the variations of facial temperature and the amplitude of RSA in children with MBS and controls when they were presented with 1-minute-long videos depicting dynamically changing facial expressions, from a neutral face to one showing disgust, surprise, anger, happiness or else remaining neutral. Given that children recognized the facial expressions used in Experiment 1 with a high degree of accuracy, we used the same sets of stimuli in Experiment 2 in order to measure the autonomic response to different emotional stimuli.

Materials and methods

Participants

A new group of 13 children with MBS (MBS group, MBS: 7 females, M_{age}=8.7 years; SD = 2.8, see table 4) participated in the study (see inclusion/exclusion criteria in Experiment 1, the emotion detection probe study, participants section). The healthy control group (CG) consisted of 16 participants (6 females, M_{age}=9.3 years; SD=1.7). Participants' legal guardians gave written informed consent for the experimental procedure, which was approved by the Ethics committee of Parma (prot. 32074). Participation in the study was voluntary and the participants were not paid. The study was conducted in line with the Declaration of Helsinki 2013.

Table 4 Demographic and clinical characteristics of participants with Moebius syndrome in experiment 2

Group	Age	Sex	Paralysis	Cranial nerves involved	Dysfunction	IQ
MBS09	11	Female	Bilateral	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS10	5.5	Female	Unilateral right	Abducens nerve (VI)	No lateral eye movements	80
				Facial nerve (VII)	Facial palsy	
MBS11	5.5	Female	Unilateral right	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS12	10	Male	Bilateral	Abducens nerve (VI)	No lateral eye movements	80
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS13	9.5	Female	Bilateral	Abducens nerve (VI)	No lateral eye movements	110
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS14	13	Male	Unilateral left	Abducens nerve (VI)	No lateral eye movements	110
				Facial nerve (VII)	Facial palsy	
MBS15	6	Female	Unilateral left	Abducens nerve (VI)	No lateral eye movements	110
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS16	7	Male	Bilateral	Abducens nerve (VI)	No lateral eye movements	80
				Facial nerve (VII)	Facial palsy	
				Vestibulocochlear nerve (VIII)	Hearing loss	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS17	8	Male	Bilateral	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS18	8	Male	Bilateral	Abducens nerve (VI)	No lateral eye movements	110
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS19	12	Male	Unilateral left	Abducens nerve (VI)	No lateral eye movements	120
				Facial nerve (VII)	Facial palsy	
MBS20	5	Female	Bilateral	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	
				Hypoglossal nerve (XII)	Fasciculations or atrophy of the muscles of the tongue	
MBS21	12	Female	Unilateral left	Abducens nerve (VI)	No lateral eye movements	100
				Facial nerve (VII)	Facial palsy	

Stimuli

The sets of stimuli comprising different facial expressions used in this study were the same as those for Experiment 1. Before measuring the impact of these stimuli on ANS reactivity, the recognition of each facial expression was carefully evaluated, as in the first study. We confirmed that the judgments of the facial stimuli were highly accurate (mean RACC = 96%) in both groups.

Procedure

Before the start of the experiment, each subject was left to acclimatize themselves for 10–20 min in a softly-lit, sound-proofed, climate-controlled room (room temperature: 23 ± 1 °C; relative humidity: 50–55%; no direct sunlight or ventilation). Five different blocks of morphed emotional faces (i.e. disgust, surprise, anger, happiness and neutral) were randomly presented to the subject (figure 1C). Subjects sat comfortably in a chair, without any restriction of their body movements.

In total, participants observed 60 video-clips divided into 5 experimental blocks. Each block was composed of different video-clips representing the same emotion. Four video-clips (two males, two females) each lasting 4s (figure 1C) were repeated three times and shown in the same block (12 facial expressions in total). Each video-clip was preceded by a fixation cross displayed in the center of the screen for 1sec. Thus, each block lasted for a period of 60sec, and was randomly presented. A baseline (i.e., a dynamic screensaver) lasting 30sec preceded each block. In order to control participants' attention, at the end of each block, an image with five-forced choice picture options appeared on the screen. It remained visible until the participant responded (figure 1C). The experimenter asked the subject to identify which of the five alternative pictures matched the emotion previously displayed in the block. Participants were instructed either to answer verbally, or to point to the chosen image. The child's answer was then noted on the experimental pre-prepared sheet.

During video-clip presentations, the participant was asked to simply observe the stimuli. Participants' fITI and ECG were recorded for the entire duration of the experiment. Thermal IR imaging was recorded by means of a digital thermal camera FLIR T450sc (IR resolution: 320 X 240 pixels; spectral range: 7.5 – 13.0 μm; thermal sensitivity/NETD: < 30 mK at 30°C). The acquisition frame rate was set to 5 Hz (5 frames/sec). A remote-controlled webcam (Logitech webcam C170) was used to film children's behavior to assure that they paid attention to the stimuli. The thermal camera was placed just above the screen used for the stimuli presentation, one meter away from the participant's face, and it was automatically calibrated and manually fixated to allow a frontal recording of the child's face.

ECG was recorded using three Ag/AgCl pre-gelled electrodes (ADInstruments, UK) with a contact area of 10 mm diameter placed in an Einthoven's triangle configuration (Powerlab and OctalBioAmp8/30, ADInstruments, UK). The answers given by the children at the end of each block were considered an index of accuracy and treated as in the emotion detection probe study (section Statistical data analyses).

Thermal data analysis

Firstly, we performed a visual inspection of the changes in subjects' thermal responses to provide a qualitative investigation of their autonomic responses throughout the experiment. Then, thermal variation, i.e., changes in cutaneous temperature, were calculated for specific facial regions of interest (ROIs)(Ebisch et al., 2012; Ioannou, Gallese, et al., 2014).

We performed a quantitative estimation of temperature variation in the following ROIs: nasal tip (Ioannou et al., 2013; Kuraoka & Nakamura, 2011; Nakayama et al., 2005), cheeks (Nakanishi & Imai-Matsumura, 2008) and forehead (figure 3 (Ioannou, Gallese, et al., 2014)). The shapes of ROIs did not vary in size across frames, and temperature was extracted only when the face was at a direct angle to the camera (Ioannou, Gallese, et al., 2014, 2014b; Ioannou, Morris, et al., 2014). The same circular shapes were used for both groups. We initially created a mask in which the ROIs were drawn (figure 3A). We then took the tip of the nose as the reference point, this being an anatomical 'landmark' that is easily identifiable in all subjects (Ebisch et al., 2012). Subsequently, tracing an imaginary straight line centered on the tip of the nose, we located the area of the forehead positioned above the two eyebrows. Further horizontal lines that passed through the center of the eyes and the tip of the nose allowed us to identify the area of the cheeks.

Once the mask with the ROIs was created, it was uploaded for each participant's first frame. Since the participants were free to move during the observation of the stimuli, one of the experimenters analyzed the movements of the participant's face frame by frame during the experiment. If the participant moved, the experimenter adjusted the position of each ROI on the participant's face, maintaining their relative positions constant. If the participant's movements did not allow accurate repositioning of the ROIs, the frame was discarded. Thus, thermal signals were extracted and processed (figure 3B) by a trained coder through the use of tracking software, developed with homemade Matlab algorithms (The Mathworks Inc., Natick, MA) and validated in (Manini et al., 2013).

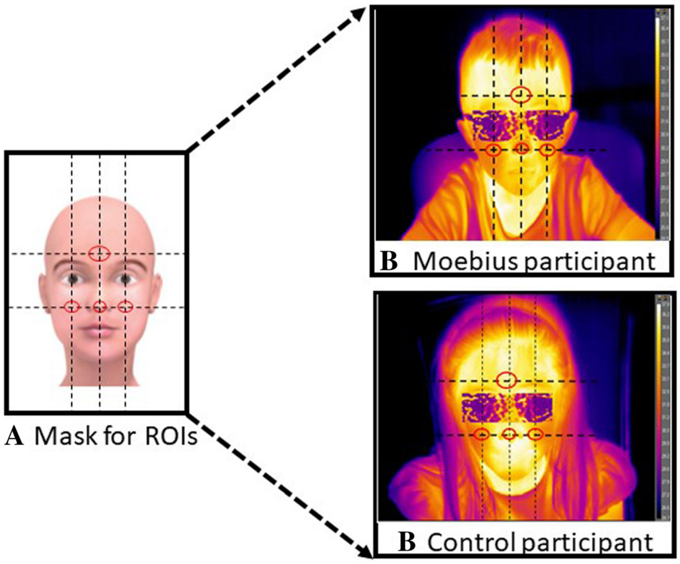

Fig. 3 To extract information of affective nature, three regions of interest (ROIs) were used (tip of the nose, forehead, and cheeks). A mask was initially created (**a**) in which the ROIs were drawn to ensure that there was no variability across the size and shape of the ROIs among participants. Taking as a reference point the nose, we traced an imaginary straight line centered on the tip of the nose to locate the area of the forehead placed above the two eyebrows. Further horizontal lines passing through the center of the eyes and the tip of the nose allowed us to identify the area of the cheeks. The same mask was uploaded on each participant's first frame and then a trained experimenter analyzed frame by frame the movements of the participant's face during the experiment. Whenever the participant moved, the experimenter adjusted the position of each ROI on the participant's face. If the participant's movements did not allow the reposition of the ROIs, the frame was discarded. In **b**, we reported two example of thermal extraction (in the top panel a child with Moebius syndrome and in the lower panel a child belonging to the control group). In the images of participants, blurred masks (glasses) are included on the children's face in order to occlude their identity

To avoid any possible noise or artifacts, thermal data were subsequently examined with PostTracking software. On average, we extracted 150 frames (30sec) for each baseline pre-block, and 300 frames (60sec) for each experimental block (neutral, happiness, surprise, anger, disgust). Non-Parametric Pearson correlations (Bonferroni adjusted) for the three ROIs yielded significant results ($\alpha = 0{,}05/3 = 0{,}02$, table 5) indicting that the three dependent variables were highly correlated.

Table 5 Experiment 2: Results of Pearson's correlations based on the three ROIs

	Forehead	Cheeks	Nose
Forehead	1.0000	0.8762	0.6882
	–	$p = 0.000$*	$p = 0.000$*
Cheeks	0.8762	1,0000	0.5812
	$p = 0.000$*	–	$p = 0.001$*
Nose	0.6882	0.5812	1,0000
	$p = 0.000$*	$p = 0.001$*	–

*Pearson correlation was significant at the 0.05 level (two-tailed). Bonferroni corrected

To eliminate temperature changes that were unrelated to the experimental conditions and to reduce inter-subject variability, thermal values were obtained by subtracting the mean thermal values of each ROI during the pre-block baseline from the mean thermal values of the ROIs during each experimental block.

Statistical analyses

First, we checked that the one-way ANOVA performed on the neutral block thermal values (neutral facial expression) in the three ROIs did not show significant differences between groups ($p>0,05$). Then, the temperature values for each emotional block (disgust, surprise, anger and happiness) were subtracted from those for the neutral block (Ioannou, Gallese, et al., 2014b). Given that the temperature values of the three ROIs (forehead, cheeks and nose) were significantly correlated (table 5), we performed a multivariate analysis of variance (MANOVA) in which the dependent variables were the three face ROIs (Ioannou et al., 2017). Thus, the effects of emotional stimuli observation on facial temperature were analyzed via a 4 × 2 MANOVA (emotion x group). The probability value was set at $p < 0.05$ for all analyses. Significant MANOVA findings are expressed using Wilks' Lambda (Λ) and effect size data (ηp^2) were also provided for additional information. The Statistical Package for the Social Sciences, version 25 (SPSS, Chicago, IL, United States) was used for all analyses.

RSA analysis

ECG data were converted and amplified with an eight-channel amplifier (PowerLab8/30; ADInstruments UK) and displayed, stored, and reduced with the LabChart 7.3.1 software package (ADInstruments, 2011). ECG was sampled at 1 kHz and online filtered with the Mains Filter. Heart period was calculated as the interval in milliseconds between successive R-waves. The amplitude of RSA [expressed in $\ln(ms)^2$] was quantified with CMetX (available from http://apsychoserver.psych.arizona.edu), a software for calculating cardiac variability that produces data with a correlation near unity with those obtained using the method of Boher & Porges (Allen et al., 2007). The amplitude of RSA was calculated as the variance of heart rate activity across the band of frequencies associated with spontaneous respiration (0.24–1.04 Hz for children below the age of 11 years and 0.12-0.40 Hz for children older than 11 years) (Allen et al., 2007). ECG data for two subjects were discarded because of technical problems. The resting RSA value was the mean of each 30sec screensaver baseline that preceded each block (2.5 min in total). RSA reactivity refers to RSA values extracted from two epochs (each of 30sec) during the 1m of each experimental block and expressed as the difference from resting RSA.

Statistical analyses

To investigate the functional modulation between vagal regulation and external, social stimuli, we first performed a one-way ANOVA to test differences in the resting RSA between groups. A 5x2 repeated mixed ANOVA was performed on RSA reactivity with emotion (neutral, disgust, happiness, anger and surprise) as a repeated measures factor and group (MBS vs CG) as a between-participants factor. When the sphericity assumption was violated, Greenhouse–Geisser degrees of freedom corrections were applied. The probability value was set at $p < 0.05$ for all analyses. Partial eta square (ηp^2) was calculated as the measure of effect size. Bonferroni post-hoc tests were conducted following the two-way ANOVA.

Pearson correlations were also calculated to assess RSA reactivity in relation to individual resting RSA in response to facial expressions and neutral stimuli (Patriquin et al., 2013). Bonferroni corrections were applied ($\alpha = 0,05/5 = 0,01$). The Statistical Package for the Social Sciences, version 25 (SPSS, Chicago, IL, United States) was used to perform all the analyses.

Results

The Kruskal Wallis test on RACC (the answers given by the children at the end of each block and considered an index of accuracy) between groups showed that scores were nevertheless significantly lower for MBS than CG (Chi-square=4.107; $p = 0.043$, MBS=92.2%, CG=97.4%). Consistent with the study hypothesis, thermal analysis showed a significant multivariate main effect of group ($\Lambda = 0.915$, $F(3, 106) = 3.27$; $p = 0.024$, $\eta p^2 = 0.085$). Specifically, children with MBS (MBS: -0.077ΔT) showed significantly lower thermal variation than the control group (CG: 0.051ΔT) while watching emotional stimuli (figure 4).

No overall significant multivariate main effects of emotion (p=0.635) or interaction with group (p=0.907) were observed.

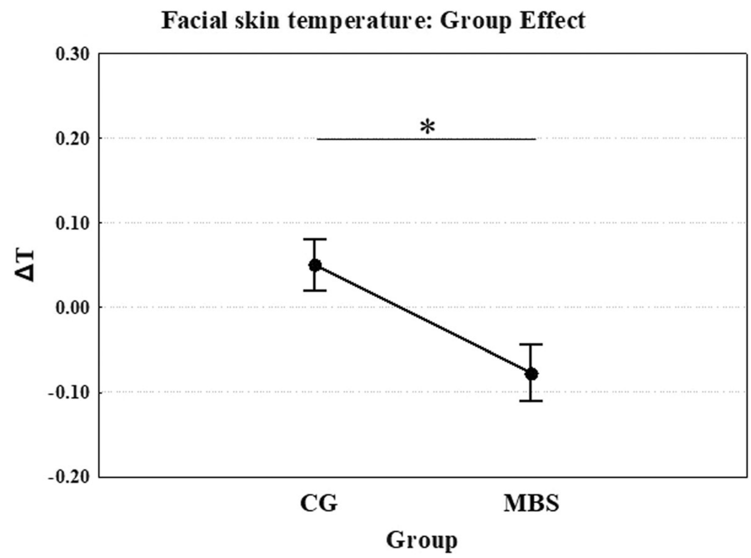

Fig. 4 Experiment 2: Mean temperature as a function of group (children with MBS and control group, CG) in all ROIs. *$p < 0.05$. Error bars represent SE (standard errors of the means)

Similar results were found when comparing the groups in terms of resting RSA. Thus, resting RSA was significantly higher in CG in comparison to MBS ($F (1, 25) = 5.805$; $p = 0.024$; $\eta p^2 = 0.188$, figure 5). The repeated mixed ANOVA performed on RSA reactivity did not show significant emotion or group main effects (p=0.526 and p=0.614 respectively), and there was no significant (group x emotion) interaction (p=0.454).

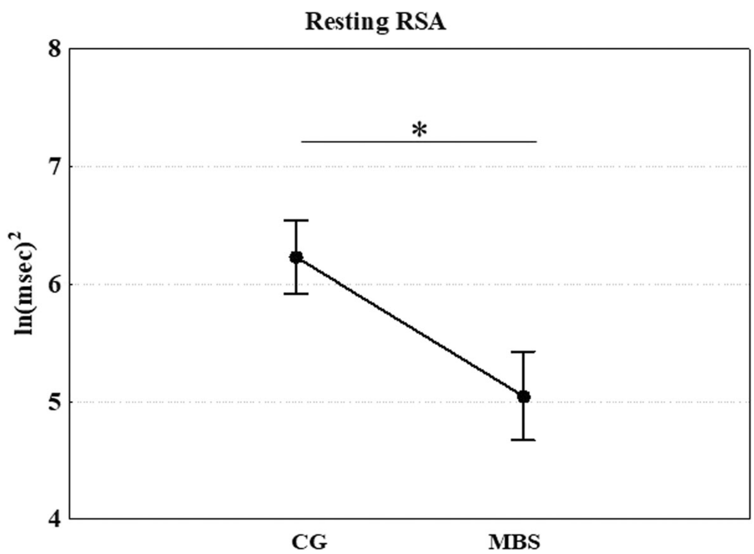

Fig. 5 Experiment 2: Mean values of resting RSA as a function of group (children with MBS and control group, CG). *$p < 0.05$. Error bars represent SE (standard errors of the means)

In order to determine whether there was a significant association between resting RSA and RSA reactivity, we performed two correlational analyses (one for each group) between the resting RSA and the RSA reactivity values for each condition. Pearson's correlations demonstrated a significant negative correlation between baseline RSA and RSA reactivity in CG only, in response to the neutral condition (r=−-0.665, Bonferroni corrected p=0.005, figure 6). No other significant correlations for either MBS or CG were found.

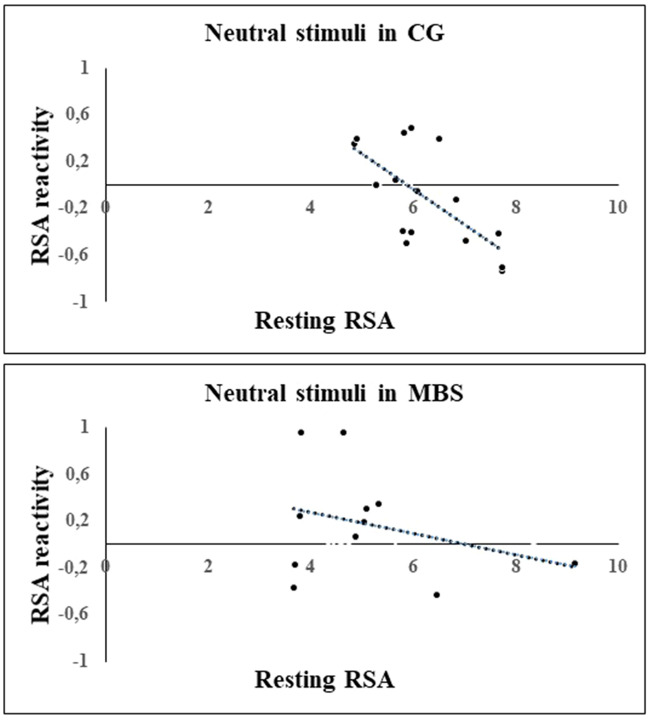

Fig. 6 Experiment 2: Plots of correlations between baseline and RSA reactivity values recorded during the observation of neutral stimuli for children with Moebius syndrome (MBS) and control group (CG) (Bonferroni corrected $p < 0.01$)

Discussion

In this second study, we focused on the contribution of the capacity for facial mimicry to autonomic regulation in response to others' emotions. We addressed this issue by studying a population of children with MBS, a rare neurological disorder that primarily affects the muscles controlling facial expressions. Individuals with MBS are born with facial muscle paralysis and an inability to produce facial expressions. This makes them the ideal population to study whether autonomic responses to emotional stimuli are affected by the inability to simulate the emotions of others from birth.

We recorded facial thermal changes and ECG during the observation of dynamic facial expressions. We found a significant difference in facial thermal responses between groups.

More specifically, consistent with previous studies, the control group showed greater thermal responses to emotional than to neutral stimuli relative to the Moebius group. By contrast, with respect to the neutral condition, Moebius children showed a skin temperature decrease, a response that is commonly associated with sympathetic activation in response to stressful, painful or frustrating situations (Manini et al., 2013; Merla & Romani, 2007). This lower autonomic response of the MBS group did not vary between the different ROIs.

Contrary to what we expected, thermal responses did not turn out to differ between the various facial expressions displayed. Our findings are, therefore, at variance with previous demonstrations of the capability of thermal IR imaging to capture physiological thermal variations in relation to different emotional states. Thus, in the study by Merla and Romani (Merla & Romani, 2007), participants were exposed to a stressful task, and the largest temperature variations were reported for subjects who were more influenced by the judgment of others. Similarly, interpersonal social contact and sexual arousal have been shown to result in an increase in facial temperature (Hahn et al., 2012; Merla & Romani, 2007).Temperature variations have also been found during stressful, fearful, painful and guilty experimental situations. Together, these studies, show that exposure to a wide range of stimuli and situations results in large variations in autonomic system reactivity.

In contrast to previous studies, the stimuli used in our study were presented for a relative short time period; and while they did, overall, elicit some arousal responses, these were minimal in magnitude and showed no specificity relative to the type of emotion. It is possible that a habituation effect due to repeated visual presentation within the same context could have leveled out potential thermal differences between emotional stimuli in our study. Future studies should explore in more depth the thermal responses of children with MBS in response to different types of emotional stimuli in order to understand whether this methodological approach is sensitive enough to detect autonomic differences between different emotions.

With regard to other indices of physiological regulation- resting RSA and RSA reactivity, our results demonstrated a significant group difference in the former that might reflect less predisposition in children with MBS to react to social stimuli and, in general, to environmental changes. Indeed, higher resting RSA indicates greater parasympathetic activation that promotes social interaction (Stephen W. Porges & Furman, 2011). Interestingly, children who exhibited high resting RSA have been shown to exhibit greater empathic concern or helping (Eisenberg et al., 1995). By contrast, low resting RSA is considered a risk factor for anxiety, depression (Rottenberg et al., 2007), trait hostility and autism (Neuhaus et al., 2014) and, more generally, can be considered a physiological response to environments that are perceived to be threatening. We also found a significant relation between resting RSA and RSA reactivity during the observation of neutral stimuli in the control group, whereas MBS group children seemed not to modulate their autonomic responses during this condition with respect to baseline level. In other words, in control participants only, the higher the RSA value at baseline, the stronger the RSA reactivity (i.e., RSA suppression) during the visualization of neutral facial expressions, a result that suggests these children recognized the neutral facial expressions as non-emotional stimuli, and consequently modulated their ANS responses accordingly.

Findings from this second experiment also indicate that, compared to the control group, MBS is associated with both lower resting RSA and more dysfunctional RSA reactivity across conditions. It is interesting to consider that deficits in emotion regulation are common to other psychiatric conditions [62–63], especially autism. Specifically, children with autism spectrum disorders have been shown to be slower in emotion recognition (Bal et al., 2010), and to have lower amplitude RSA (Guy et al., 2014). These findings emphasize the role of ANS indexes in emotion regulation capabilities and suggest that abnormal ANS responses could be the basis of reduced social skills in these children. While further data are clearly necessary in order to investigate such a possible link, it is nevertheless interesting to note that some studies indicate that these children show deficits in social interaction and self-regulation in social contexts.

The results of this study are consistent with simulation and embodiment theories of emotions (Niedenthal et al., 2010; Wood et al., 2016b). Thus, the simulation of other people's facial configuration is held to trigger matched motor programs and their associated affective states, allowing emotion recognition (Vittorio Gallese, 2003; Keysers & Gazzola, 2006). Accordingly, when the facial feedback is not available (as in the case of MBS), the response of the ASN is reduced (De Stefani, Nicolini, et al. 2019). We suggest that, without the benefit of the capacity for facial mimicry, the identification of changes in an emotional face could instead arise from a stored representation of the visual perception of the dynamic movements of the face and the memorized characteristics of the corresponding emotion, which have been learned through associative processes (i.e. in the case of a happy face, the general configuration of smiles can be identified around the corner of the lips with exposure of the teeth). This could lead children with MBS to search, at cognitive level, for those specific characteristics that somehow affect the autonomic responses associated with the processing of others' emotion. Thus, in addition to supporting the activation of shared facial motor programs, facial mimicry may contribute to the processing of visceromotor responses typically associated with the recognition of emotion (De Stefani, Nicolini, et al. 2019).

Finally, in experiment 2 we observed a significant group difference in the responses at the end of each emotional block. Although such assessment was part of a control task, children with MBS nevertheless showed some difficulty in labelling the emotion just observed. These results suggest some interesting possibilities, especially in relation to the results that emerged from the first study. Thus, in Experiment 1 we showed that children with MBS were able to recognize facial expressions presented one at a time as fast as the control group. They were also accurate in labelling each facial expression (92%), despite the fact that the level of their performance was lower than that of the control group (97%). Consequently, in Experiment 2 we expected that children with MBS would not show any difficulty in reporting what emotion they had seen, especially in view of the fact that stimuli representing the same emotion were presented several times in the course of the task (one minute of the same facial expression was presented in video sequences, each lasting 4 seconds). Instead, children with MBS showed lower levels of accuracy than controls. This highlights possible difficulties on the part of these patients in retaining information relating to the emotional aspects of facial configurations observed in the video. Interestingly, a recent study (Sessa et al., 2018) showed that in healthy subjects, in whom facial mimicry was experimentally blocked, there was an impairment of the visual working memory mechanism for facial expressions. Although our results support the hypothesis of a link between facial mimicry, ANS activity and the facial recognition process, we cannot yet specify whether the link is mediated by sensorimotor mechanisms involved in the simulation process, which are somehow impaired in MBS children, or by a purely visual memory system, or by an interaction between the two.

2.2.4 Conclusion

Our results suggest that children with MBS have a less responsive parasympathetic system during observation of social stimuli compared to the control group. We suggest that the lack of motor simulation caused by peripheral facial paralysis had an impact on the ANS reactivity of these children, implying an altered capacity for processing emotional stimuli.

The link between motor simulation and ANS reactivity is supported by previous neuroimaging studies. These have demonstrated how both the production and the observation of an emotional facial expression activate not only specific motor and premotor cortical regions, but also brain areas directly involved in both visceromotor responses and the processing of the emotional valence of stimuli, such as the anterior insula, the anterior cingulate cortex and the amygdala (Carr et al., 2003; van der Gaag et al., 2007; Wicker et al., 2003). The recruitment of both cortical motor and subcortical structures while observing others' social behavior (Ferrari et al., 2003; Moore et al., 2012; van der Gaag et al., 2007) is thought to implement a mapping of the visual representation of an action or gesture to its corresponding motor representation (Ferrari et al., 2009, 2017; Rizzolatti et al., 2014). Such sensorimotor mapping likely plays a fundamental role in the recognition of others' behaviors and emotions, at a somatomotor level, as well as at the level of bodily changes (e.g. piloerection, heart rate changes, vasoconstriction) which are typically associated with emotional responses during first person experiences. The capacity to share the inner aspects of emotions is the key to activating empathic responses and, in general, it is a necessary mechanism in the everyday regulation of social interactions (De Stefani et al., 2016; De Stefani, Innocenti, Secchi, et al., 2013; de Waal & Preston, 2017; Mermillod et al., 2017; Niedenthal et al., 2010; Wood et al., 2016). Consequently, the absence of the capacity for facial mimicry (as in the case of individuals with MBS) may impair not only facial expression recognition, but also related autonomic and somatic responses (Anon n.d.; De Stefani, Nicolini, et al. 2019; Wood et al. 2016b).

Our findings have important implications for our understanding of the emergence and development of emotional communication in infants and children. Considering that MBS is a congenital neurological condition present from birth, it is likely that the mild deficits both in emotion recognition and in ANS responses to emotion observation could also affect early social interactions between the infant and their caregivers. Thus, many studies have demonstrated the importance of the quality of the parent-child relationship in children's emotion regulation capabilities (Bariola et al., 2011; Halligan et al., 2013) and how, after birth, infant social expressiveness is accompanied by a highly organized, specific set of parental behaviours. Parents respond highly selectively to infant social cues by mirroring them and positively marking their occurrence with salient signals (e.g., smiles, eyebrow flashes) (Murray et al., 2016c). It has been also shown that such early interactions are critical for emotional attunement and self-regulation, as well as for the increase in social expressions in later development (Ferrari et al., 2013; Murray et al., 2016; Rayson et al., 2017; Tramacere et al., 2017). Other studies show that when infant social signals are perturbed by anatomical anomalies, such as cleft-lip, mothers tend to diminish their mirroring responses to infant social expressions, thereby impacting the development of infant social expressiveness (Murray et al., 2008). Thus, the biological condition of impaired facial motor activity and its impact on early social interactions might both contribute to the social deficits of Moebius patients described in several studies (Bogart et al., 2012; Strobel & Renner, 2016).

Because of the rarity of the syndrome, we could only include a small number of participants, and this precludes generalization of our results. For future studies, the research question should be addressed in a larger sample. Nevertheless, these data highlight the importance of studying the autonomic responses of children with MBS in different social contexts, where their decreased autonomic activation in response to the observation of others' facial expressions could, at least in part, account for some of the difficulties of these children during social interactions.

List of abbreviations:

Moebius syndrome (MBS); Respiratory Sinus Arrhythmia (RSA); Mirror Neuron System (MNS); Autonomic Nervous System (ANS); Functional infrared thermal imaging (fITI); Electrocardiography (ECG); Response time (RTs); Accuracy rate (RACC); MBS group (MBS); Control group (CG); Region of interest (ROIs);

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2.3 STUDY 2 - ACTION-OBSERVATION THERAPY AND HAND-MOUTH SYNERGISTIC ACTIVITY FOR FACIAL REANIMATION AFTER SMILE SURGERY: THE FIT-SAT NEUROREHABILITATION PROTOCOL

2.3.1 Background

As previously mentioned (see chapter 1.3), Moebius syndrome (MBS) more dramatic clinical deficit is the impossibility to produce mimic facial expressions, due to the bilateral non-progressive congenital weakness of the facial (VII cranial) nerve. To date, the only available treatment to partially overcome this difficulty is facial animation surgical procedures. In particular, based on the kind of facial palsy and on its evolution over time, patients may require a muscle transfer (free functional muscle transfer, FFMT) (Bianchi et al., 2013) procedure named *smile surgery*. This surgical intervention, as already described in chapter 1.3, is aimed at achieving symmetry at rest and during smile production and also at increasing oral and lower face movement modulation abilities (Bianchi et al., 2010). For patients with bilateral paralysis such as MBS, FFMT is considered as the standard procedure aimed at restoring facial animation (Zuker et al., 2000). Post-surgery rehabilitation requires a prolonged period of time, with the patient spending many months training facial movements (Kim & Byrne, 2016). With regard of rehabilitation programs, at present, considering that *smile surgery* exploits the masticatory circuitry of the masseteric innervations, clinicians have found it effective to train patients to produce muscle contractions through a teeth clenching trigger under mirror feedback (Pavese et al., 2016). This approach has been proved to be quite effective in rapidly recruiting the transplanted muscles (Murphey et al., 2018). Although, clinicians also report difficulties in dissociating the movements of muscles for chewing from those of the smiling (Lifchez et al., 2005). Long periods of rehabilitation are therefore usually required before patients learn to move facial muscles independently and to dissociate jaw closure from smiling (Manktelow et al., 2006). Moreover, some patients report poor compliance during home training because of discomfort in observing their image reflected in a mirror, probably because of the well-known negative consequences of facial palsy for self-perception (Coulson et al., 2004; Pavese et al., 2014) due to facial asymmetry and absence of facial mimicry.
The objective of the present study was to evaluate the feasibility and the effectiveness of a new rehabilitation treatment following smile surgery. In particular, it has been proposed a treatment based on two crucial properties of the sensorimotor system in order to foster the

activation of neural circuits of smile production and thus facilitating the recruitment of transplanted muscles in operated MBS patients: a) the visuomotor matching properties of the mirror neuron system (MNS)(Ferrari et al., 2017), in particular through sessions based on the principles of the Action Observation Therapy (AOT), which has been previously shown to have a clinical and rehabilitative relevance (Buccino, 2014; Buccino et al., 2006; Ertelt et al., 2007; Sgandurra et al., 2013); b) the hand-mouth cortical sensorimotor synergies (De Stefani et al., 2016; Desmurget et al., 2014; Gentilucci et al., 2001; Gentilucci, 2003; Gentilucci et al., 2012) in order to facilitate the activation of the masseteric circuitry without the actual closure of the jaw but through the clenching of the hand.

Theoretical assumptions of Facial Imitation Treatment (FIT)

Several investigations on human individuals have demonstrated the presence of Mirror Neurons activity, that is of neurons firing both when individuals execute or observe a motor act (di Pellegrino et al., 1992; V. Gallese et al., 1996) in tasks requiring observation and imitation of actions (Fabbri-Destro & Rizzolatti, 2008; Rizzolatti & Craighero, 2004; Giacomo Rizzolatti & Fogassi, 2014) and facial expressions (Carr et al., 2003; Iacoboni, 2009; Nishitani & Hari, 2000; Rizzolatti & Craighero, 2004; Tramacere et al., 2017) (Christov-Moore & Iacoboni, 2016; Ferrari, 2014; Ferrari & Rizzolatti, 2014; Wicker et al., 2003). It has also been demonstrated that the visuo-motor properties of mirror mechanisms can be exploited in neurorehabilitative treatments. For instance, in patients with motor deficits due to vascular brain injury or other neurological insults, the observation of a movement might improve movement recovery, reinforcing the activation of motor circuits which have been weakened due to the lesion (Briegel, 2012; Buccino, 2014; Small et al., 2013). This mechanism is the basis of Action-Observation Therapy, which combines exercises aimed at reducing the motor deficit with rehabilitation sessions whereby patients simultaneously observe the same exercises performed by the rehabilitator (Buccino et al., 2006).

Considering the involvement of the MNS for facial expressions, in this study we applied the principles underlying AOT to smile rehabilitation. We hypothesized that by observing an actor who is smiling, the neural circuits which control the smile in the MBS patient may facilitate the recruitment of the transplanted muscle (Figure 1a - Ferrari et al. 2017).

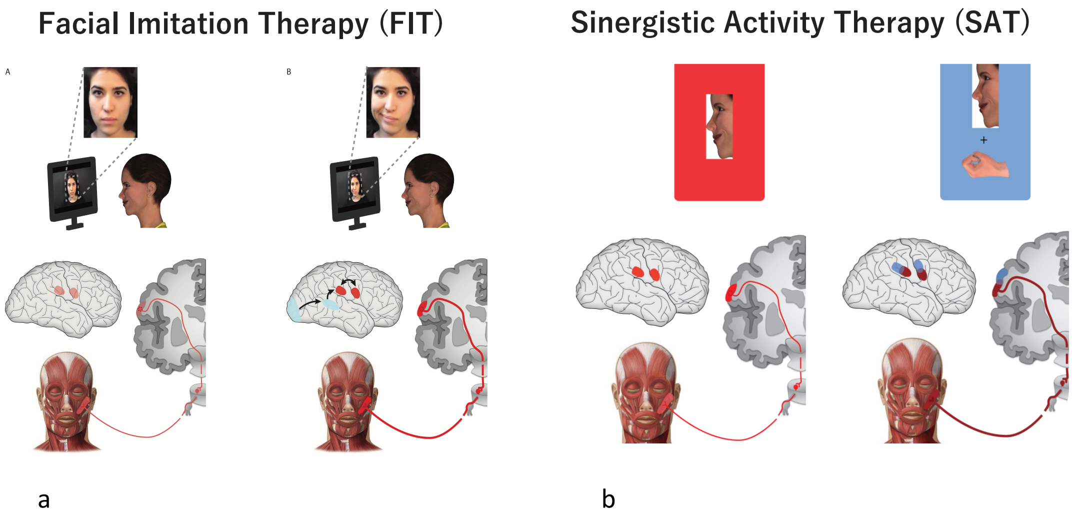


Figure 1: FIT-SAT theoretical assumptions: (a) FIT combined action observation with the direct effects of action execution suggesting that the activation of motor areas by action observation becomes reinforced by the concomitant active execution of the observed actions (Ferrari, et al., 2017); (b) the synergistic activity of hand closing while smiling should facilitate the activation of the cortical areas connected to the mouth. In other terms, we hypothesized that hand contraction would facilitate the recruitment of the Gracilis muscle as consequence of the activity of mouth motor neurons in motor cortical areas.

Synergistic Activity Treatment (SAT): theoretical assumptions

Recent investigations have challenged the classic somatotopic theories postulating the presence of distinct clusters of neuronal populations associated with specific hand muscles, fingers, or finger movements (W. Penfield & Boldrey, 1937; Wilder Penfield & Rasmussen, 1950). In fact, more recent views suggest that movements are represented in motor areas as clusters of neurons coding for different action types or goals (Graziano, 2016) involving the activation of multiple effectors. For instance, the electrical stimulation of the rostral pre-central gyrus has been demonstrated to evoke coordinated movements of the hand and mouth, and that these movements seem to be present even within the restricted repertoire of behaviors of infant primates (Graziano & Aflalo, 2007). These results are consistent with recent neuroanatomical studies of the human brain, which have shown that representations of the hand and mouth in human motor cortex are contiguous and show a high degree of overlap and functional interactions (Desmurget et al., 2014). This organization is generally believed to produce adaptive movements by optimizing neural resources associated to effectors that are jointly involved in coordinated actions. For instance, we often close our hands to grab edible objects with the aim of bringing food to the mouth. At the cortical level, the grasping movement and the mouth opening movement are represented as motor synergies for which the closure of the hand is accompanied by the opening of the mouth. These hand/mouth movements are synchronous and coordinated to maximize their efficiency. It has been demonstrated that during electrical stimulation of the sensorimotor cortex, the mouth starts to open while the closing hand moves towards the face (Desmurget et al., 2014). Furthermore, numerous kinematics studies by Gentilucci and colleagues show that the movement of the hand during grasping simultaneously affects the kinematics of the mouth during different motor tasks (De Marco et al., 2015; Gentilucci et al., 2001; Gentilucci et al., 2012). Considering hand-mouth sensorimotor synergistic activity, it has been assumed that the closing of the hand during smilie production should facilitate the activation of the cortical areas connected to the mouth via masseteric innervations, thus promoting the recruitment of the gracilis muscle independently of jaw closure (Synergistic Activity Therapy, SAT, Figure 1b - Ferrari et al. 2017).

The FIT-SAT rehabilitation protocol

The FIT-SAT treatment includes videos containing instructions and daily exercises (figure 2a) to be performed at home for up to six months. The protocol is divided into two phases. The first phase aims at increasing muscle strength with unilateral exercises avoiding teeth grinding and begins when the patient starts to recruit the transplanted muscle. This phase consists of a series of video clips of an actor performing unilateral smiles which are then imitated by the patient (figure 2b). Each video clip contains instructions concerning both the co-activation of hand closed as a fist and the specific number of repetitions that the MBS patient must perform each day. The second phase aims at synchronizing the contraction of both sides in order to obtain a harmonious movement and a natural smile. This is achieved by presenting clips of an actor smiling bilaterally and by giving instruction about the co-activation of hands.

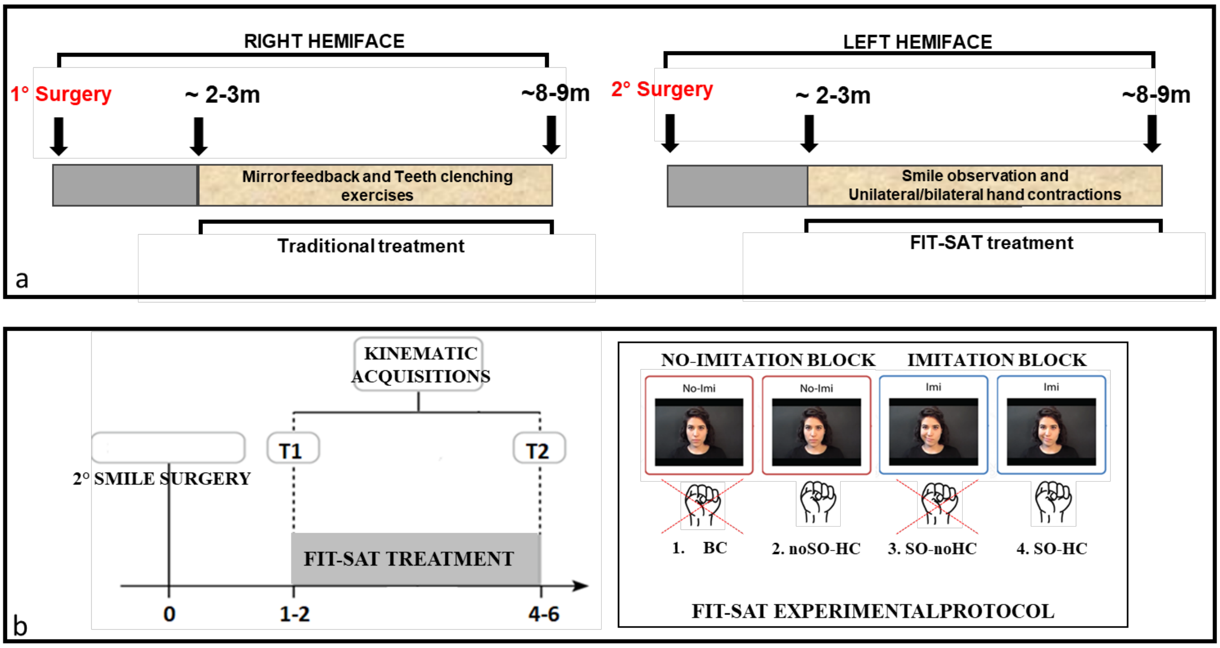


Figure 2: FIT-SAT treatment. a: The FIT-SAT treatment is performed at home for about 6 months. After the first surgery, the right side of the face is rehabilitated by teeth clenching and mirror feedback. After the second surgery, the FIT-SAT treatment begins as soon as the patient begins to recruit the muscle and is divided into two phases. In the first phase patients performed unilateral exercises while in the second phase patients performed bilateral exercises. b: Experimental condition. 1. No Smile observation and No-hand contraction (baseline condition, BC), 2. No Smile observation but hand contraction (noSO-HC), 3. Smile observation but No-hand contraction (SO-noHC), 4. Smile observation and hand contraction (SO-HC).

One of the most complex aspects of home training is ensuring that patients perform the exercises correctly. To this aim, FIT–SAT's video clips start with instructions describing the exercises, and during execution include auditory feedback in the form of an external voice that marks the timing of the observed smile to help the patient appreciate the rhythm of the smile to be performed. Thus, video clips help to sustain patient performance during home training. At each clinical assessment patients are provided with clip materials according to their clinical status.

Experimental procedures and objectives

The aim of this study was to compare the efficacy of FIT-SAT with that of the traditional treatment. In a first longitudinal investigation all patients recruited underwent a two-stage surgery procedure (FFMT), spaced at least 9-12 months apart. They rehabilitated the right side of the face with traditional treatment (Pavese et al., 2014, 2016) first and about one year later the left side with FIT-SAT. We planned two online kinematic acquisitions, one at the beginning of FIT-SAT and one at the end of treatment, to measure the three-dimensional motion of the patients' smile excursion. If the recruitment of the transplanted muscle after FIT-SAT is superior or at least comparable to that observed after traditional rehabilitation, this would be evidence that FIT-SAT is preferable as it avoids side effects associated with teeth grinding.

In a second experiment we evaluated patients' smile symmetry by an off-line processing of subjects' photos while performing gentle and maximum smile. In particular, we tested the effectiveness of the FIT-SAT in in modulating the smile by comparing patients operated through the same procedure (smile surgery) but who followed different rehabilitation treatment: FIT-SAT or traditional treatment.

2.3.2 Experiment 1 - longitudinal 3D online kinematic acquisitions: Assessing the efficacy of FIT-SAT protocol

Material and Methods

Experimental Design and Participants

A small sample, pre- and post-surgery experimental design was adopted to study the efficacy of FIT-SAT. Four bilateral patients with MBS were included. Each patient was surgically treated from 2016 to September 2018 (right and left side of the face respectively) at the Maxillo-facial surgical unit at the University of Parma Hospital. Inclusion criteria were: 1. A certified diagnosis of congenital and bilateral facial paralysis; 2. A transplanted segment of the gracilis muscle in both sides of the face, and the motor nerve to the masseter muscle used for innervation. 3. Recruitment of the right gracilis muscle subject to traditional treatment using teeth clenching. 4. Recruitment of the left gracilis muscle subject to FIT-SAT treatment. 6. Absence of congenital hands malformations; 7. Absence of any psychiatric or physical illness at the time of participation; 8. Age greater than 6 years.

All participants first underwent an operation on the right side of the face. For the rehabilitation of the right transplanted muscle they underwent traditional treatment with teeth clenching (Pavese et al., 2016). After about one year, participants underwent a second surgery on the left side of the face. The patients underwent FIT-SAT treatment(Pier Francesco Ferrari et al., 2017b) after this second surgery (table 1). Consequently, the first operated side (the right one) can be considered a “control side” as it represents activation of the gracilis muscle using traditional treatment. Written assent will be obtained after full explanation of the procedure of research, in agreement with the Declaration of Helsinki. The treatment has been approved by the Local Ethics Committee of Parma on 12nd October 2016 (Prot. 34819).

ID_num	Sex	Age	Patients classification	Type of paralisis	Type of Smile surgery	Transplanted muscle	1° Smile surgery	2° Smile surgery	FIT-SAT duration
MBS01	f	11	Bilateral Moebius	Complete bilateral paralisis	Free Muscle Transfer	Right side: gracile Left side: gracile	Right side 12-05-2015	Left side 21-01-2016	235
MBS02	f	40	Bilateral Moebius	Complete bilateral paralisis	Free Muscle Transfer	Right side: gracile Left side: gracile	Right side 03-02-2016	Left side 21-04-2017	205
MBS03	f	7	Bilateral Moebius	Complete bilateral paralisis	Free Muscle Transfer	Right side: gracile Left side: gracile	Right side 11-06-2016	Left side 31-08-2017	167
MBS04	m	8	Bilateral Moebius	Complete bilateral paralisis	Free Muscle Transfer	Right side: gracile Left side: gracile	Right side 01-07-2015	Left side 18-01-2017	147

Table 1: Patient classification: demographics and clinical characteristics of patients.

Procedure

When the left transplanted muscle innervated by the masseteric nerve gave signs of activation (approximately 2-3 months after the second surgery) the patients started FIT-SAT treatment at home and underwent the first kinematic acquisition (T1). The second kinematic acquisition (T2) occurred at the end of FIT-SAT (about 8-9 months after the second surgery) to measure the patients' progress in recruiting the transplanted muscle (figure 2a).

Kinematic data were obtained by means of an optoelectronic system for motion analysis (SMART-DX-100 system, BTS Bioengineering). This system consists of four digital infrared cameras (with a frequency of 100 HZ), which detect the 3D movement of passive markers reflecting infrared rays emitted by illuminators with a spatial accuracy of less than 0.2 mm. Two markers were applied at the corners of the mouth (right and left mouth markers, RMM and LMM respectively) and a further additional marker was placed on the nose (nose marker or reference point, RM, figure 3a). Kinematic parameters were computed from each tracked trial using a custom program developed in the RStudio 1.0.136 (https://www.rstudio.com/.).

Each kinematic acquisition consisted of 2 blocks: 1. imitation block in which an actress performed the smiles to be imitated by the patient; 2. no-imitation block in which an actress did not smiling but provided the rhythm of the smiles during patients' assessment. Each block consisted of 40 repetitions of bilateral smiles and unilateral "half smiles". After FFMT patients have active movement excursion bilaterally but they are able to move each side of their mouth independently. Thus, we asked the participants to perform a left half smile (unilateral block) to measure mouth excursion in the side rehabilitated with FIT-SAT. In both the Imitation and no-Imitation block, four experimental conditions were assessed:

(1) Smile observation and hand/s contraction (SO-HC): patients first observed a video-clip in which an actress actually executed unilateral or bilateral smiles and then they smiled while simultaneously closing their ipsilateral hand or both hands.

(2) Smile observation and no hand/s contraction (SO-noHC): patients observed/imitated unilateral or bilateral smiles maintaining their hand/s relaxed in a prone position.

(3) No smile-observation and hand contraction (noSO-HC). The actor was visible on the screen and provided an auditory feedback that marked the timing of the patients' smiles. Following the instructions of the actress on the video the patients performed unilateral or bilateral smiles while simultaneously closing their ipsilateral hand or both hands.

(4) No smile observation and no hand contraction (noSO-noHC). Patients simply performed unilateral or bilateral smiles. We refer to this condition as the baseline condition (BC, figure 2b).

Patients performed 40 left and 40 bilateral smiles (10 repetitions for each experimental condition), 80 smiles in total. Each video lasted six seconds, three seconds of instruction followed by three seconds for performing the exercise (figure 2b). Between each trial, patients could pause if they desired it.

Kinematic parameters

Bilateral smile amplitude was calculated as the maximum Euclidian distance (MMA) in millimeters between the two lip corner markers (figure 3b). This measure was expressed as a percentage of the MMA at baseline (%MMA), The MMA baseline corresponding to the Euclidian distance between the lip corner markers before movement onset (0 to 2.5 seconds, figure 3b). For all trials, the %MMA was therefore calculated as follows:

%MMA= (MMA – MMA baseline) /MMA baseline*100

In unilateral blocks, Left %MMA was the Euclidian distance in millimeters between the two lip corner markers expressed as a percentage of the MMA at baseline.

Left (or right) smile excursions (left/right SIDE) were also calculated as the Euclidian distances in millimeters between the left (right) lip corner marker and the nose marker (figure 3b). Left/right SIDE parameters were expressed as the percentage of SIDE (% left/right SIDE) with respect to the left/right SIDE baseline (the Euclidian distance between the lip corner markers measured before movement onset (0 to 2.5 seconds, figure 3a). For all trials, % left/right SIDE was therefore calculated as follows:

% left SIDE = (left SIDE – left SIDE baseline)/ left SIDE baseline *100

% right SIDE = (right SIDE – right SIDE baseline)/ right SIDE baseline *100

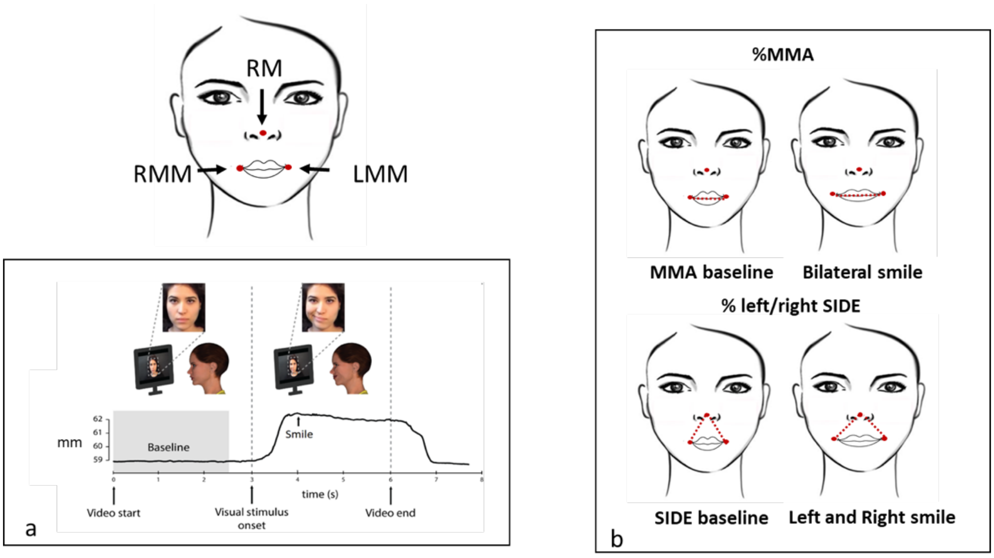


Figure 3: Kinematic parameters. a: Example of one trial; b: Three reflective passive markers (left mouth marker, LMM; right mouth marker, RMM; reference marker, RM).

We also calculated the Asymmetry Index of the bilateral Smile (AI%), which provides information to evaluate the attainment of a harmonious and natural movement. The AI was calculated with the following formula:

AI%= ((MAX_MMA – MIN_MMA) / (MAX_MMA + MIN_MMA)) * 100

A smile will be symmetrical as the value approaches 0% asymmetric as the value tends 100% (Błażkiewicz et al., 2014).

Statistical analysis

The aim of this study was to compare the efficacy of standard treatment with FIT-SAT. Right and left side of the face were operated in two phases (about one year apart). As a result, one side was rehabilitated before the other. All patients rehabilitated the right side of the face with traditional treatment(Pavese et al., 2016) first and about one year later the left side with FIT-SAT. We investigated whether at the end of FIT-SAT the patients presented:

(1) an increase in the left side of the smile (% left SIDE, rehabilitated with FIT-SAT) in comparison to that of the right side (% right SIDE, rehabilitated with the traditional treatment) between T1 and T2;

(2) an improvement in the symmetry of the bilateral smile (AI%) between T1 and T2;

(3) an increase in the excursion of the left half smile (Left %MMA) in the SO conditions (SO-HC and SO-noHC) in comparison to conditions with no smile observation-imitation (BC, noSO-noHC).

We run a linear mixed-effects model fit by maximum likelihood (LMM) to test the efficiency of the FIT-SAT treatment on the rehabilitation of the patients' smile. To select the best model for our data we used the Akaike information criterion (AIC), which offers a principled balance between goodness-of-fit and model parsimony(Symonds & Moussalli, 2011). The principal characteristic of this approach is the inclusion of random subject effects into regression models in order to account for the influence of subjects on their repeated observations. We compared random subject effects between acquisitions (T1 vs. T2) in the bilateral blocks for each kinematic parameter (% MMA, % left SIDE, % right SIDE and AI%). These random effects reflected each patient's improvement in smiling across time, and the variance of these random effects indicated the degree of variation that existed in the population of subjects.

Models of increasing complexity were compared in order to identify the best one fitting the data. We started comparing the fit of a generalized least squares (GLS) null model with fixed intercept with that of a null model with random intercept among subjects (model 1) using a maximum likelihood criterion. We then added the factor "acquisition (T1 vs. T2)" to model 1 as a fixed effect, generating model 2.

Finally, in order to test which conditions were different in unilateral excursion (% MMA), we added the factor "condition (BC, noSO-HC, SO-noHC, SO-HC)" to model 2 as a fixed effect, generating model 3. The information criteria (AIC values) together with log-likelihood statistics are reported and provide a way to assess the fit of a model based on its optimum log-likelihood value (table 2).

Data analyses were performed using RStudio 1.0.136 (https://www.rstudio.com/.) using the "Ime" function in the pack "nlme". The threshold for statistical significance was set at $p < 0.05$ for all analyses.

Results

Bilateral blocks:

On average %MMA increased at the end of FIT-SAT treatment (T2) with respect to the beginning T1 (T1=12.59 mm±0.19, T2= 14.66 mm±0.32; figure 4a) whereas %AI decreased (T1= 10.45 mm±0.52, T2= 5.59 mm±0.24; figure 4b). Similarly to %MMA, in T2 the % Left SIDE increased in the percentage of excursion in comparison to T1 (T1=0.616 mm±0.11, T2= 3.593 mm±0.24; figure 4c) whereas the average values of % Right SIDE remained almost unchanged (T1=3.544 mm±0.28, T2= 2.39mm±0.15; figure 4d).

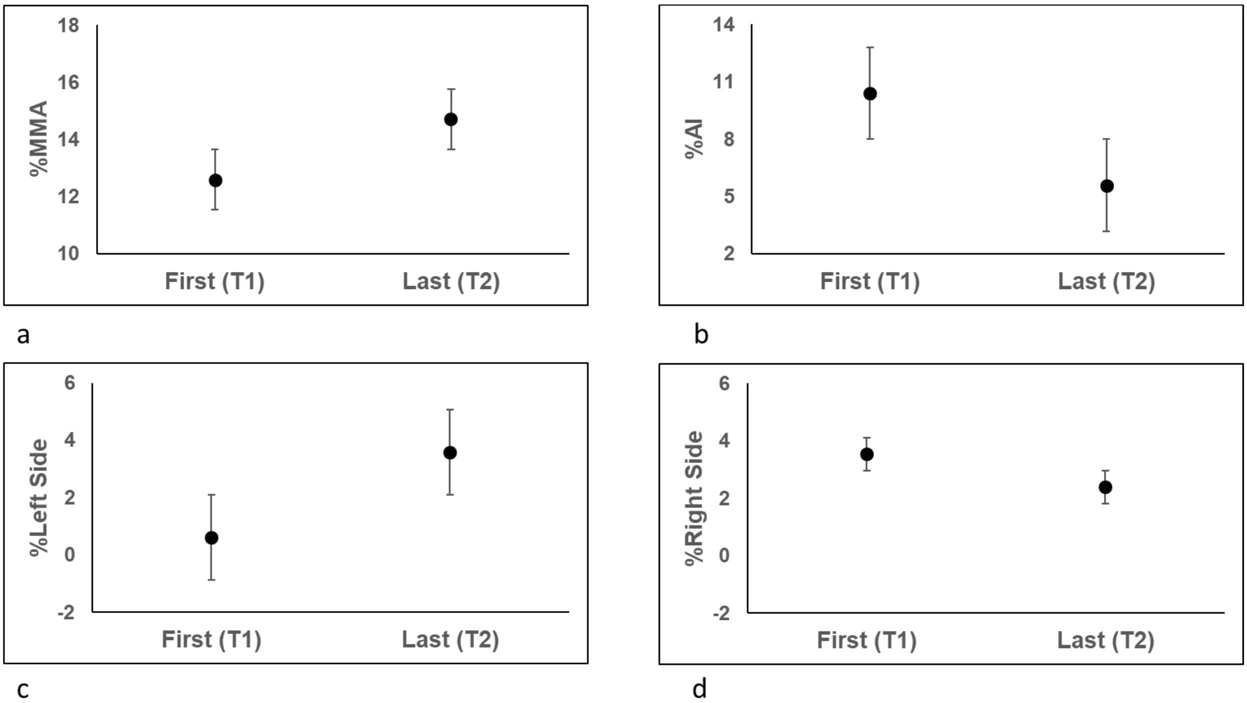


Figure 4: Results of bilateral blocks. Results in kinematic parameters (a: % MMA, b: %AI, c: % Left SIDE, d: Right SIDE). Error bars represent SE (standard errors of the means).

We run LMM with random intercepts to account for the interindividual variability and we compared models using likelihood-ratio test. We entered all kinematic parameters as the dependent variables and compared the fit of a generalized least squares (GLS) null model (m0) with fixed intercept with that of a null model with random intercept (model 1, m1). In %MMA m1 provided a superior fit than m0 (AIC_{m0}=1445.921 and AIC_{m1}=1285.435; p <.0001). We then added factor "acquisition" as a fixed effect to m1, generating model 2 (m2). The comparison between models revealed that m2 provided a better fit

(AIC_{m2}=1234.895; p <.0001, see table 2), suggesting that mouth maximal aperture increased as function of time (figure 4a).

We performed the same comparisons between models in %AI. We observed a lower AIC values in both m0 vs. m1 and m1 vs. m2 comparisons (AIC_{m0}=1595.729 and AIC_{m1}=1509.075, p <.0001; AIC_{m2}=1402.922; p <.0001, table 2). Specifically, the factor “acquisition” improves the quality of the fit compared to m0 and m1 suggesting that in T2 patients’ smiles were more symmetrical than those in T1. Thus, the best explanation for the improvement in the quality of patients’ smile was accounted by factor “acquisition” which, in turn, reflects the effect of the FIT-SAT treatment over time (figure 4b).

To analyze the effect of FIT-SAT treatment in activating the left muscle without teeth clenching, we further employed a LLM for left and right sides separately. Once again we observed that the best explanation for the improvement in the excursion of % Left SIDE was accounted by the acquisition factor (AIC_{m2}=1121.822; p <.0001). The AIC values for each comparison between models are shown in table 2.

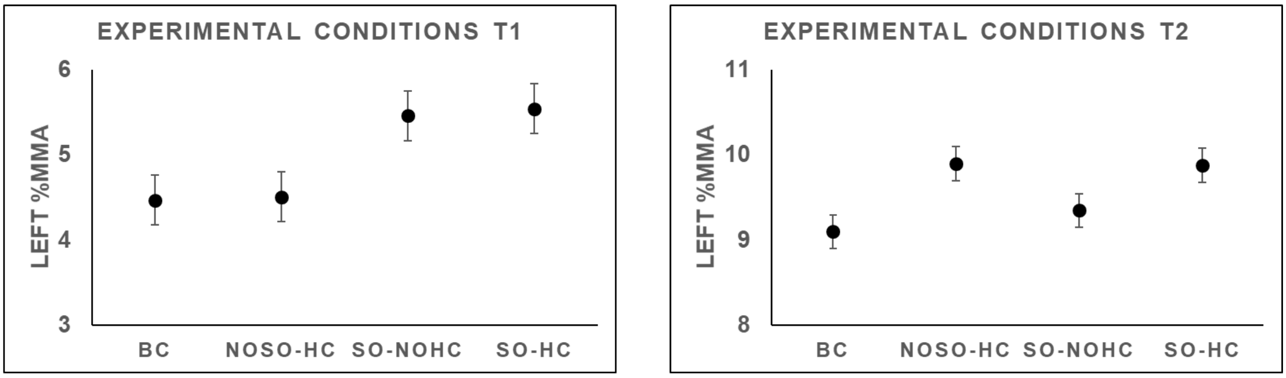
PARAMETERS	Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
%MMA	m0	2	1445.921	1453.162	-720.961			
	m1	3	1285.435	1296.296	-639.717	m0 vs m1	162.4863	<.0001
	m2	4	1234.895	1249.377	-613.448	m1 vs m2	52.53951	<.0001
%AI	m0	2	1595.729	1602.866	-795.865			
	m1	3	1509.075	1519.781	-751.538	m0 vs m1	88.65371	<.0001
	m2	4	1402.922	1417.195	-697.461	m1 vs m2	108.1539	<.0001
%Left SIDE	m0	2	1337.479	1344.727	-666.74			
	m1	3	1267.072	1277.944	-630.536	m0 vs m1	72.4075	<.0001
	m2	4	1121.822	1136.318	-556.911	m1 vs m2	147.2495	<.0001
%Right SIDE	m0	2	1330.997	1338.245	-663.499			
	m1	3	1123.332	1134.204	-558.666	m0 vs m1	209.6651	<.0001
	m2	4	1093.175	1107.671	-542.588	m1 vs m2	32.15691	<.0001
Left %MMA	m0	2	3297.119	3305.754	-1646.56			
	m1	3	3010.66	3023.612	-1502.33	m0 vs m1	288.4593	<.0001
	m2	6	2480.735	2506.638	-1234.37	m1 vs m2	535.9247	<.0001

Table 2: FIT-SAT treatment efficiency: best fit mixed-effect models. Information of the mixed-effects models used for different kinematic parameters.

Unilateral blocks:

We compared unilateral left excursion (Left %MMA) at the beginning (first acquisition) and at the end of the FIT-SAT training at home (second acquisition).

On average Left %MMA increased at the end of FIT-SAT treatment (T1=4.99 mm±0.202; T2=9.56 mm±0.214). Nevertheless, only in T1 we observed an increase in unilateral excursion between the SO-HC condition (5.55±0.40) and the other experimental conditions (HC=4.51±0.34; SO=5.46±0.40) but particularly with respect to the baseline (BC=4.47±0.44, figure 5a). To analyze the effect of FIT-SAT conditions in facilitating the unilateral left excursion we entered Left %MMA as the dependent variables and compared the fit of a generalized least squares (GLS) null model (m0) with fixed intercept with that of a null model with random intercept (m1). M1 provided a superior fit than m0 (AIC_{m0}=3297.119 and AIC_{m1}=3010.66; $p < .0001$). We then added factor "condition" as a fixed effect to m1, generating m2. The comparison between models revealed that m2 provided a better fit (AIC_{m2}=2480.735; $p < .0001$, see table 2), suggesting that FIT-SAT facilitated Left %MMA in the early phases of the rehabilitation process.



a b

Figure 5: Results of unilateral blocks. All the experimental conditions are represented in T1 (a) and T2 (b): smile-observation followed by imitation of the same smile movement and ipsilateral hand contraction (SO-HC); smile-observation followed by imitation of the same smile movement but without hand contraction (SO-noHC); no smile-observation but hand contraction (noSO-HC); no smile observation and no hand contraction (noSO-noHC, BC). Error bars represent SE (standard errors of the means).

Discussion

The aim of this experiment was to test a new neuro-rehabilitative protocol (FIT-SAT) in patients with congenital facial paralysis who underwent smile surgery procedure. FIT-SAT protocol exploits the properties of the mirror system as well as hand-mouth synergies(Desmurget et al., 2014; Graziano & Aflalo, 2007) related to the somatotopic organization of motor cortex. Results of this first experiment support the feasibility of FIT-SAT as an alternative to the traditional mirror feedback therapy. Specifically, we analyzed the excursion of the lips in four patients with bilateral paralysis. The patients rehabilitated the right side of the face with the traditional treatment involving teeth clenching (Pavese et al., 2016), whereas they rehabilitated the left side with FIT-SAT (Ferrari et al., 2017). Using 3D kinematic acquisitions, the recruitment of the left transplanted muscle was monitored by the second intervention onwards. Results showed a detectable increase in the excursion of the left unilateral smile-related movement between the first and last acquisitions, and this increase was comparable to that observed in the right unilateral movement after traditional rehabilitation. This pattern of results supports the conclusion that a hand-contraction protocol may be as effective as the traditional treatment in recruiting muscles involved in smiling. Consistent with this conclusion, we have also observed a reduction in the right unilateral movement between the first and second acquisition, suggesting that patients became better aware of the motor process involved in contracting the muscle and improved their control of the involved force.

One of the foremost goals for MBS patients undergoing post-surgical rehabilitation is to achieve a smile that is as harmonious and natural as possible. Our results indicate that FIT-SAT may be helpful in this respect as well, as we observed that smile symmetry improved between the first and last acquisition. Thus, the combined use of smile observation, smile reproduction, and contingent hand contraction resulted in a reduction of the anomalous asymmetry. In particular, a beneficial effect of contingent hand contraction was revealed by comparing unilateral left excursion in the first and second acquisitions, between the SO-HC conditions (Smile Observation and Hand Contraction) and the other experimental conditions and particularly with respect to the baseline (BC). These comparisons suggest that the contraction of the hand (SAT) associated to smile observation (FIT) was particularly effective in recruiting the transplanted muscle in the early phases of the intervention. Once the muscle has been recruited, however, the patient no longer needed to take advantage of hand contraction. This last feature of our data has promising implications for the stability of patients' improvements after termination of the rehabilitation protocol.

2.3.3 Experiment 2 – off-line smile kinematics evaluation: Assessing the efficacy of FIT-SAT in smile modulation

Materials and methods

Experimental Design and Participants

Twenty-five patients (10 male, 15 females; mean age of 20.8 ± 11.4 year) with congenital and acquired facial paralysis, bilateral or unilateral, were included in this second experiment. All patients underwent Smile surgery procedure in the Division of Maxillo-Facial Surgery, Head and Neck Department, of the University Hospital of Parma. The patients were then divided in two groups: Experimental Group (EG) composed by seven patients who completed the FIT-SAT neurorehabilitative treatment, and Control Group (CG), with eighteen patients who completed traditional treatments. Written assent was obtained after full explanation of the research procedure, in agreement with the Declaration of Helsinki. The treatment was approved by the Joint Ethics Committee of the Parma Department of Medicine and Surgery and of the Parma Hospital on 12nd October 2016 (Prot. 34819).

The experiment was designed as a prospective clinical trial using a between-subjects design.

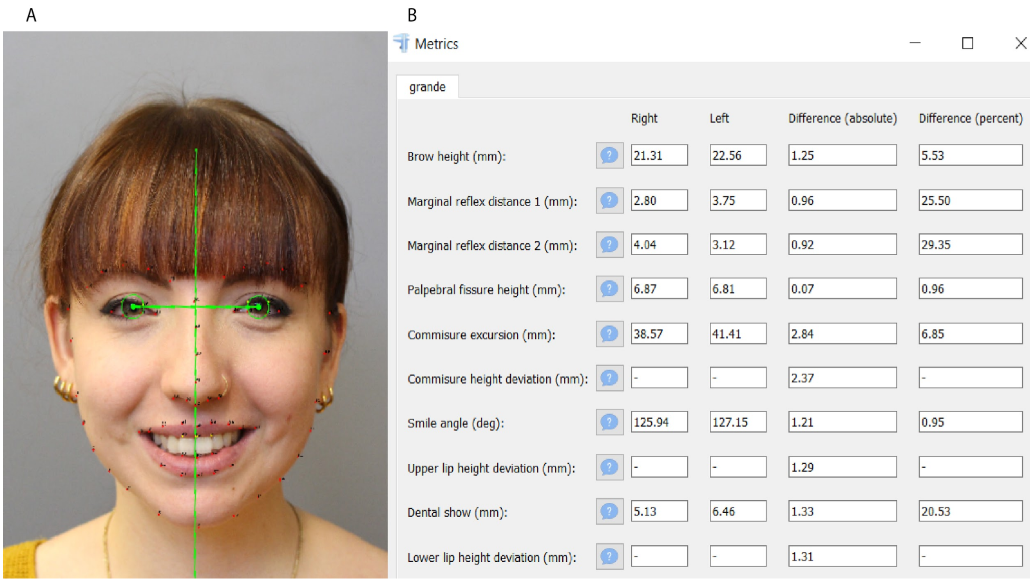


Figure 6: Graphical user interface of Emotrics. Photo of healthy subject (A) showing Big Smile condition with 68 facial landmarks (red dots), bilateral iris circumference (green circles) and facial midline (perpendicular green line). Facial measurements computed in Emotrics (B).

Procedures and Data acquisition

All participants (EG, CG) have been photographed in the following conditions: rest condition (neutral), execution of a Small Smile (SS) and Maximum Smile (MS). High definition frontal-view standardized photographs were captured with a digital camera Canon E0S 100D (18-55mm lens, 18 megapixel) at a distance of 60 cm from the face of the participants.

Data processing of the photos was carried out using Emotrics (Fig 6), an open source software based on a machine learning algorithm that calculates a full set of measurements relevant to quantify facial symmetry (Guarin et al., 2018). In particular, the software created 68 facial landmarks in specific point of the photographed face, afterwards checked and corrected by an operator (Fig. 6a). Two references midline are then added by the software: horizontal midline is defined as the linear interpupillary distance vertical midline was defined as the perpendicular line through the midpoint of the horizontal interpupillary distance.

Patients' photographs analyses focused on the rest position, as well as the small and maximum smiles (Fig. 7).

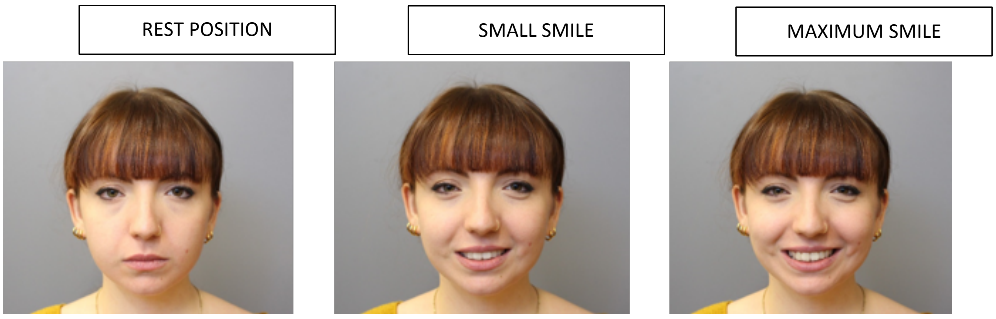


Figure 7

Smile Symmetry parameters evaluated

Based on the landmarks previously described, Emotrics than processes specific parameters allowing the evaluation of mouth symmetry: commissure excursion (CE), commissure height deviation (CHD), smile angle (SA), upper and lower lip height deviation (ULHD; LLHD).

We considered two measurements: Commissure Excursion (CE), that is the distance from the midline of the vertical/lower lip vermilion junction to the lips angle commissure, and

Commissure Height Deviation CHD, namely the vertical distance between the projection of the left and right lips commissure on the vertical facial midline (Fig. 8).

In particular, we analyzed CE and CHD absolute values, that is the difference between left and right CE. Both these parameters were considered for all the different experimental conditions (rest, small and maximum smile).

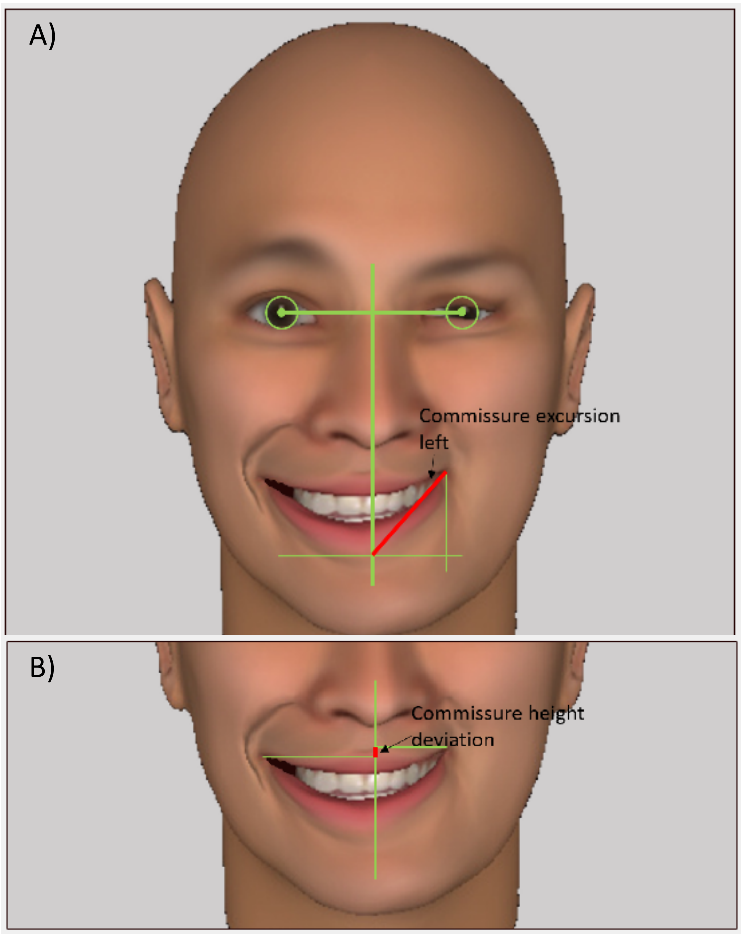


Figure 8: The Figure show the two parameters considered for smile symmetry evaluation: A) Commissure excursion; B) Commissure Height Deviation

Statistical Analysis

Statistical analyses have been conducted through the Jamovi software (version 1.1.9.0). The kinematic parameters previously explained were considered as the dependent variables of the study, while the different experimental conditions were the independent variables.

A preliminary investigation showed a non-normal distribution of the variables in most of the parameters considered (Shapiro Test, $p>0.05$).

Given the small sample size and the relevant imbalance between the groups numerosity (18 control participants versus 7 participants who finished the FIT-SAT protocol), it was therefore decided to perform a comparison between groups for all the experimental conditions (rest, SS, MS) using the Kruskal-Wallis non-parametric test, which can be applied when the aforementioned assumptions are not respected.

Results

The analysis of the photos carried out with the Emotrics software allowed to extrapolate smile metrics during the three experimental conditions (rest, small and maximum smile) of patients belonging to the experimental group (patients who followed the FIT-SAT protocol) and to the control group (patients who performed traditional rehabilitation approaches). Analyses have been focused on the rest condition (neutral face) and two dynamic expressions: the small smile, which required a controlled lips excursion (modulation effect) to produce a faint smile, and maximum smile, in which patients contracted the gracilis muscle to achieve a full-face smile.

Results about lips symmetry during resting posture revealed that Commissure excursion values during static posture (baseline) were significantly lower in the experimental group (FIT-SAT) than in control group (traditional treatment) (χ^2 3.989; df 1; $p = 0.046$) (Figure 9; EG mean = mean = 2.112 ± 1.053; CG mean = 4.883 ± 0.657).

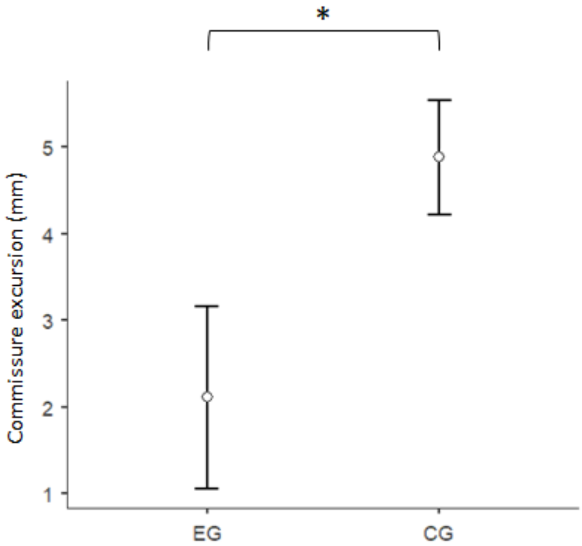


Figure 9: The figure reports the significative difference between Experimental Group (EG – patients who followed FIT-SAT protocol) and Control Group (CG – patients treated with traditional programs). Lower Commissure excursion values indicates minor difference between the two side of the mouth, that is better lips symmetry at rest (neutral facial expression).

Similar results emerged for the small smile dynamic condition, where the EG group compared to the CG reported significantly lower values for both parameters considered (Figure 10), namely Commissure Excursion (χ^2 4.234, df 1, p = 0.04; EG mean = 1.678, ± 1.667; CG mean = 6.009 ± 1.039) and Commissure Height Deviation (χ^2 4.487; df 1; p = 0.034; EG mean = 1.554 ± 0.922, CG mean = 4.082 ± 0.575).

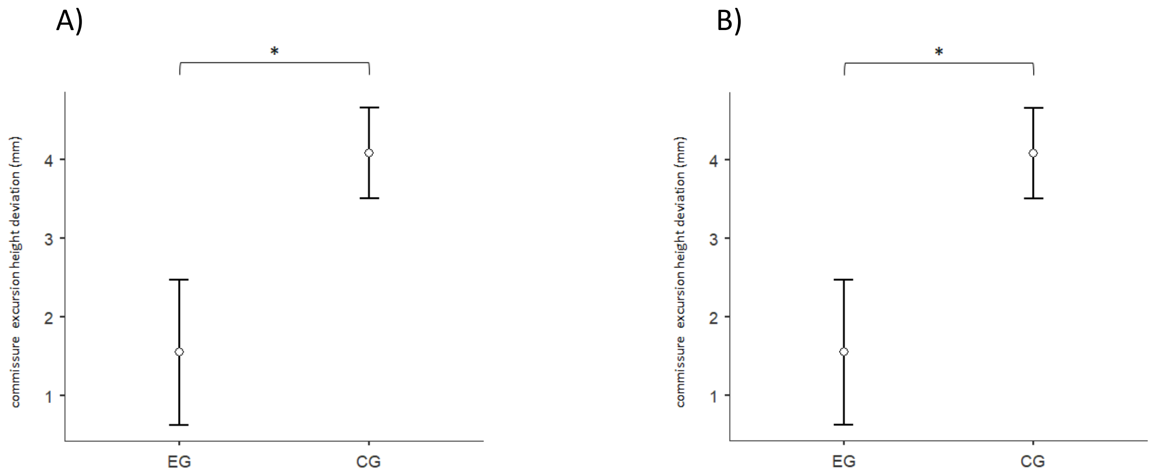


Figure 10: The figure reports the significative difference between Experimental Group (EG – patients who followed FIT-SAT protocol) and Control Group (CG – patients treated with traditional programs) for both Commissure excursion and Commissure Height Deviation absolute values. Considering that the lower CE and CHD values the minor the difference between the two side of the mouth during smile production, Experimental Group reported better symmetry values during the small smile condition with respect to control group participants.

Finally, no differences emerged during the maximum smile condition, not for the Commissure Excursion nor for Commissure Excursion Height Deviation (Table 3).

	Group	Commissure Excursion	Commissure Height Deviation
Mean	EG	2,474	2,98
	CG	4,971	4,139
Standard error	EG	0,691	0,515
	CG	0,964	0,604

Table 3: The Table shows the two groups Mean and Standard Error values of all the parameters considered for Maximum Smile condition evaluation. No significative difference emerged in this condition between CG and EG. Low CE and CHD values for indicates good smile symmetry for both groups.

Discussion

All the patients recruited in this study, both those with congenital paralysis and those with acquired paralysis, underwent smile surgery in order to increase lower facial motility to partially recover facial mimicry as well as to improve phonetics and food management skills. All the participants followed post-surgery rehabilitation program: some of them performed traditional approaches (Control Group) while others a new *mirror system*-based neurorehabilitation protocol, namely the FIT-SAT.

At the end of the rehabilitation treatment, the smile posture of the participants was evaluated at rest and during the execution of the smile using the Emotrics software. This software allows the analysis of facial posture by means of high-definition photos through the localization of facial reference points and then returning several facial metrics. Therefore, patients were photographed during a resting position (neutral face), while maintaining a controlled excursion of the lips, so as to produce a slightly hinted smile (Small Smile), and while maintaining the maximum excursion of the smile (Maximum Smile).

The purpose of this study was to evaluate the qualitative restoration of symmetry both at rest and during the execution of a small and large smile in patients who underwent different post-surgery rehabilitation programs (FIT-SAT or traditional). Two parameters about mouth symmetry were considered, namely the commissure excursion (CE) and the commissure height deviation (CHD).

Considering that the lower CE and CHD absolute values the minor the difference between the physical configuration of the two side of the mouth, these results clearly have shown the presence of a better smile symmetry of the patients who followed the FIT-SAT protocol (experimental group) compared to control group (traditional approaches) both during the resting condition (neutral face) (Fig. 16) and during the production of a gentle smile (Small Smile condition).

Better effectiveness of FIT-SAT protocol in terms of lips symmetry already during the rest condition (neutral face) could be considered of particular relevance. Given dramatic psychological impact of facial weakness, improving patients' facial physical appearance could crucially affects patient's quality of life. Moreover, FIT-SAT protocol resulted in better mouth symmetry even during the production of a gentle smile condition. This result could be discussed in terms of a better ability to control the excursion of the lips. In other words, patients who followed the FIT-SAT rehabilitation procedure compared to control group are more able to control the force of contraction of the transplanted muscle while smiling bilaterally, managing to obtain good symmetry even in the execution of faint and detailed lip movements.

The Facial Imitation Therapy (FIT), based on AOT (Action Observation Therapy), through the observation of the smile correctly performed by another individual, would allow the activation of a smile simulation mechanisms which in turn would facilitate the patients' execution of the smile. In particular, these processes would allow patients to identify and define motor boundaries and adequate smiling modalities that are clearly absent when patients observe themselves in the mirror during traditional rehabilitation approaches. Moreover, the Synergistic hand-mouth Activity Therapy, because of the previously mentioned synergistic sensorimotor organization of hand and mouth cortical representations, would have facilitated a better controlled recruitment of the transplanted muscle via masseteric circuitry activation without the actual clenching of the jaw.

In fact, teeth clenching procedure used in the traditional approach have been shown to be effective in producing a full-range extension of the transplanted muscle (Pavese et al., 2016) .Coherently in this experiment there were no difference in mouth symmetry values during the maximum-smile condition between FIT-SAT and traditional rehabilitation outcomes.

The results here discussed showed that the FIT-SAT neurorehabilitation protocol is more effective than traditional approaches in terms of better facial symmetry at rest and while performing small smiles. In other words, FIT-SAT protocol seems to facilitate a better ability in modulating the force of contraction of the transplanted muscle (s) during the execution of a smile. This ability reflects one of the main objectives of the exercises included in the FIT-SAT rehabilitation protocol, that is to increase patients' sensorimotor-awareness allowing them to implement an amount of force necessary to contract the muscles of the face in order to accurately control the extent of the excursion of the lips. This competence could then allow patients to adapt different kind of smiles based on the various social contexts. In fact, during social interactions the act of smiling indicates a disposition to relational contact and varies according to the circumstances. For example, when we meet a person for the first time, a barely hinted smile usually is a precise social signal with an adequate "welcoming" meaning. Conversely, an open-mouthed laugh can be exhibited in particularly stimulating situations or in the context of closer relationship. Therefore, the results of this second experiment is a further proof of the effectiveness of the FIT-SAT post-surgery neurorehabilitation protocol not only in facilitating the recruitment of the transplanted muscle for smile production, but also in giving the patients the possibilities to produce several degrees of smile intensity, thus adequately modulating it based on different personal communicative intentions and in relation to various social contexts.

2.3.4 Conclusion

Peripheral facial palsy, involving a lesion of cranial nerves involved in facial mimicry, is typically correlated to important functional and aesthetic deficits. Patients with congenital unilateral or bilateral facial palsy show reduced or absent expressivity, they either cannot smile (when affected bilaterally) or find it very difficult to smile (unilateral paralysis). In addition, they cannot grimace or close their eyes normally. Finally, because of the lack of strength in their lip muscles, they also have problems with chewing, swallowing, and speaking. Surgical interventions aim to reduce the symptoms and to restore a degree of facial mobility (i.e. facial reanimation (Bianchi et al., 2010)). Despite the strong negative impact of facial palsy on psychosocial functioning and quality of life (Strobel & Renner, 2016), however, current approaches to post-surgery treatment remain largely unsatisfactory. Following muscle transplant, traditional rehabilitation programs aim to activate newly formed motor circuit**s** under the control of the masseteric nerve. Thus, patients are initially encouraged to practice biting in front of a mirror (Pavese et al., 2016). However, the practice of teeth clenching, although extremely effective in recruiting the transplanted muscles (Murphey et al., 2018), leads to difficulties in separating chewing from smiling and remains divorced from mimicry processes**,** which play an important part in social interactions. As an additional problem, clinicians report poor compliance with prescriptions involving home training under mirror feedback, presumably due to the negative consequences of facial palsy for self-perception (Coulson et al., 2004).

Results of the first study indicate that hand contraction and smile observation may be as efficacious as traditional teeth clenching treatment, while bypassing patients' difficulties in working with the mirror and allowing a correct dissociation between chewing and smiling. To the best of our knowledge, this study is the first to apply an AOT based rehabilitation approach (Buccino, 2014; Buccino et al., 2006, 2011) to patients with facial paralysis who undergo smile surgery (Bianchi et al., 2010; Bianchi et al., 2009) and to integrate knowledge derived from neuroscience such as hand-mouth synergy with the clinical rehabilitation needs of these patients (De Marco et al., 2015, 2018; Michel Desmurget et al., 2014; M. Gentilucci et al., 2001; Graziano, 2016). Although this preliminary data is encouraging, further confirmation will be necessary with a greater number of patients and with experimental designs including assessments of FIT-SAT after the first muscle transplant.

To this regard, the second experiment confirmed have that FIT-SAT protocol foster better outcomes in terms of smile symmetry and also showed its effectiveness in allowing better smile modulation compared to typical treatments.

Facial paralysis has a devastating psychological impact, affecting patients' self-perception and social interactions. Therefore, the results of the second experiment are of particular relevance showing the efficacy of FIT-SAT protocol in improving facial appearance and giving to patients previously characterized by a complete facial paralysis not only the possibility to produce a better smile in terms of symmetry but also to extend the degrees of intensity of smile production in relation to different context, thus significantly improving their social skills and quality of life.

To this end, a final consideration is about the social function of smiling. The absence of a spontaneous smile is what brings most problems to patients suffering from facial paralysis since it impairs communication and social interaction (Ho et al., 2012). In these patients, smile production cannot be controlled by a sensitive nerve, which means that they must control the smile consciously. Nevertheless, some authors have reported that, over time, some MBS patients develop an ability to activate their facial muscles in social situations, especially if they underwent smile surgery at an early age (Hontanilla & Cabello, 2016; Lifchez et al., 2005a). These reports have been used to propose that greater brain plasticity in younger patients leads to the achievement of a spontaneous smile after neural reorganization of involved motor processes (Hontanilla & Cabello, 2016; Lifchez et al., 2005a). We hypothesize that FIT-SAT could favor this process. The motor and premotor cortex have been demonstrated to be part of a visuomotor coupling mechanism (i.e. the mirror neuron system (G. Rizzolatti et al., 2001)). During the observation of an action/gesture our motor system resonates with that of the model because the observer is automatically recruiting the same motor programs of the model. Motor resonance mediated by the above-mentioned sensorimotor mirror system could support basic functions such as action perception, understanding, and imitation of the observed agent (G. Buccino et al., 2001a), including mimicry which normally occurs during face-to-face interactions (Stel & Vonk, 2010). Thus, FIT-SAT may improve not only the recovery of motor function, but also the spontaneity of the smile normally occurring in everyday social situations. In fact, when the patient smiles at another person who responds with eye contact (Ferri et al., 2014; Pönkänen & Hietanen, 2012) and by smiling back, there is a powerful reinforcement both consciously and unconsciously, which likely aids the learning process as the patient can realize that the movement was indeed recognized as a smile. Such a hypothesis is supported by studies on mother-infant interactions, showing that infants tend to increase social expressiveness when their mothers mirror their facial expressions (Murray et al., 2016a). Moreover, such mother mirroring has an impact on the development of cortical motor circuits involved in facial expression perception (Rayson et al., 2017). However, future follow-up studies are needed to investigate the validity this hypothesis, that is the effectiveness of FIT-SAT protocol in promoting automatic and spontaneous smile.

To this regard, another crucial point is about timing of intervention, that, as previously mentioned, could affect smile surgery and rehabilitation treatment outcomes in terms of smile spontaneity (Lifchez et al., 2005b). A large number of researches have suggested that brain possibility to undergo anatomo-functional changes due to experience (neuroplasticity) is particularly active during early period of life (childhood and adolescence). Although several studies have already shown that cortical re-organization following interventions in patients with peripheral body impairment could occur even in adulthood, to date it is still debated if such plasticity is present even in the case of congenital peripheral body disease. These aspects will be further discussed in the last study of this doctoral thesis, aimed at investigating sensorimotor cortical reorganization in MBS patients who underwent smile surgery at different ages.

List of Abbreviations

CG: Control Group

EG: Experimental Group

MBS: Moebius syndrome

FFMT: free functional muscle transfer

FIT: Facial Imitation Treatment

SAT: Synergistic Activity Therapy

AOT: Action Observation Therapy

2.4 STUDY 3 - USE IT OR LOSE IT: ATYPICAL SOMATOTOPIC (RE)ORGANIZATION IN PATIENTS WITH CONGENITAL FACIAL PARALYSIS AFFECTED BY MOEBIUS SYNDROME. INSIGHTS FOR SENSORIMOTOR NEUROPLASTICITY PROCESS

2.4.1 Background

Several studies have shown that peripheral facial paralysis or hand amputation could cause a reduction of the related somatotopic maps in the cortex, usually with parallel invasion of the nearby hand or facial representations respectively (Clarke S et al., 1996; Giraux et al., 2001; V. S. Ramachandran & Hirstein, 1998; Rijntjes et al., 1997; Uysal et al., 2016). Moreover, regaining of typical somatotopic organisation has been reported after hand transplant in a former amputee, resulting also in peripheral perceptual improvement (Farnè et al., 2002; Giraux et al., 2001). However, if such neuroplasticity could be extended to congenital body deficits is still debated.

In this study, for the first time it has been evaluated face-hand sensorimotor representations in patients with congenital peripheral facial palsy, affected by Moebius Syndrome (MBS) (Kadakia et al., 2015), who underwent surgery to increase lower face motility at different ages. In this respect, in a single case report study, Lifchez and collegues (Lifchez et al., 2005) showed better outcomes in terms of smile production in MBS patients who underwent surgery early in life, suggesting a probable better cortical reorganisation due to more effective plastic processes. In line with this results, considering that behavioural and motor experiences could affect brain organization (Clarke S et al., 1996; Giraux et al., 2001; V. S. Ramachandran & Hirstein, 1998; Rijntjes et al., 1997; Uysal et al., 2016), the leading hypothesis of this investigation is that the congenital facial palsy could induce an atypical facial sensorimotor organisation and that early surgical intervention could result in greater cortical reorganization. In particular, aim of this study is to evaluate if early surgery could induce greater sensorimotor reorganization compared to late surgery, resulting in better face somatotopic awareness.

2.4.2 Materials and methods

Participants

In this study we involved 18 patients affected by MBS, an extremely rare congenital disorder (estimated prevalence 1 in 50.000 to 1 in 500.000 live births), characterized by uni- or bilateral paralysis of the mimic facial muscles and impaired horizontal eyes movement due to an altered formation of the facial (VII) and the abducens (VI) cranial nerves (Bianchi et al., 2009; Kadakia et al., 2015). All MBS participants underwent an operation for lower face reanimation.

For the purpose of this study, two different MBS groups were selected based on whether the surgery was performed at age below or above 11 years (early surgery - ES, and late surgery - LS) (Table 1). Based on patients' age at the moment of the test we also recruited 22 adult (mean age = 25,1 sd = 2.58) and 12 young (mean age = 12.1 sd = 2.2) control subjects. All participants gave their informed written consent after full explanation of the procedure, which is in accordance with the 1964 Declaration of Helsinki. The study was approved by the Local Ethics Committee of the University of Parma.

A) MBS - LATE SURGERY

SUBJECT ID	AGE	SEX	LATERALITY	DATE OF SURGERY	YEARS FROM SURGERY	AGE AT SURGERY
mbsLS01	61	f	RIGHT	2000	18	43
mbsLS02	18	m	BILATERAL	2015	3	15
mbsLS03	34	m	BILATERAL	2007-2008	10.5	23.5
mbsLS04	52	m	BILATERAL	1998	20	32
mbsLS05	25	f	LEFT	2005	13	12
mbsLS06	18	m	BILATERAL	2011-2013	6	12
mbsLS07	17	f	RIGHT	2015	3	14
mbsLS08	22	m	LEFT	2012	6	16

B) MBS - EARLY SURGERY

SUBJECT ID	AGE	SEX	LATERALITY	DATE OF SURGERY	YEARS FROM SURGERY	AGE AT SURGERY
mbsES01	8	f	BILATERAL	2016; 2017	1.5	6.5
mbsES02	13	f	BILATERAL	2010; 2011	7.5	5.5
mbsES03	13	m	LEFT	2015	3	10
mbsES04	12	f	LEFT	2012	6	6
mbsES05	12	f	BILATERAL	2013; 2014	4.5	7.5
mbsES06	15	f	BILATERAL	2009; 2010	8.5	6.5
mbsES07	7	f	RIGHT	2017	1	6
mbsES08	12	m	BILATERAL	2013; 2014	4.5	7.5
mbsES09	15	f	RIGHT	2009	9	6
mbsES10	21	f	BILATERAL	2003 - 2004	14.5	6.5

Table 1: A)MBS Late Surgery Group (LS), operated after 11 years (mean age at surgery: 20.9 sd = ± 11.2; mean age at the moment of the test = 30.8 sd= ± 16.9) and b) MBS Early Surgery Group (ES), operated before 11 years (mean age at surgery = 6.8, sd= ± 1.2; mean age at the moment of the test = 12.8, sd= ± 3.8). All MBS patients underwent facial surgery to increase their lower face motility. Most of them (10 ES and 7 LS) underwent the *smile surgery* procedure (Bernardo Bianchi et al., 2009d; R. Zuker et al., 2000). This procedure involves the transplant of a leg muscle (i.e. *gracilis*) on the face, then connected to the corner of the mouth and activated through the masseteric innervations. This allows MBS patients to produce a smile by exploiting the preserved chewing neural circuitry(Bernardo Bianchi et al., 2009d). Considering the complexity of this surgical procedure, based on thin nerves interconnection and regrowth, this intervention is suitable for individual of at least almost 6 years of age(Bernardo Bianchi et al., 2009d).

Experimental procedure

In order to evaluate participants' facial and hand tactile sensitivity we performed standardized testing procedure using a clinical tool for sensitivity evaluation analogous to the Semmes-Weinstein monofilaments (Semmes et al., 1960; Weinstein, 1993). This tactile testing procedure is a non-invasive assessment often used in neurologic clinical practice for peripheral sensitivity evaluation. Originally developed in 1960 (Semmes J., Weinstein S., et al., 1960), it consists in a set of twenty nylon monofilaments of different standardized weight from 0.008 g to 300 g, which means different amount of force needed to fold the filament. The assessment consists in applying the monofilament to the test site perpendicularly until it bends for about one second. Patients are instructed to keep their eyes closed and to say "yes" each time they sense the monofilament touch. Initially used to measure sensory loss in the hand of patients with brain injury, currently the test is used by clinicians also to detect peripheral neuropathy (e.g to identify patients at high risk for ulceration or amputation or to assess feet sensation of patients with diabetes mellitus) (Feng et al. 2009). Considering the standardized weight of the monofilaments and the validated assessment procedure, this test is suitable also to perform specific experimental task evaluating the participant sensitivity accuracy and threshold for a specific body part.

Using Semmes-Weinstein monofilaments tool-kit we then performed an experimental procedure based on a previous study conducted by Farné and colleagues on a former amputee (Farnè et al., 2002).

Touches have been delivered to the face and the hand (Figure 1) of the subjects in two experimental conditions: Single Stimulation (SS; only one touch was delivered to the hand or to the face) and Double Simultaneous Stimulation (DSS; both face and hand touches were simultaneously delivered).

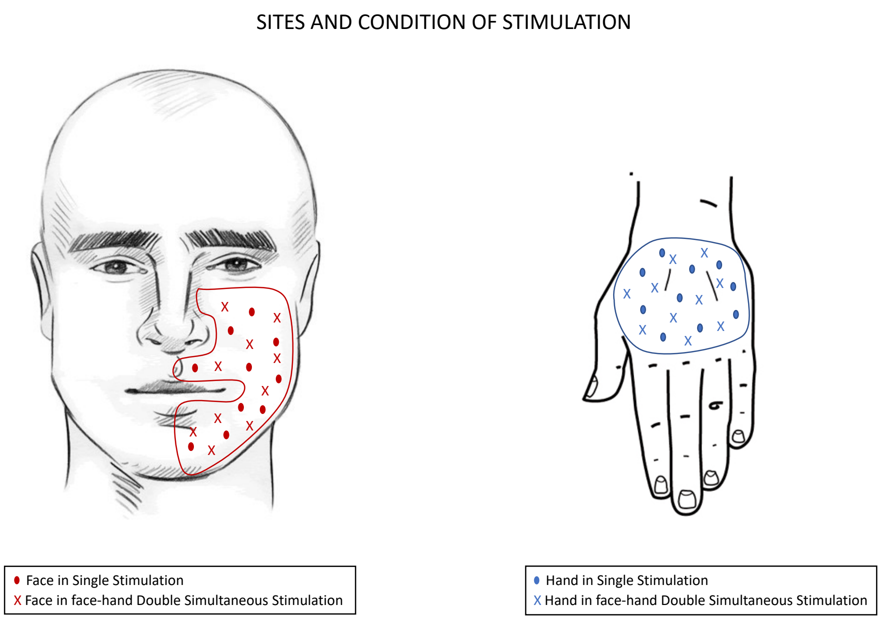


Figure 1: The figure shows the stimulated sites during the tactile testing procedure: the lower face, apart from lips, ears and nose, and the dorsum of the hand

SS and DSS were randomly administered in four blocks: two ipsilateral blocks (Right face – Right hand; Left face – Left hand) and two contralateral (Left face – Right hand; Right face – Left hand). Each block consisted of 20 trials (10 SS, 10 DSS), plus 5 catch trials in which there were no touches, in order to check any attentional bias. For adults participants, we added four further control blocks involving the stimulation of the face in DSS with the neck, that is physically close to but somatotopically far from the face (Harding-Forrester & Feldman, 2018; Wilder Penfield & Rasmussen, 1950). During the tactile stimulation procedure participants were sitting in front of two experimenters (first experimenter and assistant), palm down on a table and with the eyes closed. At the beginning of each block, the subjects were informed of the block condition (ipsilateral or contralateral) and the side of the body parts that was going to be involved (left or right). The experimenter said loud "start" before starting the trials administration by following one of the five random sequences. The chosen sequence was displayed by the assistant on a laptop screen. Each touch was delivered silently and lasted around 1 second, following the monofilaments instruction manual. The subjects were instructed in answering by reporting the body part in which they felt the touch: "hand", "face", "both" (or "face", "neck" "both" in the face-neck blocks). The assistant took notes of the answer of the subject on an excel sheet in which the sequence was reported and checked that each trial was correctly administered by the experimenter: touched delivered incorrectly were correctly repeated.

Before the experimental testing, a preliminary Monofilament Selection procedure has been performed in order to choose the right monofilament (selection criterion: accuracy >70%) for all the sites of interest. Accuracy scores lower than 70% in SS face or hand or neck conditions has been considered as an exclusion criterion: of the original sample (10 adult MBS patients, 11 young Early Surgery MBS patients, 24 adult controls and 12 young controls) 2 MBS patients operated during adulthood, one MBS patient operated during childhood and two adult controls were excluded from the study.

2.4.3 Statistical Analysis and Results

Considering how many times the subjects were able to correctly recognise the touch delivered by the experimenter, a proportion of total accuracy for hand, face and neck was calculated for both single and double simultaneous stimulation condition. In DSS each "both" answers were counted for both sites accuracy scores, while "hand" or "face" or "neck" answers were respectively separately counted. For 8 MBS patients with unilateral paralysis we took into account just the affected side for group analysis. Afterwards, the accuracy scores were transformed in the arcsen values following the formula "arcsin of the square root of the proportion of accuracy ($\arcsin\sqrt{(prop)}$)", in order to have a normal distribution of the data suitable for statistical analysis.

All statistical analyses have been conducted through Jamovi software for statistical analysis (version 1.1, 2019).

Considering the low and unbalanced numerosity of the groups, non-parametric analyses have been conducted on the arcsen transformed accuracy scores of the different experimental conditions. Within subject Friedmann test analysis revealed for both MBS and control participants no block or side effect. Therefore, we merged left and right and Ipsilateral and contralateral accuracy scores, so that we obtained an overall index of accuracy for all the stimulated sites in SS and DSS. A Kruskall-Wallis rank-based between group ANOVA test showed a significative difference just for face accuracy scores during face-hand DSS ($p=0.01$, $\varepsilon^2=0.22$) (Figure 2). Dwass-Steel-Critchlow-Fligner pairwise comparisons test following a Kruskall-Wallis rank-based between group ANOVA showed that LS MBS participants reported lower scores of face accuracy during face-hand DSS compared to controls ($p=0.016$) and ES ($p= 0.013$). Moreover, a within-subject Freedman non-parametric ANOVA test showed that face DSS scores were significantly different from hand DSS scores for both LS and adult controls: while controls showed higher facial performances compared to hand, LS participants reported an inverse trend with higher scores for the hand (Figure 2).

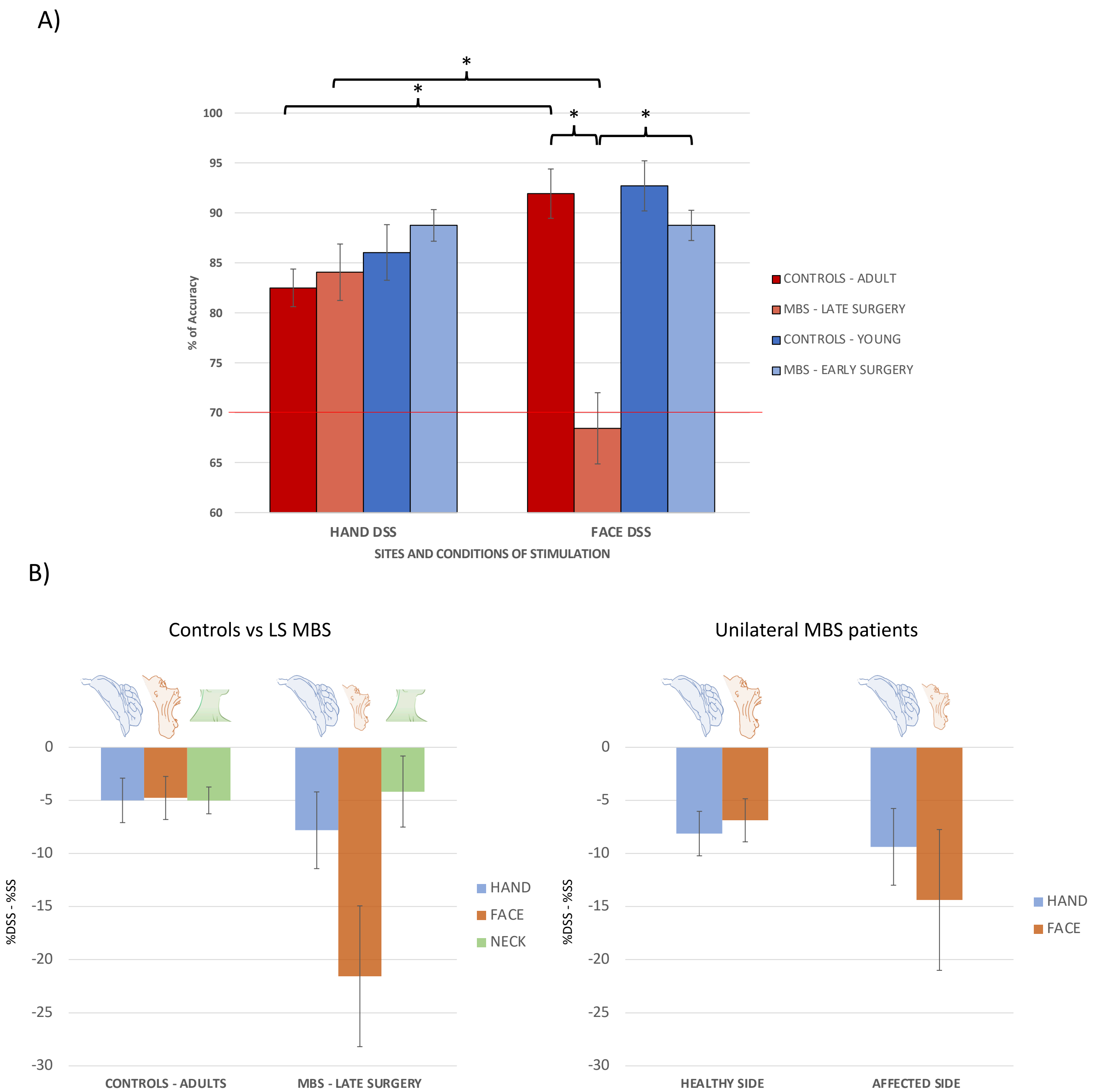


Figure 2. a) shows the group averages of the overall accuracy index of the stimulated sites during the DSS experimental conditions. The red line represents the threshold criteria required for choosing the correct monofilament for each site of interest in SS. b) This figure shows LS weaker facial performances compared to hand. More specifically, it reports the group average of LS and adult controls face, hand and neck accuracy obtained by subtracting DSS percentages scores from SS: scores approaching zero indicate high levels of accuracy, meaning that the performance in SS is qualitatively similar to the performance in DSS. It is also reported the performance of unilateral MBS patients, comparing the healthy and the affected side, with the latter being less accurate with regard to the face.

Furthermore, a non-parametric Kruskall-Wallis rank-based between group ANOVA test analysis showed no significative differences between groups for face accuracy while simultaneously stimulated with the neck (face-neck DSS) (p= 0.13), while the difference between groups was confirmed for face accuracy in face-hand DSS condition (p=0.003)(Figure 3). Moreover, a non-parametric Within subject Friedman test revealed a significative difference between face-hand and face-neck accuracy scores in MBS LS participants but not for Control subject.

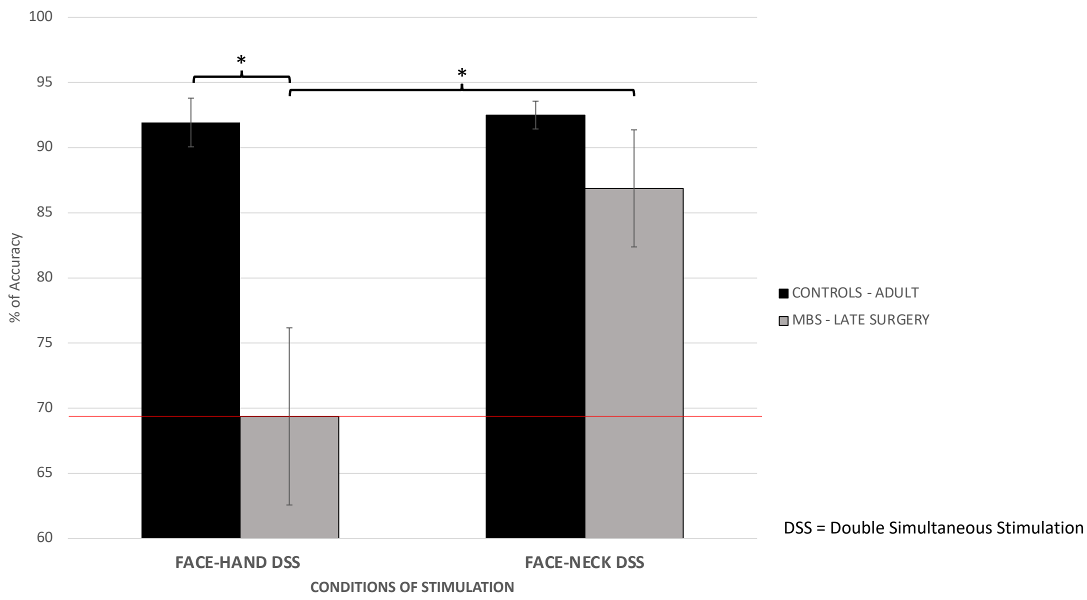


Figure 3: The figure show accuracy scores about face simultaneous stimulation with the hand or with the neck of both MBS patients operated late in life and control subjects. Red line indicates the cut-off accuracy percentage (70%) about face and hand single stimulation used as an inclusion criterion during the participants preliminary testing examination.

We than performed a signal detection analysis considering the accuracy scores of the two facial sides of the unilateral MBS participants, in order to compare healthy versus affected side. In particular using a d' value, that is a standardized index resulting from the proportion of correct answers weighted on the percentage of false positive responses, and considering that lower d' values correspond to lower accuracy, MBS participant operated later in life report worst performances for the affected side compared to the healthy one (Figure 3). Coherently with the previous results already discussed, MBS participants operated during childhood did not show the same pattern, reporting less or no difference between the two side of the face in terms of perceptive accuracy (Figure 4).

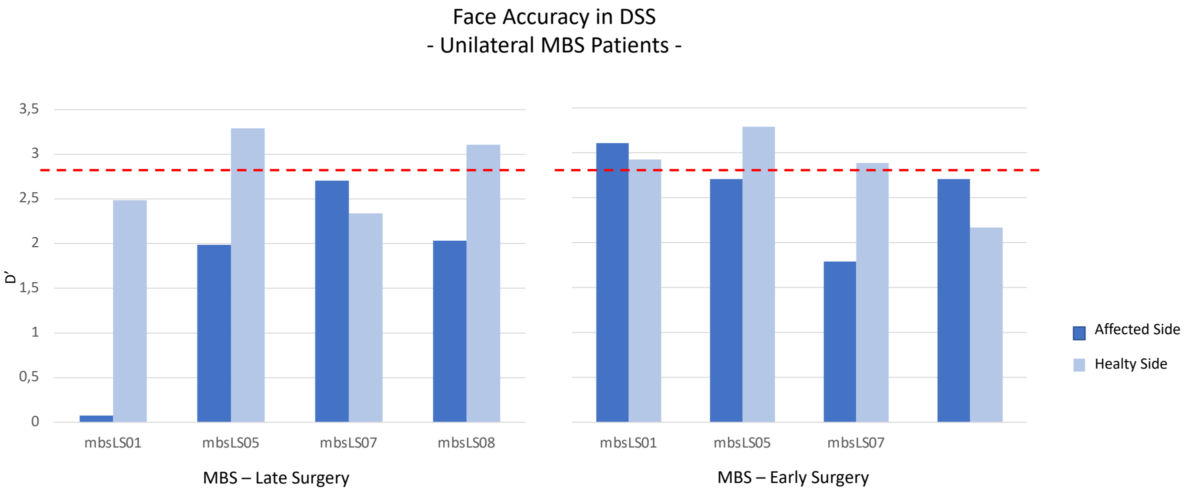


Figure 4: The figure reports d' values for oth healthy and affected facial side of late surgery and early surgery unilateral MBS participants. From a qualitative point of view, LS MBS (MBS01, MBS07 and MBS08) reported worse facial accuracy while simultaneously stimulated with the hand for the affected side compared to the healthy one and a greater difference between Affected and Healthy side D' values than Early Surgery MBS participants. Red dotted line represents the D' values average of the healthy side accuracy.

Finally, in order to test more in detail if the timing of the intervention affects MBS participants' sensorimotor functionality, we performed a correlational analysis on all MBS participants considering hand and face accuracy scores in SS and DSS in relation to "age", "age at surgery" of the participant, and "years from surgery" at the moment of the test (Table 2).

Correlation Matrix

		AGE	Y.F.S	A.A.S	FACEdss	HANDdss	FACEss	HANDss
AGE	Pearson's r	—						
	p-value	—						
Y.F.S	Pearson's r	0.850 ***	—					
	p-value	< .001	—					
A.A.S	Pearson's r	0.959 ***	0.664 **	—				
	p-value	< .001	0.003	—				
FACEdss	Pearson's r	-0.799 ***	-0.536 *	-0.842 ***	—			
	p-value	< .001	0.022	< .001	—			
HANDdss	Pearson's r	-0.463	-0.495 *	-0.389	0.282	—		
	p-value	0.053	0.037	0.111	0.257	—		
FACEss	Pearson's r	0.024	-0.201	0.143	-0.307	-0.025	—	
	p-value	0.925	0.424	0.573	0.215	0.922	—	
HANDss	Pearson's r	0.101	-0.165	0.231	-0.334	0.321	0.131	—
	p-value	0.691	0.513	0.355	0.175	0.194	0.605	—

* p < .05, ** p < .01, *** p < .001

Table 2: The table represent the correlational matrix reporting all the parameters of correlational analysis. In particular the correlation index and p value are reported about hand and face accuracy scores in SS and DSS in relation to "age", "age at surgery" (A.A.S) of the participant, and "years from surgery" (Y.F.S) at the moment of the test.

A significant negative correlation emerged between "age at surgery" and face accuracy in DSS (Pearson correlation, r=-0.82; p=<0.001 – corrected alpha value p=0.007) (Figure 5): the later the patients underwent the surgery, the less accurate they were in detecting touches delivered on the face while simultaneously stimulated on the hand. Interestingly, no significative correlation emerged between years from surgery at the moment of the test and face-DSS scores.

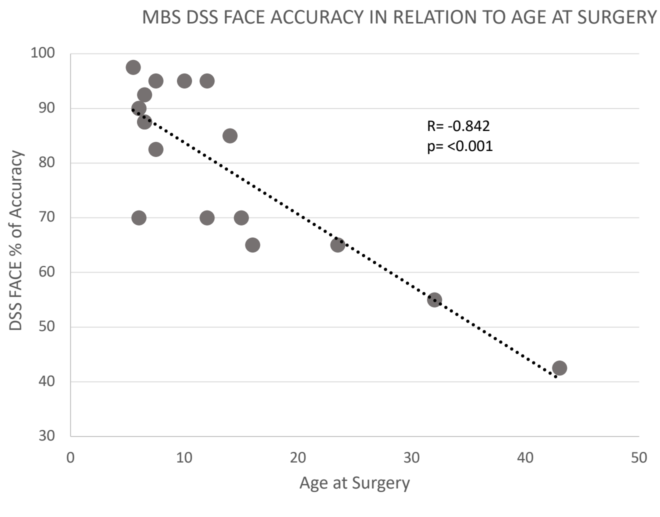


Figure 5: The figure shows the significant negative correlation between the age in which MBS patients underwent *smile surgery* and face accuracy scores in DSS with the hand.

Discussion

Results showed that MBS patients operated late after childhood (LS) reported facial extinction, that is omitting the touches delivered to the face during simultaneous hand stimulation. Interestingly, patients operated early in life (ES) reported no face extinction.

On one hand, these results suggest that congenital facial immobility could cause a shrinkage of the facial somatotopic field in favour of an over-represented hand region. Moreover, interventions in early age could benefit of higher use-dependent plastic processes (Knudsen, 2004), ending up to a sensorimotor organisation similar to that of normal population.

Considering also the absence of group difference about SS performances, these results clearly indicate that LS showed deficits that were specific and not related to a peripheral perceptual impairment. Furthermore, with regard to LS participants, no differences emerged during face-neck DSS compared to controls, suggesting that face extinction depends upon face-hand topographical relationships at a central rather than peripheral level.

Such results are compatible with the somatotopic anatomo-functional sensorimotor organization where the face and hand territory are closely represented and functionally highly interconnected (Desmurget et al., 2014; Graziano & Aflalo, 2007; Kaas et al., 2013). Challenging the classical sensorimotor topography characterized by segregated moduli for each body part first identified by Penfield(Wilder Penfield & Rasmussen, 1950), it has been shown a high level of overlap, interaction and synergies between neuronal populations coding for hand and face sensorimotor representations (Desmurget et al., 2014; Kaas et al., 2013). Thus, the co-occurrence of face-hand stimulation could then imply a perceptual rivalry because of the simultaneous activation of face-hand neuronal populations, resulting in an ambiguous signal difficult to correctly disambiguate. Coherently, also control subjects reported slight lower performances for face and hand accuracy in DSS than in SS (Figure 1 - b and see SI). The weaker facial awareness during DSS in LS MBS patients suggests that the hand inputs prevail over the face one.

In line with these results, one of the prevailing assumptions about the reorganization of cortical maps relies on synaptic plasticity characterized by the weakening of an unused brain network and the parallel strengthening of a more active adjacent one (Buonomano & Merzenich, 1998), due to horizontal cortico-cortical synaptic connections (Buonomano & Merzenich, 1998). Coherently, the face-hand "remapping hypothesis" (Vilayanur S. Ramachandran & Rogers-Ramachandran, 2000) posits that the inactivity of one of the two body parts induces an augmented functionality of the other one due to the unmasking of pre-existing silent synaptic connections between face and hand cortical regions. In fact, studies on patients who underwent limb amputations showed that the cortical hand regions were invaded by face representations (Farnè et al., 2002; Giraux et al., 2001; Vilayanur S. Ramachandran & Rogers-Ramachandran, 2000). Similarly, some studies on patients with peripheral acquired facial paralysis or face transplants reported an inverse pattern with referred face sensations while they were touched on the hand (Clarke S et al., 1996) and with cortical regions typically dedicated to the face responding to hand stimulations and hand movements (Rijntjes et al., 1997; Uysal et al., 2016).

Furthermore, our findings resonate with other studies suggesting that somatosensory maps are not static but rather dynamic since early stage of development. In fact, even if the cortical somatotopy seems to be approximately already settled precocious after birth(Desmurget et al., 2014; Graziano & Aflalo, 2007; Saby et al., 2015), several researches have proposed that since the last months of gestational development the actual sensorimotor organisation mainly depends on activity-dependent synaptic competition processes (Ferrari et al., 2013; Stoeckel et al., 2009) resulting in the pruning of the unused pathways(Buonomano & Merzenich, 1998; Holtmaat & Svoboda, 2009).

Within this theoretical framework, brain facial representations formation in MBS patients could be thought as not completely deficient due to the normal facial peripheral tactile sensitivity and partially preserved lower face motility (e.g. chewing, opening and closing of the jaw). This is consistent with our results showing high accuracy level in recognizing touches singularly delivered to the face or simultaneously with the neck. Although, LS - MBS patients' unawareness of touches delivered to the face during DSS with the hand suggests the presence of unbalanced organisation of face-hand representations in favour of the hand. Probably, due to competing process occurring during brain development, MBS patients' neuronal populations typically coding for facial or for both hand and facial inputs respond just (or mainly) to hand. Thus, the congenital facial impairment, directly involving only subcortical and peripheral structures (VII cranial nerves nuclei and branches)(Jaradeh et al., 1996; Sadeghi et al., 2020), indirectly could affect cortical sensorimotor organisation, ending up in a reduced amount of neural population involved in facial sensory and motor processing, with a parallel augmented hand region (Figure 6b).

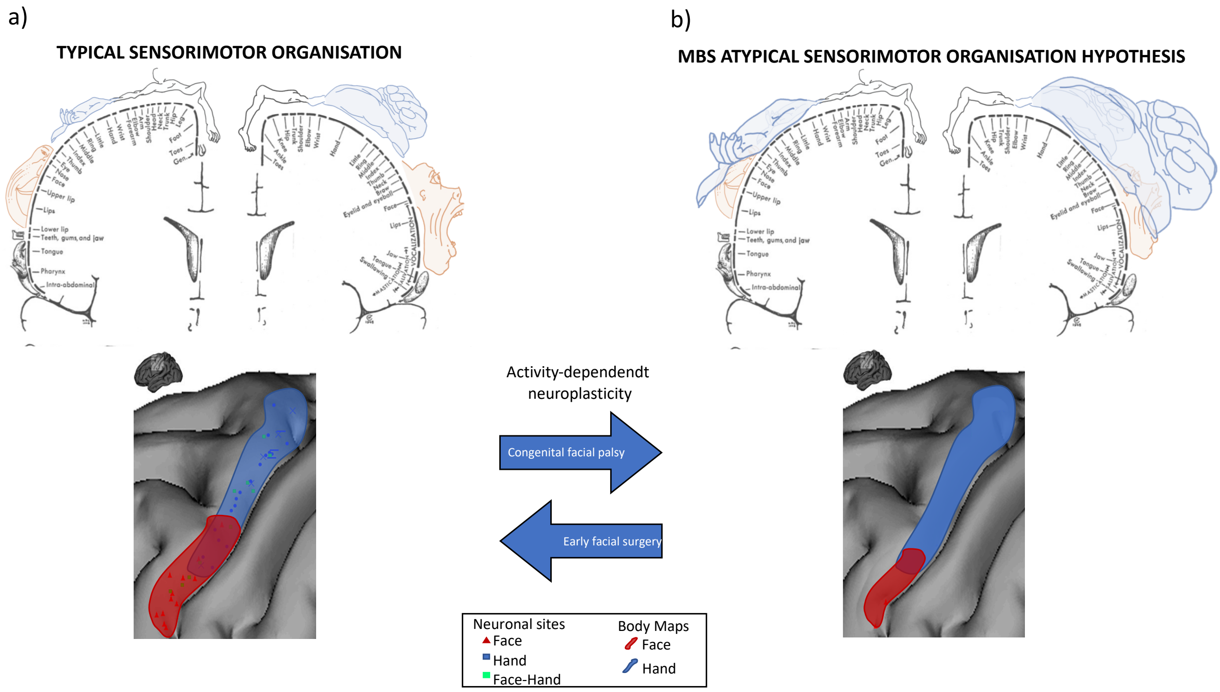


Figure 6: Adapted from Penfield (Wilder Penfield & Rasmussen, 1950) and from Desmurget(M. Desmurget et al., 2014): the left panel of the figure a) reports the typical human neuronal sites in sensorimotor cortices involved in hand, mouth/face and hand-face representations. The right panel b) represent the atypical MBS cortical organisation hypothesis, with neuronal population usually coding for face and face-hand representation mainly responding to hand because of diminished facial inputs due to congenital facial paralysis. Blue arrows indicate the activity-dependent neuroplasticity process leading to the atypical MBS sensorimotor organization and the reverse pathway leading to cortical re-organisation after early smile surgery intervention.

Another crucial point highlighted from our results is the absence of facial extinction in those patients who underwent facial surgery during childhood.

Notably, it has been shown that experience-dependent plasticity could cause not only deficient reorganization because of peripheral deficit, but also cortical remapping following interventions even in adulthood. In fact, for example, hand transplant in a former amputee or rehabilitation interventions in dystonic hand deficits led to the regaining of normal hand somatotopic representations, resulting also in peripheral perceptual improvement(Candia et al., 2005; Farnè et al., 2002; Giraux et al., 2001). Therefore, after surgery the facial cortical maps of all MBS patients were expected to regain the presumable previously lost typical territory, resulting in an increased face somatosensory sensitivity. However, our results suggest that deep facial somatotopic remapping did not occur in adults but only in those patients who underwent surgery early in life (Figure 2-a; Figure 6). In particular correlational analysis has shown that face-DSS accuracy progressively decrease as age at the moment of surgery increase. Interestingly, no significative correlation emerged between face-DSS scores and years passed from surgery at the moment of the test (Table 2). This clearly indicates that is not the amount of time the patients could produce the smile per se but the specific moment (namely, during childhood) in which they acquired this new facial movement that affect their facial somatotopic awareness.

To conclude, these results suggest that in MBS patients who underwent surgery after childhood the perceptual rivalry between face and hand induces face extinction, indicating deficient facial sensorimotor representations even following facial reanimation intervention. Conversely, the control-like performances of MBS patients who underwent surgery during childhood entails a greater cortical reorganisation probably due to more effective use-dependent neuroplasticity process (Figure 6).

2.4.4 Conclusion

Even if many studies have shown that sensorimotor cortical reorganisation could occur also in adulthood, this appears rather marginal in patients with congenital disease, as hinted by the lower facial awareness during face-hand DSS reported by MBS patients operated later in life (i.e. late adolescence/adulthood) and probably characterized by limited large-scale cortical somatotopic remodelling (Hensch & Bilimoria, 2012; Makin & Bensmaia, 2017). Further studies should investigate if more intense, standardized and environmentally enriched rehabilitation protocols could foster better outcomes in terms of smile production and brain reorganization even in patients operated during adulthood (Ferrari et al. 2017; Hensch and Bilimoria 2012).

As previously mentioned, behaviourally relevant experience shapes brain functionality especially during some specific developmental windows of time, namely sensitive periods (Knudsen, 2004). Neurodevelopmental researches have demonstrated that sensorimotor development goes on until late childhood, reaching its peak in terms of cortical thickness around 7 and 10 years of life (Shaw et al., 2008). Interestingly, in our study, MBS patients who underwent facial surgery before 11 years of age reported control-like performances in terms of facial somatotopic awareness, with no facial extinction during face-hand DSS. Therefore, giving the possibility to produce a new facial gesture during a developing period of life, in which brain and behaviours are still being organized, could entail greater changes at both synaptic and behavioural level. We could speculate that early facial reanimation interventions could thus foster the formation of new and wider facial circuits, partially exploiting the previously chewing dedicated networks for the production of a new communicative gesture with emotional value, that is smiling. Even if further studies are indeed needed in order to clarify the actual brain organization before and after surgical intervention in congenital facial palsy, the results of this study clearly indicate that early surgery could be considered crucially effective in fostering better outcomes in terms of patients' brain response to treatment.

3. GENERAL DISCUSSION

To date, facial sensorimotor representations involvement in emotion processing has been widely demonstrated (Carr et al., 2003; Ferrari et al., 2017; Ferrari et al., 2003; Van der Gaag et al., 2007). Several investigations have also investigated emotion processing in clinical conditions affected by facial amimia, in particular in patients with congenital facial paralysis due to Moebius Syndrome (MBS). MBS is an extremely rare neurological disease mainly characterized by a congenital disfunction of the VII (facial) cranial nerve and relative brainstem nuclei, responsible of mimic facial expressions. Because of the rarity of the syndrome, there are not many studies on this topic and results are far from conclusive (for a review see chapter 1.3 and De Stefani, Nicolini, Belluardo, Ferrari, 2019). In particular, it is still debated if congenital mimic facial weakness could have led to a deficient organization of the sensorimotor system, with a consequent impaired simulation mechanism during facial expression observation and emotion processing.

In line with previous results (Bogart and Matsumoto, 2010), Vannuscorps and collogues (2020) have recently suggested that sensorimotor simulation is unnecessary for emotion recognition in MBS patients, reporting that two patients out of 11 showed control-like performances in several facial expressions of emotion recognition task. Nevertheless, these conclusions seem quite drastic based only on two MBS patients reporting no deficit in facial expressions recognition. Considering normal variability among clinical populations, it is quite not surprising to find some MBS individuals reporting no or less emotion deficit than others. Moreover, these studies involved mainly adult patients presented with explicit emotion recognition task.

In contrast, other researches have clearly demonstrated the presence of mild or intense deficient performances in facial expressions and emotional stimuli recognition, especially with regard of complex and more ecological protocols (Giannini et al. 1984; Calder at al. 2000) (e.g. dynamic, morphed and or blended facial expressions), and evaluating implicit responses related to emotion processing (such as autonomic activity)(De Stefani et al. 2019; Nicolini et al. 2019).

One of the leading hypothesis of this doctoral thesis was that MBS individual's congenital impossibility to produce complex facial expressions with emotional value could have led to a hypo-functioning brain facial networks involved in emotion processing thus affecting MBS capacity to recognize accurately facial stimuli, especially during developing period of life, where compensatory strategies to process facial expressions of emotion are not yet consolidated.

This hypothesis was confirmed by the results of first study, showing MBS children emotion processing impairments, not only in terms of difficulties in facial expressions recognition, but also in terms of a weaker autonomic activity in response to dynamic facial expressions (De Stefani, Ardizzi, et al., 2019).

A crucial aspect about the mirror neuron system functionality is that individual own sensorimotor representations is exploited for a non-verbal and automatic processing of sensory information. Such mechanisms have been described in terms of motor or emotional resonance. The emotional resonance consists in a simulation process where the observation of others' facial expressions activate in the observer both cortical sensorimotor and subcortical structures involved in visceromotor responses, such as the anterior insula, the anterior cingulate cortex and the amygdala (Carr et al. 2003; Ferrari et al. 2017; van der Gaag et al. 2007; Wicker, Keysers, Plailly, J. P. Royet, et al. 2003). Face, indeed, is one of the universal and most efficient channels of communication between individuals, promoting social interactions and emotion regulation (Darwin, 1872; Ekman, 2009; Friesen & Ekman, 1978). MBS individuals impaired facial mimicry could than affect their social interactions because of difficulties both in emotion communication, thus making difficult for other individuals to understand their intentions and inner feelings, as well as in understanding emotion expressed by others.

Early motor experience and social face-to-face interactions are fundamental for the actual anatomo-functional organization of facial sensorimotor networks involved in emotion expression/recognition (Ferrari et al., 2013; Rayson et al., 2017; Vanderwert et al., 2015). Thus, the normo-typical development of the facial component of the mirror neuron system could be thought as particularly altered by MBS patients' impossibility to produce facial expressions.

To this regard, the only strategy to partially overcome MBS facial impairment is the smile surgery procedures, giving them the possibility to increase facial animacy by voluntary producing a smile via a transplanted muscle. It is worth noting, however, that the actual and spontaneous smile during social interactions is not recovered following surgical procedures. The limited recovery of facial expressiveness suggests that there are clear developmental constraints in the plastic changes that might occur after surgery. Notably, some authors have shown that peripheral body injuries or motor activity are not sufficient to induce large-scale cortical reorganization *per se* (Hensch & Bilimoria, 2012; Makin & Bensmaia, 2017).

Intense training, enriched context fostering the actual peripheral stimulation or the enactment of a new behaviour, and even pharmacological treatment seem to be crucial aspects promoting plastic process, especially during adulthood (Hensch & Bilimoria, 2012). In other words, for example in the case of surgical interventions, even if the production of a new motor act is reached, its actual usage allows to the patients only a partial recovery of the full array of facial expressions, thus limiting wide plastic reorganization. Coherently, MBS patients usually report difficulties in spontaneously smiling after surgery, usually requiring a long period of post-surgical training and practice (Bianchi et al., 2009). Moreover, traditional rehabilitative approaches, usually involving self-mirroring and jaw clenching in order to recruit the transplanted muscle connected to masseteric circuitry (Pavese et al., 2014), have reported important limitation in promoting smile production (e.g. smile/chewing dissociation or low patients' compliance).

To this end, a second crucial aspect discussed in this doctoral dissertation is related to MBS individuals' brain and behavioral functional reorganization following smile surgery.

In particular, the aim of the second study here discussed was to evaluate the effectiveness of a new mirror-system based neurorehabilitation protocol for smile production following smile surgery procedure, namely the Facial Imitation Therapy (FIT) - Synergistic Activity Therapy (SAT) protocol (Ferrari et al., 2017). This rehabilitative approach is based on Action-Observation Therapies and hand-mouth sensorimotor synergies, involving the observation of smile performed by an actor and also simultaneous clenching of the hand as a fist during smile production by the patient. Longitudinal smile kinematics investigations conducted in two experiment involving MBS patients (experiment 1) and both MBS patients and individuals with acquired peripheral facial paralysis (experiment 2) who underwent smile surgery have shown that FIT-SAT protocol is particularly effective in facilitating the activation of the transplanted muscles on the face, especially during the first post-surgical period, and then allowing the production of an harmonious and symmetric smile. Moreover, FIT-SAT protocol, compared to traditional approach, resulted more effective in promoting better modulation of the smile, that is allowing a better control of the transplanted muscle. Although further researches are needed in order to investigate the actual usage of the smile in more ecologically-relevant contexts, these findings suggest that FIT-SAT protocol could foster better outcomes in terms of smile production because of a more effective recruitment and accurate control of the neural structures responsible for smile execution.

To this regard, the last study of this thesis investigated facial sensorimotor reorganization following smile surgery procedures by means of facial somatotopic awareness. Previous studies have shown that relevant behavioral experiences during early period of development, namely *sensitive periods* (Knudsen, 2004), are fundamental in order to narrow and define the actual brain organization. Indeed, experience-dependent neuroplasticity processes are already active during gestational brain formation and then continue during childhood. Coherently, the results of this study have shown that MBS patients operated early in life reported better facial sensorimotor reorganization compared to patients operated later after childhood.

In particular, MBS individuals operated later in life have reported lower facial awareness during a face-hand simultaneous tactile sensitivity test compared to patients who performed smile surgery before 11 years of age. Although several researches have shown cortical reorganization following interventions even in adulthood, this appear rather marginal in congenital disease.

Probably, interventions following acquired peripheral body deficits could induce cortical reorganization by restoring previously well settled brain circuits. On the contrary, interventions on adult with congenital body disease could be thought as less effective because they would impact on pre-existing weak brain representations. Since the last period of gestational formation and in the following developmental stages, activity-dependent neuroplasticity process narrows and refines brain anatomo-functional organization (Knudsen, 2004) Thus, in the case of Moebius Syndrome, the impossibility to produce complex facial gestures since the first period of gestation (see chapter 1.3) could have probably led to a hypo-functioning face sensorimotor representation since birth. Furthermore, MBS patients operated during adulthood could have intrinsic limitation in brain responses to treatment because of the lack of mimic facial expressiveness for the most of their entire life.

Conversely, increasing lower face motility in young MBS patients during early sensitive stages of brain development could foster more effective synaptic and behavioural changes leading to the formation of new and wider facial circuits, partially exploiting the previously chewing dedicated networks which involves the trigeminal nerve, for the production of a new motor repertoire with communicative and emotional value, namely smiling.

Embodied simulation theories have posited that emotion recognition is based on a direct and automatic “matching” of the observed facial expression with the observer sensorimotor representation itself (Gallese & Goldman, 1998), due to the automatic activation of the facial component of the mirror neurons system. Similarly, developmental researches showing sensorimotor predisposition allowing early face-to-face mother-infant interaction implicitly entails the presence of inner facial sensorimotor representation at birth subsequently refined by the exposure to caregivers' facial expressions during development (Ferrari et al., 2013; Murray et al., 2016; Rayson et al., 2017; Tramacere et al., 2017). The augmented facial motility following early surgery could probably allow anatomo-functional cortical reorganization not only because of activity-dependent neuroplasticity processes related to smile production but also because of an augmented sensorimotor responsivity during the observation of facial expressions performed by others, thus inducing a cascade of processes leading to a dramatic somatotopic and functional reorganisation.

3.1 Final conclusions, future perspectives: how facial sensorimotor representation contribute to simulative processes and interpersonal consciousness?

As a whole, the findings discussed in this Ph.D. thesis confirmed that limitations to produce complex facial gesture since gestational development could affect the actual MBS patients facial sensorimotor organization (study 3), supporting also the hypothesis of deficient motor simulation processes as a core deficit for emotion processing in congenital facial palsy (Study 1).

Although, these findings could not be considered conclusive in order to disentangle between different facial mimicry simulative model hypotheses, namely the ones focused on the proprioceptive sensations driven by peripheral motor activity (facial feedback) or direct central simulative models based on sensorimotor mirror neuron system activity (see chapter 1.3). For example, some authors have posited that peripheral body impairment could affect subcortical structures rather than cortical sensorimotor representations (Makin & Bensmaia, 2017). Among several theoretical models, Wood and co-workers (2016) have posited that simulative processes subserving facial mimicry could be mediated by multi-hub network involving visual (extrastriate pathways for face processing), sensorimotor (premotor, primary motor and somatosensory cortices) and subcortical structures related to autonomic and visceral responses. Thus, several brain structures allow an individual to adequately elaborate the complex sensory information of an observed face as well as its emotional meaning. In particular, individual facial sensorimotor representations would be re-activated during facial expressions observation thus allowing an immediate sharing of the affective state experienced by another one (both in terms of autonomic and behavioral components). Furthermore, sensorimotor activity is thought to be modulated by visual inputs and also recursively modulate visual processing due to backward feedback projections. This multi-modal integration process could then lead to a more vivid and accurate elaboration of the observed face.

Interestingly, some authors postulated that multi-modal integration between different cortical and subcortical structures represents one of the prevailing neurobiological mechanisms of consciousness (Edelmann, 2003; Edelman et al., 2011). In other words, these scholars posit that consciousness depends on neuronal network formation based on integration processes occurring in the range of milliseconds and involving differentiated cortical and subcortical neural structures activity. Moreover, the selection of the connectivity pattern between different structures leading to the formation networks underlying conscious process depends on experience-dependent plasticity, following a principle named "neuronal darwinism": based on genetical constraints and predisposition, neurons that fire together wire together; experience, moreover, affect the strength of neuronal connections.

In line with this theoretical account, we might speculate that sensorimotor, visual and subcortical networks integration in relation to emotional processing has been defined across evolution and then refined during ontogenetic development of an individual (Ferrari et al., 2013).

Giving that conscious perception seems to rely on different brain structures multi-modal integration, it follows that the presence of an impaired functionality of one of the neuronal hubs involved in the network could affect the connectivity pattern underlying individual conscious perception processes. Within these theoretical framework, MBS individual impossibility to produce facial gestures could have led to deficient facial sensorimotor representations (study 3), also affecting the formation of typical neural networks related to socio-emotional processes.

Thus, facial sensorimotor deficient representations could probably affect individual conscious perception of an emotion experienced by himself as well as by others, thus altering a crucial aspect for social interactions that is interpersonal consciousness (defined as "awareness that one features, oneself, in another person's consciousness", Peacocke, 2014). In fact, on the one hand, the probable hypo-activation of facial sensorimotor representation could then result into the already observed diminished emotional arousal in terms of low autonomic modulation during emotion experiencing (Nicolini et al., 2019). Furthermore, deficient facial brain representations could cause an impaired sensorimotor resonance during the observation of others facial expressions. In turn, MBS facial sensorimotor hypo-functionality could affect even facial visual processing because of the recursive modulatory interaction previously mentioned between sensorimotor and visual networks (Wood et al., 2016), thus impacting on the conscious elaboration of the observed facial expression, and thus resulting in an altered emotion recognition ability and lower autonomic modulation in response to facial expressions (study 1; De Stefani et al., 2019).

Future studies are needed in order to better clarify these hypotheses, in particular about the actual impaired simulative mechanisms in MBS. In fact, it still remains partially unclear if MBS patients' impossibility to produce mimic facial gestures could preclude proprioceptive feedback signals rather than affecting cortical sensorimotor organization. Furthermore, even if their facial awareness seems to be critically affected by facial paralysis (study 3), this finding could support both peripheral and central simulative model hypothesis. Both approaches, indeed, entail a crucial role of cortical facial representation (sensitive – facial feedback hypothesis, rather than sensorimotor – embodied theories).

In order to further explore this aspect, future studies should investigate the correlation between emotion processing performances and actual sensorimotor activity in patients with congenital facial palsy. To this end, fMRI investigations could be considered an elitist approach to expand experimentally these aspects. In fact, several brain imaging studies have allowed the identification of specific brain networks and structures involved in both sensorimotor and autonomic activity related to emotion during the production and observation of emotional facial expressions. Based on the results discussed in this doctoral thesis, it is possible to hypothesize an atypical brain activity of MBS patients during facial expressions observation.

Finally, the third study here discussed (study 3) suggest that brain and behavioral changes induced by surgical interventions could occur, especially when performed during childhood, probably because of more effective activity-dependent plasticity process. Although direct evaluation of sensorimotor activity following FIT-SAT rehabilitation protocol has not been yet carried out, the results here reported about the effectiveness of FIT-SAT neurorehabilitation protocol in promoting better smile production compared to traditional treatments (study 2), suggest that intense and adequate training could foster sensorimotor cortical reorganization even in adult patients affected by congenital disease. Nevertheless, in order to further investigate this issue, it would be particularly interesting to conduct brain imaging studies aimed at evaluating MBS individual's sensorimotor activity as well as emotion processing performances before and after surgical and FIT-SAT rehabilitative interventions.

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This is (not) the end

Probably it's not for chance that I have finished this doctoral thesis reading the same pages of a book I have read at the end of my master's degree. "New eyes always allow to see something new", even looking at the same things. My passion for neuroscience as well as for psychology and ethology has increased during these years, thus helping me in going forward even when the mountain seemed too high to be climbed.

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