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Processing emotions in children with Moebius syndrome.

A behavioral and thermal imaging study

Coordinatore:
Chiar.mo Prof. Vittorio Gallese

Tutore:
Chiar.mo Prof. Pier Francesco Ferrari

Dottorando: Ylenia Nicolini

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To my family.

Abstract

According to the “facial feedback hypothesis”, the proprioceptive feedback from facial muscles while mimicking face movements is crucial in regulating emotional experience. Facial mimicry, intended as a spontaneous reaction of individuals when observing emotional faces, plays a key role in understanding others’ facial expression of emotions. Studies on facial expression processing and emotion understanding have revealed that the neuronal bases of facial mimicry are underpinned by a mirror mechanism, named “mirror neuron system” (MNS), which is active during both observation and imitation of facial expressions. The emotion recognition process is therefore based on the activation in the observer of a neural motor representation similar to that present in the observed one. In addition to this, a tight connection between motor and autonomic responses has been proposed. Neuroimaging studies, in fact, showed that the observation and first-person experience of an emotional state involve a more widespread network composed of the anterior insula, the anterior cingulate cortex and the amygdala, whose functions are strictly associated with the processing of the emotional valence of stimuli and to visceromotor responses to emotions.

Individuals with deficits in the motor programs involved in expressing emotions could therefore be impaired in the face-based recognition process or, according to simulation theories, in responding to emotional stimuli through the activation of similar somatomotor and visceromotor responses. In this regard, Moebius syndrome (MBS) patients represent an optimal model to test this hypothesis. MBS is a rare congenital neurological disorder affecting the VI and VII cranial nerves, resulting in facial paralysis. MBS patients are therefore unable to perform any sort of facial movement, or, depending on the severity of the damage, have extensive deficits in the motor control of facial muscles.

The aim of this doctoral thesis was to investigate emotional processing in MBS children.

Physiological measurements were performed by means of a functional infrared thermal imaging (fIRT), a dynamic, contactless, non-invasive method that allows to map bodies' skin temperature distribution in order to quantify autonomic nervous system (ANS) responses to emotional stimuli.

The first study highlighted that MBS children (mean age = 5.5) display lower scores of emotion recognition and exhibit a weaker thermal response to emotional (video cartoon) stimuli than healthy children of comparable age.

The second study showed that children with MBS (mean age = 9.0) do not show differences in response times on emotion recognition, however they perform a higher number of errors in labelling the facial expressions than age and gender matched controls.

The third study confirmed that MBS children (mean age = 8.7) have difficulties in categorizing emotions presented as dynamic facial expressions, and revealed that, unlike controls, they are characterized by a lower thermal response to emotional faces than to neutral faces.

Overall, these findings support embodied simulation theories, according to which the motor control of the facial musculature and the facial peripheral feedback are critical for emotional expression recognition. In addition, impairments of facial movements can attenuate the intensity of emotional experience both when stimuli represent a more general emotional context, as the one depicted by animated video cartoons, and when a more specific type of stimuli (i.e. faces displaying different emotions) is presented. The differences in thermal responses between MBS patients and controls are probably ascribed to a differential and diminished activation of the autonomic responses associated to emotions.

Contents

1 Introduction

"Blushing is the most peculiar and most
human of all expressions."

Charles Darwin

1.1 The facial expression of emotions

The scientific study of the facial expression of emotions received remarkable contribution when Charles Darwin first published his work on *The Expression of Emotions in Man and Animals* in 1872. In his treatise on the expression of emotion, Darwin examined the facial expression of animals, in particular of primates, aimed at discovering the origins of expressive movements in man. His assumptions about emotions were the cornerstone of his theory of evolution and suggested that emotional expressions are adaptive, biologically innate and framed in a phylogenetical continuum between different species. Specifically, Darwin first claimed that some emotions have a universal facial expression and that certain facial muscles were created so as to let humans express their feelings.

Early studies testing Darwin's ideas were inconclusive and highlighted the perspective that facial expressions were culture-specific; in other words, that every culture was characterized by its own verbal and gestural means of communication beyond its own language of facial expressions (Cole and Ekman, 1972).

Since the second half of the 20th century, convincing evidence that there are basic facial expressions of emotions was provided. The first study, which demonstrated a link between certain facial expressions and emotional states was conducted by Tomkins (Tomkins and McCarter, 1964). Subsequently, after long debates among psychologists (Scherer and Wallbott, 1994; Mesquita and Frijda, 1992), cognitive sciences endorsed the concept that these basic expressions are universal and created by specific configurations of facial muscles (Ekman and Friesen, 1971; Ekman, 1993; Izard, 1971, 1994). In detail, a number of studies proposing emotional universality examined cross-cultural facial emotion recognition and convincingly demonstrated the universality of a set of six (Ekman and Friesen, 1971; 1994) or seven (Biehl et al., 1997) facial expressions of emotion, including anger, disgust, fear, happiness, sadness, surprise and contempt.

According to the Darwinian perspective, the ability to distinguish and spontaneously produce emotional facial expressions is biologically innate; this capacity is present in non-human primates who are able to express the same facial expressions considered universal among humans (de Waal, 2003) and even in congenitally blind individuals who can produce the same facial expressions as sighted ones do (Cole et al., 1989; Galati et al., 2003).

The ability to recognize and interpret emotions from facial expressions is a main component of the nonverbal communication system and is a critical social ability for the development and the retention of human relations. The face is a visible signal of others' social intentions and motivations, and facial expression represents a critical variable in social interplays, especially during first social exchanges. The same facial musculature that is present in human adults exists in newborn babies and is totally functional since birth (Ekman and Oster, 1979); the importance of decoding emotions from facial expressions is actually crucial from the first interactions between the mother and the infant and has important implications for emotional development.

Figure 1. Example of facial expressions of emotion in human and nonhuman primates (taken from "The expression of the emotions in man and animals" by Charles Darwin).

"What does it mean to be born a mammal, with the emotional legacy that makes me capable of caring of others, breeding with the ovaries of an ape, possessing the mind of a human being.[…]"

Sarah Blaffer Hrdy

1.2 Development of emotional expressions through mother-infant social communication

As Darwin (1872) first noticed, "The movements of expression in the face and body, whatever their origin may have been, are in themselves of much importance for our welfare. They serve as the first means of communication between the mother and her infant; she smiles approval, and thus encourages her child on the right path, or frowns disapproval" (pp. 365–366). Infants' sensitivity to facial expressions is very important early in life, as it is a fundamental feature to learn about environment. Newborns' ability to decipher emotional facial expressions is particularly crucial during their first social interactions and is believed to be essential for children's development into adulthood (Bowlby, 1958; Trevarthen, 1998; Nagy, 2006). Newborn babies are active participants within early social exchanges with their mothers and they display basic facial expressions that are similar to or the same as those of adults (Schmidt and Cohan, 2001).

During the first year of their lives, infants' basic need is to be protected and cared for by a caregiver. Their behaviors thus vary according to the context and their caregiver's behavior, and are informative about their needs and emotions (Weinberg and Tronick, 1994). This relationship between the infant and the sensitive caregiver develops through an affective preverbal communication channel, which is primarily represented by facial expressions of emotion (Bowlby, 1988; Izard, 1978; Trevarthen, 1977).

The infants' facial expressions are the first regulators of the mother-infant relationship and are essential to orient maternal responses. Weinberg (1989) highlighted that positive facial expressions co-occur when the infant is gazing at the mother, is gesturing and emitting non-distress vocalizations, while negative facial expressions occur when infants are complaining and looking away from their mothers. By producing the same messages through multiple channels, infants would increase the likelihood that the caregiver will appropriately respond to these signals (Weinberg and Tronick, 1994). Indeed, infants are sensitive to the timing and quality of maternal affective response, a process that mediates reciprocal affective relationship between mother and infant (Weinberg and Tronick, 1997). A large amount of data on neonatal perception of social stimuli demonstrated that neonates prefer human over nonhuman stimuli (Johnson et al., 1991), and that they favor mother's voice (DeCasper and Fifer, 1980) and face (Pascalis et al., 1995).

Figure 2. Face-to-face interaction between mothers and babies.

As infants develop, they coordinate facial displays with their caregivers; during these face-to-face interactions, sensitive mothers tend to imitate their children's facial expressions and gestures, promoting a social dialog that shapes infants' self-development and self-regulation (Emde, 1992; Nagy, 2006; Feldman et al., 1999). The central feature of face-to-face exchanges is maternal attunement to what the infant is expressing with an exaggerated mimicry (Papousek and Papousek, 1989); the child, for its part, looks attentively at the mother's face and couple his/her own expressive behaviors with the mother's expressions (Trevarthen, 1998). Stern (1985) defines these emotional interactions as a "reflection" or "empathic correspondence" of the caregiver toward the children's expressions of affection. Frequent face-to-face interactions within the first few months of life have profound effects on both mothers' capacity to evaluate infants' distress and on children's cognitive (Trevarthen, 1998; Striano and Rochat, 1999), social and emotional (Fonagy et al., 1991; Kochanska et al., 2002) and language development (Bruner, 1986).

During face-to-face interactions, newborns are also capable to imitate facial expressions (including expressions of fear, sadness and surprise) (Meltzoff and Moore, 1977). It has been shown that children even play an active role in initiating facial displays, thereby making the synchronized reciprocal imitation a source of dyadic exchange between mother and infant. In this scenario, infants are not just passive individuals that learn from maternal solicitations, but they actively engage and stimulate maternal responses thus demonstrating early capabilities of inter-subjective emotional communication (Trevarthen and Aitken, 2001). During this period of time, children learn to differentiate themselves from others and to acquire a social understanding of their unique emotional and behavioral features as social beings (Rochat, 2001; Rochat and Striano, 2000).

"Our civilization is still in a middle stage, scarcely beast, in that it is no longer guided by instinct, scarcely human in that it is not yet wholly guided by reason."

Theodore Dreiser

1.3 Neurobiological basis of emotions

As mentioned in the previous chapters, perception, experience and processing of emotion are vital for survival in a social environment. Over time, there has been increasing interest in the examination and understanding of the neurobiological processes underlying emotion perception. In this regard, various findings have increased knowledge of the nature of neural correlates of processes related to the identification of an emotional information and generation of emotional experiences.

James Papez (1937) was the first to propose a scheme for the central neural circuitry of emotion - now known as the "Papez circuit". Papez suggested that sensory input into the thalamus diverged into separate streams of "thought" and "feeling". The thought stream was transmitted from the thalamus to the sensory cortices, especially the cingulate region, and continued to the hippocampus, to the mammillary bodies of the hypothalamus and then back to the anterior thalamus. The feeling stream, instead, was transmitted from the thalamus directly to the mammillary bodies, allowing the generation of emotions, and then upwards to the cingulate cortex. At a later time, a more widely supported model of the brain regions involved in emotion was proposed by Paul MacLean (1949).

The scientist viewed the brain as divided in three parts; the first part is the ancient reptilian brain, which would seat primitive emotions such as fear and aggression. The second part is the "old" mammalian brain or "visceral brain", which elaborates the social emotions. Finally, the "new" mammalian brain consists mostly of the neocortex, which interconnects emotion with cognition. MacLean's idea was that emotional experiences involve the integration of sensations from the world with information from the body and proposed that such integration was the function of the visceral brain, in particular the hippocampus. Three years later he introduced the term "limbic system" for the visceral brain. Alexander and colleagues (1990) subsequently described a "limbic" circuit comprising ventral regions of the anterior cingulate gyrus, the ventromedial prefrontal cortex, the ventral striatum and the dorsomedial nucleus of the thalamus as a brain network involved in emotional processing. Afterwards, additional neural regions including amygdala and anterior insula (AI) (Calder et al., 2001) were shown as crucial for the identification of emotional stimuli. The amygdala is a small region situated within the anterior part of the temporal lobe; the insula, instead, is a part of the cerebral cortex, it is localized at the base of the lateral fissure and it shares extensive, reciprocal anatomic connections with the amygdala (Augustine, 1996). A number of studies conducted on non-human primates (Amaral, 2002) and on patients with amygdala lesions (Adolphs et al., 2002; Adolphs et al., 2005) as well as neuroimaging studies (Ghashghaei et al., 2007) highlighted the importance of the amygdala in face identification (Davis and Whalen, 2001) and emotion recognition of expressions displayed by others (Morris et al., 1998). A further role of the amygdala in response to unpleasant auditory, olfactory and gustatory stimuli (Royet et al., 2000), and in the memory for emotional information (Calder et al, 2001; McGaugh, 2004; LeDoux, 2003) was emphasized. Furthermore, a number of investigations, which focused on the role of the anterior (agranular) insula, highlighted its function in the identification of displays of disgust in others (Wright et al., 2004; Phillips et al., 1998) and its involvement in the autonomic response (Wicker et al., 2003). In detail, Critchley et al. (2003, 2004) found strong correlations between insula activation and autonomic arousal (heart rate and heart rate variability), anxiety, and visceral changes associated with facial emotional processing (Critchley et al., 2005). In fact, insula activation seems to be involved in many emotional processes, including pain perception (Gelnar et al., 1999; Peyron et al 2000), anticipation and viewing of aversive images (Phan et al., 2006; Simmons et al., 2004), the making of judgments about emotions (Gorno-Tempini et al., 2001) and positive versus negative (fear especially) emotional processing (Buchel et al 1998; Morris et al 1998). In addition to the amygdala and AI, other regions including the ventral region of the anterior cingulate gyrus and ventromedial and ventrolateral regions of the prefrontal cortex are involved in the response to emotional stimuli (Etkin et al., 2011). The ventral anterior cingulate gyrus is crucial for autonomic function and emotional behavior. A few neuroimaging studies have demonstrated activity within this structure in mood induction and during anxiety associated with the anticipation of pain (Vogt, 2005). The ventromedial prefrontal cortex and the medial region of this structure, the orbitofrontal cortex, directly connected with the amygdala, mediate decision-making behaviors (Euston et al., 2012), and are involved in the representation of the reward value of a stimulus, and this seems functional for the judgments of pleasant and unpleasant odors, flavors and tactile stimuli (Rolls, 2000). Eventually, the ventrolateral prefrontal cortex, which is located laterally to the orbitofrontal cortex, has shown to be activated during the response to emotional stimuli and during mnemonic retrieval (Petrides, 2002).

Together with the numerous investigations of the central nervous system (CNS), which suggested the involvement of multiple brain regions in emotional elaboration (for a review see Borod, 2000), a separate body of psychophysiological research has demonstrated that peripheral processes in the form of autonomic (i.e., visceral) and somatic (e.g., motor, expressive) events are also associated with emotional processes. The controversy about the possibility that peripheral changes contribute to the experience of emotion began more than a century ago when William James (1884) proposed that specific somatovisceral patterns can not only precede, but also generate the experience of emotion. After an early critique of James's position (Cannon, 1927) arguing that different autonomic patterns do not elicit different emotions, but different emotions produce different autonomic patterns, many theorists (Schachter and Singer, 1962; Damasio, 2001) have commented on the role of physiological changes controlled by the autonomic nervous system (ANS) in emotional experience. The ANS includes two main branches, the sympathetic (SNS) and parasympathetic (PNS) nervous systems, both of which innervate visceral organs, blood vessels, and glands. The effects on a target organ of SNS and PNS activation are generally antagonistic.

Until today, scientific consensus on whether there exists a relation between emotions and the organization of ANS activity has not been reached yet. In this regard, the principal debate is about the specificity of autonomic responding to basic emotions: happiness, surprise, fear, anger, disgust and sadness. On the one hand, Feldman-Barrett (2006) sustain that it is not possible that there are kind of emotions with a unique autonomic signature, but rather that a distinction can be observed between positive and negative states. On the other hand, Stemmler (2004, 2009) argues that emotions require to differentiate autonomic activity for body protection and behavioral adjustment because they have differential goals; from his perspective, behavioral preparation needs a specific autonomic activity which is necessary before any behavior is initiated and this process should activate through ANS depending on behavioral demands. In this scenario, the existence of multiple "discrete" emotions would predict emotion-specific autonomic patterns. As an example, vascular resistance and blood flow to the hands were shown to be greater during anger than during fear (Ekman, Levenson, and Friesen, 1983; Levenson, 1992), while disgust appeared to involve both an increase in parasympathetic activation and some degree of sympathetic activation (Rozin, Haidt, and McCauley, 1999). A final intermediate position is sustained by Cacioppo and colleagues (1997, 2000), who conducted meta-analyses of physiological responses to emotion; the authors support some degree of autonomic emotion specificity and a more substantial valence-specific patterning with negative emotions related to stronger ANS responses than positive emotions.

Although controversies concerning an autonomic differentiation among emotions are still burning and different studies reported mixed results, major reviews and a number of studies present in literature sustain some degree of specificity, especially when major emotion categories are taken into account (Cacioppo et al., 2000; Kreibig, 2010; Kreibig et al., 2007; Rainville et al., 2006).

Interestingly, reports of physiological responses (cardiovascular, respiratory or electrodermal measures) in basic emotions were recently summarized in a qualitative review from Kreibig (2010); more specifically, the author showed an overview of principal changes in physiological parameters by gathering together the response direction reported by the majority of studies taken into consideration (at least three).

Considering negative emotions, physiological changes to the emotion of "anger" were featured by sympathetic activation and increased respiratory activity, which was distinguished by faster breathing. To the contrary, two overlapping patterns of response were observed with the emotion of "disgust": from the one hand, disgust stimulated by contamination (e.g., pictures of dirty toilets, cockroaches, maggots, foul smells, facial expressions of expelling food) elicited both the activation of the sympathetic and parasympathetic systems and faster breathing as well; on the other hand, disgust provoked by scenes of mutilation, injuries, blood was characterized by a pattern of sympathetic cardiac deactivation and heart rate (HR) deceleration, increased electro-dermal activity, and faster breathing. Some other studies investigated physiological response to "fear", which was typically induced by the presentation of threatening pictures, film clips, real-life manipulations; overall, these studies reported cardiac acceleration accompanied by sympathetic activation, increased myocardial contractility, vasoconstriction, and increased electro-dermal activity. This kind of response could be accompanied by an increased respiratory activity featured by a decreased expiratory time, aimed at lowering carbon dioxide blood levels. The most of negative emotions involve increased activation of the sympathetic branch of the ANS (see Cacioppo et al., 2000), which facilitates physical exertion needed for "fight or flight" reaction, a physiological response occurring when a danger or a harmful event is threatening the individual. Concerning studies that focused on autonomic response to "sadness", a pattern of sympathetic-parasympathetic co-activation was observed. A few investigations, however, considered sadness accompanied by a cry-status. In these cases, a sympathetic activation was associated with crying (activating) sadness; in particular, this kind of response was featured by increased cardiovascular sympathetic control and variations in breathing rates. Non-crying (deactivating) sadness was instead characterized by sympathetic withdrawal. Similarly to all other negative emotions, a decrease in electro-dermal activity was reported with non-crying sadness.

Concerning positive emotions, physiological responses to the emotions of “happiness” and “surprise” were examined. Happiness was generally featured by increased cardiac activity, vasodilation, increased electro-dermal activity and greater respiratory activity. Particularly, responses to happiness presented a central cardiac activation, common to previously described negative emotions; vice versa, a peripheral vasodilation was observed during happiness. Any patterning of physiological response could be obtained from the analysis of studies concerning “surprise” because of the limited number of investigations on this emotion. Nevertheless, a few general observations common to both negative and positive emotions, including surprise was HR increase; additionally, an inspiratory pause and increased breathing frequency were present in surprise, as well as in anger and fear.

According to the observations previously outlined, autonomic response is a crucial component of the emotional response, thus making critical the use of multiple ANS measures and therefore of physiological parameters so as to evaluate how the organism is responding to differential kind of emotional stimuli and situations. Concerning physiological measurements, patterns of cardiac activity are most of all determined by the dynamic interaction between acceleratory SNS activation and deceleratory PNS activation (Berntson et al., 1994). The deceleratory (parasympathetic) function is provided by the vagus nerve (X cranial nerve). This cranial nerve is a primary component of the ANS, it exits the brainstem and has branches that control the striated muscles of the face and head (e.g. facial muscles, eyelids, muscles of mastication) and a number of visceral organs (e.g. heart, gut). In this regard, a contemporary theory (Polyvagal theory) has proposed that positive emotions associated with physical contact, pleasant stimuli and social bonding are featured by enhanced activation of the vagal component of the PNS system (Porges, 1997). Based on Porges’ theory (2007) the mammalian ANS system would have evolved to support the survival, reproduction and social engagement of species. Through various stages of phylogeny, mammals, primates in particular, evolved a functional neural network, which regulates visceral state and supports social behavior. In this scenario, the association between intense emotional experience and vagal withdrawal would be functional. The vagal branch mediates a set of responses when environment requires a sudden coping strategy; if the individual is threatened, vagal tone is inhibited triggering a set of reactions (e.g. increased blood flow to the limbs, HR acceleration) that promote “fighting or fleeing” behaviors; to the contrary, when the subject is safe, a “vagal brake” is functioning, encouraging social behavior and interactions, as well as homeostatic functions (e.g. HR deceleration). The Polyvagal theory sustains that a good control of the vagal brake and an efficient regulation of the ANS would favor social interaction, engagement with others and an optimal recognition of emotional faces (Porges, 1996, 2003, 2007). In infants, individual differences in vagal responses reflect emotional expressiveness (Stifter et al., 1989), empathic behaviors (Fabes et al., 1994), social competence (Eisenberg et al., 1995), attentional abilities (Suess et al., 1994). Adults with atypical vagal tone, instead, have been demonstrated to have a predisposition to hostility, aggression (Mezzacappa et al., 1997), anxiety (Thayer et al., 1996) and depressive state (Carney et al., 1995).

"[…] there is no "gold standard" measure of emotional responding.
For theories of emotion, this means that there is no
"thing" that defines emotion, but rather that
emotions are constituted by multiple, situationally
and individually variable processes."

Iris B. Mauss and Michael D. Robinson

1.4 Methodological tools to investigate emotions in human and nonhuman primates

Over time, a number of different kinds of measurements of emotional responding have been developed. Many brain studies, as an example, have provided evidence for a close link between brain states and emotional responses. In this regard, many researches have indicated that the physiological correlates of emotions can be found in the central nervous system (CNS) and this perspective has been supported by measuring patterns of brain activity through electroencephalography (EEG) and other neuroimaging methods (e.g. functional Magnetic Resonance Imaging, fMRI).

EEG consists of an electrophysiological recording of electrical activity of the brain on the scalp; it is characterized by an optimal temporal resolution, but it has a limited spatial resolution (Dale and Sereno, 1993). EEG measures voltage changes resulting from ionic current flows within the neurons of the brain. In detail, it measures contrast activation in the anterior versus posterior regions of the brain in combination with a distinction between left-sided and right-sided hemispheric activation.

Numerous studies have examined the relationship between emotions and asymmetries in electroencephalographic activity over the frontal cortex, suggesting that asymmetries in frontal EEG activity (especially in the alpha (8–12 Hz) band) are widespread and linked to changes in emotional state (Coan and Allen, 2003). Early studies of frontal asymmetry highlighted that greater left-sided activation was related to a more intense experience of positive than negative emotions (Tomarken et al., 1990; Davidson et al., 1990). For example, Ekman and Davidson (1993) discovered that voluntary facial expressions of smiles produced higher left frontal activation. To the contrary, left frontal activity tends to decrease during voluntary facial expressions of fear (Coan et al., 2001). Some other studies proposed that frontal EEG asymmetries, in addition to mediate tendencies reflecting emotional responding, are linked to propensity to approach (Davidson, 2004); together with these observations, it was found that greater left-sided activation predicted dispositions toward approaching behaviors (Sutton and Davidson, 1997). Overall, these investigations indicate that greater left frontal activity tends to index approach-oriented emotional states (e.g. anger, joy), while greater right frontal activity influences withdrawal-oriented emotional states as disgust, fear or sadness (Coan and Allen, 2004; Coan and Allen, 2001; Harmon-Jones and Sigelman, 2001). At present, even if the EEG remains the tool of choice with respect to real time registrations of human neural activity, a large amount of obstacles remain studying brain EEG responses in relation to discrete emotions. In this regard, EEG recordings have not yet produced response patterns sufficient to distinguish brain activity for specific emotions.

Neuroimaging studies including fMRI can locate brain activation in far more specific regions than EEG. For this reason neuroimaging methods have been proposed as better suited than EEG to reveal emotion specificity in the brain (Panksepp et al., 1998). fMRI measures the uptake of oxygen in the blood (Detre and Floyd, 2001); in detail, it registers activation of a particular brain area based on greater blood flow to that area (BOLD signal).

In has long been proposed that emotions involve the limbic system (Papez, 1937; LeDoux, 1996). A certain amount of studies conducted through fMRI technique have reported cerebral blood flow increases in cortical, limbic and paralimbic regions. In this regard, a strong relation between fear stimuli and amygdala activation (Murphy et al., 2003, LeDoux, 2000) has been highlighted. However, this brain region is not only critical to fear-related processing. Few more studies have indeed observed amygdala activation in response to uncertainty and ambiguity (LeDoux, 1996; Whalen, 1998) in addition to negative emotions (Cahill et al., 1996), suggesting a widespread involvement of this brain region in responses to unexpected inputs (Barrett and Wager, 2006). Together with many authors who have postulated the amygdala involvement with fearful stimuli, some others have suggested that amygdala is related to dispositional affective style (Devidson and Irwin, 1999), to reward processing and positive emotional states (Mather et al., 2004; Murray, 2007).

Similarly to what observed in amygdala, a wide variety of negative emotions activated the insula as well (Phan et al., 2002). Beyond its association with disgust stimuli, the insula is also thought of as a brain "alarm center", which integrates internal somatic cues with emotional experience (Damasio, 1999; Phillips et al., 1997). fMRI studies have also shown the role of other brain regions in emotional responses; as an example, the medial prefrontal cortex would be crucial for emotional decision making and emotional self-regulation (Damasio et al., 1996; Davidson et al., 2000), while the orbital prefrontal cortex has a role in the evaluation of emotion-related reinforcement contingencies (Rolls, 2004).

Emotions are also accompanied by a more peripheral physiological response, which is linked to autonomic nervous system (ANS) activation. The ANS is a physiological system responsible for both modulation of peripheral functions like digestion, homeostasis, effort, attention (Berntson and Cacioppo, 2000) and emotional responding. ANS activation can be assessed through measurement of electrodermal activity (EDA) (i.e. sweating) or cardiovascular (i.e. circulatory system) responses.

EDA, previously known as skin conductance, galvanic skin response (GSR), electrodermal response (EDR), psychogalvanic response (PGR), skin conductance response (SCR), sympathetic skin response (SSR) or skin conductance level (SCL), started to be measured in the 1970s and first studies were published in the 1980s (Venables et al., 1980). In the 1990s, progresses in computer processing commuted the use of polygraph (used to record EDA signal) with the introduction of digital data recording and electronic analysis. In fact, EDA measurement consists of a registration of changes of skin conductance at the skin surface and denotes activation of the sympathetic axis of the ANS, thus providing information about alterations in sympathetic arousal (Critchley, 2002). If the parasympathetic nervous system promotes a bodily rest condition, the sympathetic nervous system stimulates metabolism to cope with challenges; in this regard, a series of physiological responses, which can be elicited by various emotional states, include increases of heart rate, blood pressure, sweating, piloerection, vasomotor changes. These autonomic sympathetic changes altering sweating and blood flow affect EDA by modifying skin conductance; EDA sensors are usually placed on human extremities (i.e. fingers, palms, soles of the feet) and measure changes of electrical conductance between two points by sending a small amount of current through that specific body area. The change of electrical properties of the skin is related to the activity of sweat glands; the production of sweat and thus the moistening of the skin in relation to a particular stimulus, it is responsible for the change in conductance level (Nakasone et al., 2005). Skin conductance measure has been shown to be a reliable index of autonomic expression of emotions. As an example, some studies showed that EDA could be modulated by valence and arousal with both visual and auditory stimuli (Bradley and Lang, 2000; Lang et al., 1998). Moreover, a number of researches demonstrated that electrodermal response increases according to the arousal of emotional stimuli (Bradley and Lang, 2000) and that the same effect is observable by using emotional pictures (Winton et al., 1984) and emotional words (Manning and Melchiori, 1974). Furthermore, Lang and colleagues (1993) found a positive correlation between arousal and conductance values in more than 80% of subjects.

Another of the most frequently used measures of ANS activity is heart rate (HR). It has been shown that sympathetic stimulation, occurring in response to exercise or stressful situations, determines an increase in HR or cardio-acceleration; on the contrary, parasympathetic activity, primarily linked to the function of internal organs, triggers a decrease in HR or cardio-deceleration, providing a regulatory balance in physiological autonomic function. Indeed, ANS function can be indexed non-invasively and more evidently by heart rate variability (HRV), the variation over time of the period between consecutive heartbeats, which is generally dependent on the extrinsic (neural) regulation of the HR. HRV (i.e., the amount of HR fluctuations around the mean HR) can reflect the heart's ability to adjust to changing situations by quickly responding to unpredictable stimuli. It represents thus a measure of the continuous interaction between sympathetic and parasympathetic (vagal) influence on HR and reveals the capacity to self-regulate emotional reactions. Separate rhythmic contributions from sympathetic and parasympathetic ANS activity modulate the heart rate (RR) intervals in the electrocardiogram (ECG). Higher frequency range of HRV (0.15–0.4 Hz) reflects cardiac parasympathetic control and vagal tone (Berntson et al., 1997). On the contrary, reduced HRV frequency range (0.04–0.15 Hz) is associated to sympathetic activity and has been reported in a number of psychiatric illnesses including depression and anxiety (Kemp et al., 2010; Demenescu et al., 2010). A number of studies have also focused on HRV as a marker of emotion regulatory abilities. Individuals with greater emotion regulation capacity have been shown to have greater levels of resting HRV (Appelhans and Luecken, 2006). In addition, HRV appears to increase during successful performance on emotion regulation tasks (Butler et al., 2006; Smith et al., 2011). Porges' Polyvagal theory (1997, 2001, 2007), based within an evolutionary framework, causally relates ANS flexibility represented by HRV with regulated emotional responding. In his theory, Porges describes the connection between the quality of social behavior and physiological state. In mammals, the myelinated vagus (tenth cranial nerve) works as a "vagal brake" in social contexts, changing visceral state by speeding up or slowing down the heart (Porges et al., 1996). A further measure of ANS activity on emotion regulation is thus represented by the effect of the dynamic influence of the cardiac vagal tone, which can be monitored by quantifying the amplitude of the respiratory sinus arrhythmia (RSA). RSA refers to the rhythmic fluctuations in heart rate that are associated with respiration. During inspiration, vagal tone is attenuated and heart rate accelerates, while during expiration vagal activity is restored, causing heart rate to slow. The amplitude of RSA is thus a way to quantify vagal tone (Porges et al., 1994). In detail, high amplitude RSA indicates that the myelinated vagus has a strong influence to the heart, which reduces the sympathetic activity. RSA is typically studied when individuals are in a resting or baseline state. Concerning RSA reactivity in response to stressors or emotional stimuli, a decrease in RSA from baseline indicates a vagal withdrawal. The term recovery is instead used to evaluate an individual's response after a stressor and his capacity to return to initial baseline levels (Santucci et al., 2008). In this regard, a certain amount of studies has demonstrated that better regulation of RSA was related to better social engagement (higher positive engagement) (Bazhenova et al., 2001). Basically, studies examining RSA levels support a connection between RSA and emotional experience (Beauchaine, 2001). Investigations on infants, children and adults found decreases in RSA during negative emotional experience (Gottman et al., 1995; Friedman and Thayer, 1998), while in infants, increased RSA has been associated with positive affect (Bazhenova and Porges, 1997). Similarly, a better vagal regulation has been linked to fewer behavioral problems in ASD children (Vaughan Van Hecke et al., 2009), while resting RSA below the median has been reported in children suffering from depressive symptoms (Shannon et al., 2007).

As described above, a number of parameters have been considered and explored as emotion indicators, including EDA and galvanic skin response, heart rate and related HRV and RSA. On the one side, the advantage of these physiological emotion indicators is that they are principally regulated by the ANS and are not consciously controlled. On the other side, a limitation to this kind of physiological approach is the requirement for sensors and devices that are in direct contact with the subject.

1.4.1 The use of Thermal Imaging for investigating the autonomic activity in relation to emotions

In recent years, it has been demonstrated that during arousal specific physiological signs materialize on the face as well. In this regard, a thermal imaging methodology aimed at extracting thermal signals has been developed. Infrared Imaging (IR), also known as thermal IR imaging or infrared thermography (IRT) is an ecologic, non-invasive, contactless technique that permits to map bodies' temperature distribution. Any object whose temperature is above absolute zero Kelvin (-273.15°C) emits radiation at a particular rate and with a distribution of wavelengths. This wavelength (λ) distribution depends on the temperature of the object and its spectral emissivity ε (λ), which is the object's ability to reflect radiation. The spectral emissivity is based on whether the body is a black body, grey body, or a selective radiator (i.e. an object that emits electromagnetic radiation whose spectral energy distribution differs from that of a black body with the same temperature) (Abbas et al., 2011). Human skin, for example, behaves as an almost blackbody with an emissivity of 0.96–0.98, which means that skin reflects only minimal radiation from surrounding objects. Given that IR radiation is invisible to the human eye, it can be detected and visualized by special IR cameras.

Actually, IR imaging has a number of practical applications in industry, construction, military and civil services, animal husbandry, biology, zoology, ecology and human and veterinary medicine (McCafferty, 2007). These cameras convert the invisible IR radiation emitted by an object into a monochrome or multicolored image on a monitor screen, in which the various shades or colors represent the thermal patterns across the object's surface (Maldague, 2001; Murthy et al., 2009). A peculiarity of infrared cameras is their ability to record the temperature of each pixel within the image, which is important for emotion research. In fact, these numerical values are used to calculate the overall temperature of the areas of the face that are believed to be associated with particular emotions. These areas are named "regions of interest" (ROIs). The most used ROIs are eyes, cheecks, forehead, mouth and nasal tip. Indeed, recent researches took advantage of the use of thermal IR imaging to understand and scrutinize affective and psychophysiological human states without the use of specific contact sensors. As previously mentioned, the ANS is actively involved in the bioheat exchange through its unconscious control of the heart rate (Garbey et al., 2007), metabolic breathing (Pavlidis et al., 2007), tissue metabolism, perspiration (Ebisch et al., 2012; Pavlidis et al., 2012), cutaneous temperature variations (Merla et al., 2004; Hahn et al., 2012), and cutaneous blood flow (Puri et al., 2005). Thermal IR imaging thus consists of an efficient tool to observe emotional responses related to ANS. Since the face is naturally exposed to social interactions and communication, thermal imaging for psychophysiological studies is mostly conducted by registering the individual's face. In particular, thermal IR imaging studies of ANS are based on the characterization of the thermal signal in facial regions of autonomic valence (nose or nasal tip, perioral or maxillary areas, periorbital, and supraorbital areas associated with the activity of the periocular and corrugator muscle, and forehead), to monitor the modulation of the autonomic activity (Khan and Ward, 2009; Nhan and Chau, 2010).

To confirm and validate the reliability and feasibility of this method, thermal data were first compared with other data simultaneously recorded by means of standard methods such as electrocardiography (ECG), piezoelectric thorax stripe for breathing monitoring, skin conductance or galvanic skin response (GSR). In this regard, various studies have demonstrated that thermography and GSR have a similar detection power to other standard methods used for assessing autonomic activity (Coli et al., 2007; Shastri et al., 2009; Kuraoka and Nakamura, 2011, Pavlidis et al., 2012; Di Giacinto et al., 2014; Engert et al., 2014). EDA responses and skin conductance measures, in particular, have been shown to correlate with active sweat glands, which activation can be observed through facial thermography. As in the palm area, sweat glands are strongly functioning in the maxillary, perioral, and nasal tip regions revealing a possible sympathetic response, which is measurable both with GSR and thermal imaging instruments (Pavlidis et al., 2012; Krzywicki et al., 2014).

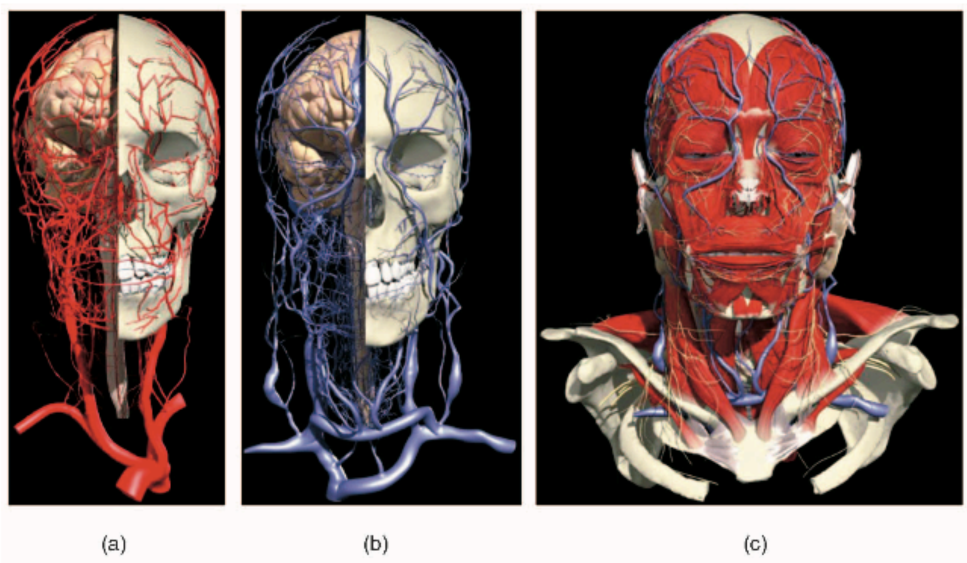

Figure 3. Map of superficial blood vessels on the face; (a) arterial network; (b) venous network (c) arteries and veins together. Figure taken from Buddharaju et al., 2007.

Thermal imaging cameras have been used to visualize changes in the body temperature of nonhuman primates exposed to various stressors. Nakayama and colleagues (2005), for example, examined changes in facial temperature in rhesus macaques (*Macaca mulatta*) during threatening situations, where nasal temperature decreased significantly within 10-30 s from the presentation of the negative stimulus. In particular, the upper nasal area showed a temperature decline as soon as 10 seconds after presentation of stimuli, with the cooling spreading across the rest of the nasal region. The decrease in nasal temperature, a process linked to subcutaneous vascular constriction (Ioannou et al., 2014a), was identified as an indicator of a change from a neutral to a negative emotional state in nonhuman primates. In a further experiment, Kuraoka et al. (2011) confirmed that temperature in the nasal region of macaque monkeys decreases during negative emotional states. In particular, the authors administered to the monkeys a number of video-clips in three different experiments: in the first one the monkeys visualized a raging individual; in the second one they observed three distinct emotional expressions; in the third they were exposed to faces or voices representing a threat. As previously observed, threatening stimuli elicited a decrease in nasal skin temperature and, in addition, simultaneous perception of both facial expressions and vocalizations induced a stronger thermal response then did the visual or acoustic channels alone.

Concerning great apes, until now only a few studies used thermal IR imaging to investigate chimpanzees' internal psychological/emotional states. In this regard, Kano et al. (2016) tested facial thermal variations in chimpanzees that were randomly presented with playback sounds or videos of fighting conspecifics. The nasal temperature of chimpanzees linearly decreased in two minutes and then recovered to baseline in the subsequent two minutes during the experimental, but not with control conditions. Furthermore, in a recent study conducted by Dezecache et al. (2017) nose and ear temperatures of wild chimpanzees of Budongo Forest (Uganda) were measured when the animals were exposed to naturally occurring vocalizations of conspecifics. A significant nasal temperature decrease after exposure to conspecifics' vocalizations was observed as well, while a corresponding ear temperature increase was highlighted.

Thermal infrared (IR) imaging has proved to be a reliable tool even to investigate psychophysiological responses and emotional states in humans. As an example, this method has been adopted in a variety of studies including stress and stressful situations. In a recent research, Pavlidis and colleagues (2012) tried to measure stress by analyzing perspiratory responses on the perinasal area by means of IR thermal imaging. In detail, they demonstrated that distress signs can be assessed by lower temperatures of the perinasal area along with the activation of perspiration pores. In another case, in particular considering human-computer interaction, a Stroop task was used to discover signs of frustration (Puri et al., 2005; Zhou et al., 2007); the authors observed that compared with the rest, stress increased blood volume into supraorbital vessels, which dissipated convective heat. Similarly, Calvin and Duffy (2007) exploited IR imaging to conduct a study of occupational ergonomics in order to evaluate the mental workload of professional drivers. Subjects were exposed to simulator driving tasks both in the city and on the highway while they were cognitively involved with a mental loading task (MLT). Compared with predriving temperatures (baseline), thermal differences were found in the nasal temperature across all conditions; in particular, a nose temperature decrease was observed during the simulated city drive, while no significant changes were seen on the forehead.

As previously outlined, thermal IR imaging has also been used to identify facial heat patterns associated with emotional response (e.g. Robinson et al., 2012). Concerning humans' positive emotions, Hahn and colleagues (2012) found that overall facial temperature increased following social contact with the opposite sex partner, suggesting that thermal changes can be a sensitive index of arousal during interpersonal interactions. In a similar study, Merla and Romani (2007) analyzed thermal imprint in participants that were viewing an erotic movie compared with subjects that were watching a sport movie (5 min duration each); their results showed a thermal increase of the forehead, lips, and periorbital regions.

IR thermal imaging has also been used to discriminate discrete emotions in humans. Ioannou et al. (2013) examined the thermal signature of guilt in children; by means of a "mishap paradigm" children were led to believe that they had broken the researcher's favorite toy. Nasal temperature decreased after the "mishap" and recovered to baseline after soothing. Consistently with primate research, the temperature of the nasal area was recognized as an indicator of general arousal in humans (Pavlidis et al., 2012). In this regard, a further thermal imaging study highlighted that the nasal tip temperature in infants tends to decrease after laughter (Nakanishi and Imai-Matsumura, 2008).

In another human study aimed at inducing fear, Merla and Romani (2007) administered unexpected subpainful mild electric stimuli to the subject's median nerve. Thermal signals of the face revealed a thermal reduction and sweating on the perioral area, forehead, and palms. In line with these observations, Shastri et al. (2009) observed a thermal decreasing of the upper lip and surrounding regions and a temperature increase on supraorbital and periorbital regions of the face in response to experimentally (by means of natural sounds) induced startles. In agreement with this, Pavlidis et al. (2001) obtained similar findings after exposing subjects to a loud startle of 60 dB while they were sitting in a quiet dark room.

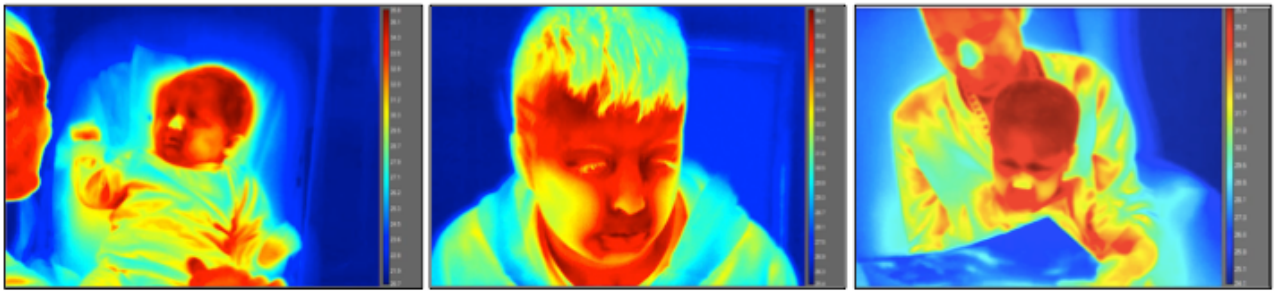

Figure 4. Example of thermal images showing the facial thermal imprints.

Some more studies investigating human emotions focused on empathy. In particular, Ebisch et al. (2012) measured thermal response between children and their mothers in a distressful situation; in order to induce distress and quantify the thermal response in both the mother and the child, a toy preplanned to break in the child's hands was used as stressor, while the mother was observing the child through a mirror. During the entire experiment, both participants presented simultaneous temperature decreases on the maxillary and nasal regions of the face. In contrast, during the soothing phase, after the toy was restored, both maxillary and nasal regions increased in heat. A subsequent experiment included an additional group of female participants who presented a slower empathic reaction than the mothers' one (Manini et al., 2013).

Summing up, negative emotions such as stress or fear tend to decrease nasal skin temperature, basically due to the reduced blood flow caused by vasoconstriction of subcutaneous vessels. As previously mentioned, pain, startle, distressful contexts, play, empathy with someone in a stressful situation, and mental load associated with tasks execution decline the nasal temperature. In contrast, sexual arousal, intimate touching, interpersonal proximity are responsible for a nasal temperature increase due to the increased blood flow.

IR thermal imaging thus provides a reliable tool that enables to infer psychophysiological responses even without the need of wires or electrodes attached to the skin, both of which could make any kind of interaction uncomfortable. Although this is a very significant advantage in order to make the measurement the more naturalistic as possible, a few limitations have to be taken into account. Temperature measurement needs a controlled setting, with no direct ventilation and temperature and relative humidity set at comfortable values for the subject. The cutaneous temperature is in fact continuously adjusted with relation to environmental conditions; therefore, thermoregulatory or acclimatization processes have always to be taken into account to avoid artifacts throughout the experimental session (Merla et al., 2004). Furthermore, despite the number of advantages provided by this technique, the thermal signal as a result of perspiration, muscles activity and metabolism is rather slow. Kuraoka and Nakamura (2011) compared thermography with SCR and stressed the fact that IR thermal imaging has a longer latency than SCR. If temperature changes can be visualized within 3 seconds by means of SCR, at least 10 seconds are required with thermal imaging. Nevertheless, this technology is still under development and the need to determine if heat patterns mean discrete emotions or dimensional responses is still ongoing. In view of all this, in order to explore more in detail emotional responses and to deepen knowledge on the use of this methodology it would be useful to integrate it with other techniques so as to obtain different kinds of physiological responses within the same paradigm.

In the light of previous considerations concerning reciprocal emotional communication, it remains to consider that of special relevance to emotional processing and especially to imitative capabilities is a subset of action-coding neurons, the mirror neurons (MNs), whose importance and functioning will be treated in the next chapter.

"…we understand action because the motor representation of that action is activated in our brain."

Rizzolatti, Fogassi, and Gallese

1.5 The role of the motor system and of the mirror mechanism in emotional processing

During the last decade, neurophysiological studies have discovered a neural system associated with action understanding and imitation (Iacoboni et al., 1999). This set of neurons, defined mirror neurons (MNs), is a particular class of visuomotor neurons, which were originally identified in the prefrontal cortex (area F5) and subsequently in the inferior parietal lobule (Gallese et al., 2004) of the monkey (di Pellegrino et al., 1992; Rizzolatti et al., 1996, Gallese et al., 1996). Such neurons become active both when the monkey performs a specific action (e.g. when grasping an object) and when it observes the same or a similar action executed by an other individual (monkey or human); generally, mirror neurons do not fire when the action is performed in the absence of the target and do not respond to the observation of an object alone (Gallese et al., 1996; Rizzolatti et al., 1996). Therefore, MNs can translate the observation of a biological meaningful action into the corresponding cortical motor representation.

The spatial and temporal resolution reached by depth single-cell electrode recordings cannot be achieved in humans because these methods of brain investigation are quite invasive. Nevertheless, a number of techniques such as transcranial magnetic stimulation (TMS), electroencephalography (EEG), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been used to explore the same neural network in humans. In this regard, it has been demonstrated that during action observation there is a strong activation of premotor and parietal areas, the human homologue of the macaque's F5 area, thus confirming the existence of a mirror neuron system matching action perception and execution in the human brain as well (for a review see Rizzolatti, Fogassi, and Gallese 2001; Rizzolatti and Craighero 2004; Gallese, Keysers, and Rizzolatti 2004). fMRI studies in humans, in particular, have identified areas with mirror-like properties in the ventral premotor cortex (vPMC), inferior frontal gyrus (IFG) and the posterior parietal cortex (Iacoboni et al., 1999).

The initial hypothesis about the functional role of MNs focused on action recognition. Considering that they fired during actions (such as grasping an object) of the self and of other individuals, MNs could provide a neural mechanism not cognitively mediated for recognizing the actions of others. Subsequently, further investigations emphasized MNs' role of connection between the "self" and the "other", their importance to understand observed actions in terms of one's own action programs, and their relevance in social cognition including the capacity to "read minds" (Gallese and Goldman, 1998), to communicate through gestures (Rizzolatti and Craighero, 2004), to imitate (Rizzolatti et al., 2001).

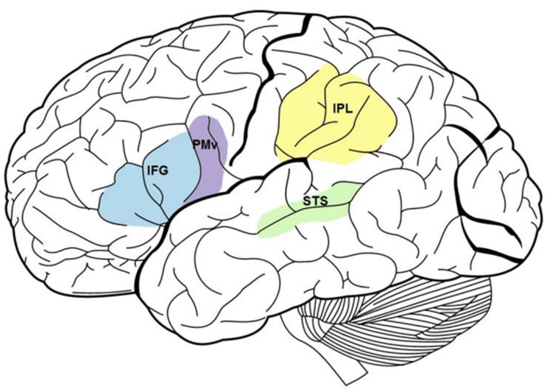

Figure 5. Lateral view of the brain with frontal (IFG & PMv) and parietal (IPL) mirror neuron system regions highlighted. The superior temporal sulcus is also labeled. (IFG = inferior frontal gyrus; PMv = ventral premotor area; IPL = inferior parietal lobule; STS = superior temporal sulcus). Figure taken from Werner et al., 2012.

Indeed, even if first studies in monkeys and humans focused on MNs' role in the comprehension of goal-directed actions, more recent investigations highlighted their involvement in decoding facial expressions. Interestingly, neurons in the monkey's premotor cortex have been observed to respond to communicative affiliative facial gestures (Ferrari et al., 2003); in line with these results, Carr and colleagues (2003) showed that premotor and parietal cortex were both activated during facial expression observation and execution in humans, thus sustaining the idea of the involvement of MNs in understanding facial expressions. In addition, the authors found that two limbic areas were implicated in this mechanism, that is the insula and the amygdala were active both during the observation and execution of facial gestures. Similarly, Wicker et al. (2003) showed that the AI and the anterior cingulate gyrus were active both when the subjects were experiencing and when they were observing the emotion "disgust". The majority of social neuroscience literature on emotions used the observation of pain in others as a model paradigm to evoke empathic responses (de Vignemont and Singer 2006; Decety and Lamm 2006; Singer and Leiberg 2009). In this regard, Singer and colleagues demonstrated, through an interactive empathy-for-pain paradigm, that experiencing pain activates part of the neural network that is also activated when we are in pain ourselves. In a first experiment (2004) the authors recruited couples and measured in an fMRI scanner the response of a volunteer either when the person was receiving pain herself or when she was perceiving pain in the partner, delivered via pain electrodes on the back of the volunteer's or the other person's hand. The results suggested that parts of the so-called "pain matrix" - bilateral AI and dorsal anterior cingulate cortex (ACC) - were activated when she experienced pain herself as well as when she saw the cue indicating that the partner was experiencing pain. In a more recent study, the authors demonstrated that brain responses in AI and ACC were not restricted to a beloved partner, but also present when an unknown person was in pain (2006). Subsequently, imaging studies in which subjects were viewing pictures or videos of faces displaying pain (Lamm et al., 2007) or of body parts in painful contexts (Cheng et al., 2007; Morrison and Downing, 2007) revealed similar findings. As a matter of confirmation, a recent study conducted on nonhuman primates demonstrated that electrical stimulation of the insula evoked affiliative facial expressions as well as disgust-related/ingestive behaviors (Caruana et al., 2011). The limbic system, including the insula, the amygdala and the cingulate gyrus is thus directly linked with emotional processing as well as with the control of visceral sensations and autonomic responses. Therefore, in concert with the activity in limbic centers, MNs could also mediate the understanding of emotional states of others through a mechanism able to match the perceived action/gesture/facial movement with the internally felt emotional significance. More in detail, this mechanism is able to transform the observed emotional context into a viscero-motor response analogous to the one felt when an individual experiences the same emotion. Overall, these results suggest that humans' capacity to empathize with others is conveyed by the activation of the same neural circuits underpinning own emotional experiences (Gallese, 2007).

These issues have been explored in infant development by means of a less invasive technique, EEG, which can be used to investigate responses associated with MNs in human infants and children (Oberman et al., 2005; Marshall et al, 2011). Studies concerning cerebral activation during execution and observation of actions revealed that specific frequency bands within the alpha range (9-13 Hz in adults; 5-9 Hz in infants) desynchronize in newborns (Kessler et al., 2006) and older infants (Marshall et al., 2011). This suppression (mu rhythm) has been linked with the activation of mirror areas and can be considered a marker of MNs activity (Arnstein et al., 2011; Muthukumaraswamy et al., 2004). In this regard, mu rhythm suppression has been recorded during neonatal imitation in newborn monkey macaques (Ferrari et al., 2012); during execution and observation of lipsmacking (LPS) (affiliative facial behavior) and tongue protrusion (TP) facial gestures, suppression in the 5-6 Hz frequency band was observed, highlighting that a mirror mechanism can underlie the synchronous dyadic communication between infant and caregiver.

The mirror network may thus be at the basis of human and non-human primate infants' abilities to respond to their mothers and to synchronize with them through a complex face-to-face communication. As mentioned in a previous chapter, human mothers stimulate active engagements and elaborate emotional exchanges including mutual gaze, body contact and exaggerated facial and vocal expressions (Stern, 1985; Tronick and Cohn, 1989; Trevarthen, 1974). Neonates as well display multiple facial expressions and gestures, which are crucial for responding and initiating facial patterns within this dyad, where the primary caregiver's capacity to engage in an interactive communication is fundamental to regulate the infant's internal state and arousal. Newborns' early imitative capacities, which were studied in monkeys (Ferrari et al., 2006; 2012; Paukner et al., 2011) and chimpanzees (Bard, 2007) as well as in human newborns (Simpson et al., 2014) can indicate MNs' functioning and therefore they could be a source of information about early development of this network.

This kind of studies would be particularly relevant in infants with neural pathologies that affect cranial nerves and implicate congenital facial paralysis. As an example, Moebius syndrome (MBS) patients suffer from the inability to perform facial expressions since birth. The features and aetiology of this rare syndrome will be treated in the next chapter.

1.6 The Moebius syndrome (MBS): principal features, aetiology and treatment

1.6.1 Understanding the syndrome

The present doctoral thesis will be centered on the analysis of emotional processing and of autonomic functioning in children affected by Moebius syndrome (MBS).

Moebius syndrome (MBS), also referred to as Moebius sequence, is a rare congenital syndrome affecting approximately 1 in 50.000 to 1 in 500.000 live births (Rasmussen et al., 2015) with no gender predominance (Lindsay et al., 2010; Kulkarni et al., 2012). The disorder presents with varying phenotype and severity, resulting in unilateral (less frequent) or bilateral facial paralysis due to altered development of the facial (VII) and abducens (VI) cranial nerves (Abramson et al., 1998; Bianchi et al., 2009; Cattaneo et al., 2006; Sjo¨green et al., 2001; Terzis et al., 2003; 2011; Verzijl et al., 2005a; Briegel, 2006).

The clinical findings of Moebius syndrome (MBS) were originally identified by von Graefe (1880), and further characterized by Moebius in 1888. Over the last century, the Moebius sequence definition has expanded to include varying cranial nerves, with or without craniofacial dysmorphisms and other congenital abnormalities (Verzijl et al., 2003; Verzijl et al., 2005). Subsequently, more strict criteria have been outlined to include only those cases with both congenital paresis of the VI and VII cranial nerves (Verzijl et al., 2003; Webb et al., 2012), even though XII, X, IX, III, VIII, V, IV and XI cranial nerves can also be affected (in order of decreasing frequency) (Kulkarni et al., 2012; Shashikiran et al., 2004; Singham et al., 2004). Some moebius patients retain residual lower facial muscle activity, possibly due to aberrant innervation from other cranial nerves (Verzijil et al., 2005).

1.6.2 The abducens (VI) and facial (VII) cranial nerves: an overview

There are 12 pairs of cranial nerves that arise from the brain stem and innervate the head. Some of the cranial nerves are part of the CNS (Olfactory - I - and Optic – II -), the others are part of the somatic or visceral parasympathetic system. Each cranial nerve has a name and a number associated with it. For the aim of this thesis, the description of cranial nerves will focus on the abducens (VI) and on the facial (VII) cranial nerves.

The VI cranial nerve originates in the abducens motor nucleus, which is located at the dorsal aspect of the pons near the floor of the fourth ventricle, surrounded by the motor fibers of the VII nerve (Ezer et al., 2012). Its function, together with the oculomotor (III) and the trochlear (IV) nerves, is associated with the movements of the eyes. In particular, it supplies the lateral rectus muscle, thus coordinating the action of both eyes to produce horizontal gaze. A VI cranial nerve paralysis can result in convergent strabismus with the inhability to abduct the eye and in reduced or absent convergence of eye movements (Roldan-Valadez et al., 2015; Rucker et al., 2014).

The VII cranial nerve has multiple origins: it emerges from the motor nuclei, in the dorsal part of the pons; from the superior salivary nuclei, in the dorsolateral part of the pons; and from the geniculate ganglion. It leaves the brain stem as two separate nerves, the intermediate and the facial nerves. Its branchial motor fibers are adjacent to, but separated from, the remaining fibers, which in turn carry visceral and sensory information.

The VII cranial nerve mainly consists of five segments (Binder et al., 2010): (1) the intracranial segment; (2) the internal auditory canal segment; (3) the labyrinthine segment; (4) the tympanic segment; and (5) the mastoid segment. The motor root of the facial nerve consists of fibers from the motor nucleus, which supply the muscles for facial expressions. Its motor component also supplies the stapedius muscle of the middle ear, a muscle that prevents excessive movements of the stapes due to loud sounds. VII nerve's functions involving visceral and sensory channels are to conduct taste sensation from the anterior tongue and oral cavity and to effect parasympathetic secretions from the salivary, lacrimal, nasal and palatine glands (Zhang et al., 2013).

Lesions to the facial nerve could therefore be responsible for the paralysis of the muscles of facial expression (upper and lower face), loss of the corneal reflex, loss of taste from the anterior two-thirds of the tongue and hyperacusis (increased acuity to sounds) as a result of stapedius paralysis (Roldan-Valadez et al., 2015).

1.6.3 Clinical perspective

In the light of a previous description of the cranial nerves that are commonly damaged by MBS, maldevelopment of VI and VII nerves in this symptomatology results in complete non-progressive unilateral or bilateral facial palsy and impaired abduction of the eyes (Singham et al., 2004). Additional clinical dysfunctions of the syndrome include incomplete eye closure during sleep, drooling and difficulties in sucking during infancy (Singham et al., 2004); with development, a "mask-like face" (with associated inability to smile), horizontal gaze palsy and strabismus are also common. Further congenital abnormalities are sometimes associated with the syndrome, including sensorineural hearing loss, craniofacial malformations (epicanthic folds, micrognathia), limb anomalies (such as club feet and missing or underdeveloped fingers or hands), Poland syndrome (underdevelopment of the pectoralis muscle combined with hand malformation), and hypoglossia (weakness or malformation of the tongue) (Terzis and Noah, 2002; Verzijl et al., 2003). Cardiovascular abnormalities are rarely present, but can include dextrocardial, atrial, or ventricular septal defect, transposition of the great vessels, and total anomalous pulmonary venous connection (Suvarna et al., 2006; Deda et al., 2001; Thapa, 2009). Mostly, patients present normal intelligence, but approximately 10% of patients have mental retardation, and another 30–40% may be diagnosed with autism (Bandim et al., 2003; Meyerson, 2001).

Figure 6. Photographs of two children affected by Moebius syndrome. Taken from Singham et al., 2004.

1.6.4 Aetiology

Although MBS aetiology is still unknown or unclear, theories of the underlying intrauterine environmental factors and genetic causes have been considered. At present, the most widely accepted theory concerning this pathogenesis sustains the interruption of blood supply during the initial development of the fetus. In particular, MBS would be induced by the obstruction of vascular supply resulting from the disruption of the primitive subclavian arteries before establishment of the vertebral arteries (theory of "subclavian artery supply distribution sequence" (SASDS) by Bavink and Weaver, 1986). Indeed, the nascent hindbrain is originally supplied by the primitive trigeminal arteries; however, during the sixth week of gestation the blood supply changes from these arteries to the vertebral arteries that start supplying the brainstem through the basilar artery. In this context, timing is crucial during the formation of the vertebral arteries and the regression of the primitive trigeminal arteries so as to ensure blood supply to the cranial nerve nuclei (Terzis and Anesti, 2011). This possible pathogenic sequence is supported by the findings from radiological imaging, including ultrasonography, computed tomography, and magnetic resonance imaging in patients with MBS, which showed calcifications and hypoplasia of the brainstem (Singham et al., 2004; Dooley et al., 2004).

The vascular disruption could be caused by various theratogens acting on the embryo during the first trimester, such as abuse of benzodiazepines (Courtens, 1992) and misoprostol (an abortifaciant drug) (Puvabanditsin et al., 2005; Pachajoa and Isaza, 2011), thalidomide (Elsahy, 1973), alcohol (Martinez-Frias et al., 2001) or cocaine (Puvabanditsin et al., 2005) exposure during pregnancy. Infection, hyperthermia, hypoxia, and vasculitis can also interfere with blood flow (Terzis and Anesti, 2011).

Additional minor theories have been proposed of the underlying pathophysiology of the syndrome, which include peripheral nerve injury with degeneration and atrophy of the facial musculature (Towfighi et al., 1979). On the one side, muscles and brain problems of the syndrome would be a consequence of irregularities of peripheral nerves during development; on the other side, muscles would be the primary problem, causing a degeneration of the nucleus of peripheral nerves and of the brain.

The genetics underlying MBS remain unclear; nevertheless, even if most cases of MBS are sporadic, some familial trends have been seen with both autosomal dominant and recessive patterns

(Legum et al., 1981; Becker-Christensen and Lund, 1974; Schröder et al., 2013). In particular, when linked to musculoskeletal anomalies, MBS is not likely to be familial and the risk of hereditariness is as low as 2%. The risk increases to 25-30% with clinical features suggesting a genetic etiology such as isolated facial palsy, deafness, ophthalmoplegia, and digital contractures (MacDermot et al., 1991).

The individual moebius phenotype is slightly variable, thus suggesting genetic heterogeneity in the syndrome. Genetic studies have mainly focused on potential chromosomal loci, selecting candidate genes and analyzing the genomes of MBS patients for mutations in these genes. These studies are particularly difficult due to genetic heterogeneity.

The most implicated loci include 1p22, 3q21-q22, 10q21.3-q22.1, and 13q12.2-13 (Van der Zwaag et al., 2002), which are linked to particular homeobox genes (including HOXA1, HOXB1, and SOX14), necessary for spatial and temporal development of the brain. The effects of these genes can also influence other genes; mutations in homeobox genes may therefore determine the varying phenotypes characterizing the syndrome. Further candidate genes, which are crucial for neuronal development and could be involved with the syndrome, are PLEXIN-A1, GATA2, EGR2, BASP1 and TUBB3. Interestingly, the gene FLT1/VEGFR1, which is linked to an aberrant vascular growth, is probably implicated in the syndrome, thus sustaining the previously mentioned SASDS theory (Kadakia et al., 2015).

Eventually, a recent study from Tomas-Roca and colleagues (2015) reported *de novo* mutations affecting two genes, PLXND1 and REV3L, in six MBS patients. The neuropathological deficiencies found in MBS patients correlated with those found in mutant mice models. In this regard, analysis of Plxnd1 and Rev3l in knock-out mice demonstrated that mutations in these genes were responsible for a lowered number of motoneurons in the facial branchiomotor nucleus; in addition, developmental anomalies, such as craniofacial bone abnormalities or vertebral defects were present both in knock-out mice and in MBS patients carrying a PLXNDl mutation.

In the light of previously reported data, it seems appropriate to emphasize that MBS is characterized by considerable genetic heterogeneity with multiple genes involved and several moebius cases with different anomalies of variable severity.

1.6.5 Treatment

Treatment of patients affected by MBS is based on a multidisciplinary approach depending on symptoms. Surgery can correct the eye deviation, protect the cornea through tarsorraphy and improve the deformities of jaw and extremities. A “smile surgery” aimed at restituting facial expression, facilitating smiles, reconstructing the lower lip incontinence or oral hypotonia is also being performed. In this sense, the reinstatement of even a small degree of voluntary facial movement can be rewarding in terms of interacting with others.

Figure 7. Preoperative picture of a 7-year-old patient with congenital unilateral right facial palsy at rest (a) and smiling (b). Taken from Bianchi et al., 2016.

Plastic surgery for facial reanimation has been pioneered by Dr. Ronald Zucker (Zucker et al., 2000). In this regard, the emergence of microsurgery was fundamental for developing this kind of clinical interventions. The most common operation for restoring facial expression in patients with MBS is the "neurovascular free muscle transfer" (Singham et al., 2004; Bianchi et al., 2010). The most frequently used muscles are the gracilis and the pectoralis minor. The gracilis muscle is ideal since it is easily accessible, dispensable (so that its harvest leaves no functional deficits), and distinguished by appropriate vasculature for free transfer (O'Brien et al., 1990). Only a segment of the muscle, approximately one-third to half, is needed to produce the appropriate amount of movement. During the gracilis muscle transfer, two teams operate simultaneously to elevate the muscle and prepare the face. After being transferred to the face, the muscle is revascularized by microvascular repair of the gracilis muscle's artery to the facial artery and of the gracilis' veins to the facial vein. The muscle is then positioned and sutured securely to the oral commissure and the upper lip, and to the masseter muscle (muscle of mastication).

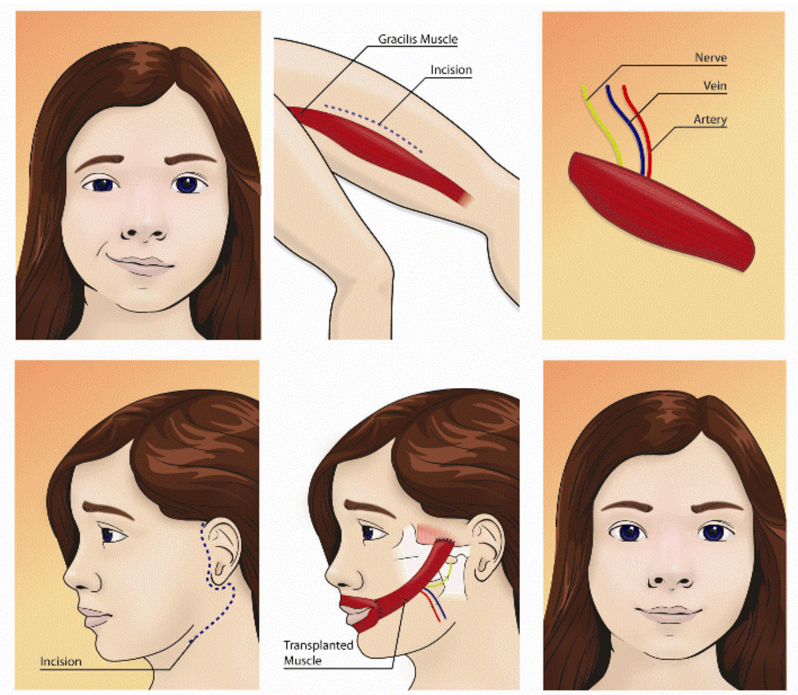

Figure 8. Example of surgical treatment performed on MBS patients to restore facial animation.

With time, the motor nerve to the masseter muscle (Trigeminal nerve, 5th cranial nerve) innervates the gracilis muscle to reanimate the face (Bianchi et al., 2010). Trigeminal nerve provides very strong innervation to this muscle, allowing patients to easily control smiling as a result of speech therapy and cerebral plasticity. In fact, after surgery it's very important for patients to undergo smile training by a speech-language therapist using mirror exercises. This is crucial in order to achieve a spontaneous and symmetrical smile. Surgery is usually performed after the age of 4.5-5, so that the vessels and cranial nerves have grown enough to sustain muscle transfer. The age of the patient does not influence the outcome of the clinical operation, however adults' reinnervation takes longer (5-6 months) than children's one (3.5 months) (Bianchi et al., 2010).

1.7 The interactivity of a smiling brain: a mirror mechanism perspective

This final introductive chapter wants to unify the multiple perspectives previously taken into account, so as to obtain a better understanding of the MBS by means of a multidisciplinary approach. In detail, the aim of this overview is to consider the syndrome's features as damaged VI and VII cranial nerves and associated eye movements deficits and paralysis of the muscles of the face in the light of recent neuroscientific findings.

As previously mentioned, the understanding of basic aspects of social cognition and communication depends on implicit activation of neural structures involved in our personal experience of actions and emotions. When we see someone performing an action or a facial gesture, the same motor circuits that are activated when we perform the same gesture are concurrently triggered, even if we do not reproduce the observed action/facial expression. This implicit action simulation, based on mirror neurons network, represents a bridge between others and ourselves. According to this view, the double pattern of activation of the same somatosensory-related brain regions suggests that our capacity to experience and directly understand the experience of others could be mediated by this mechanism (Rizzolatti et al., 1996; Ferrari et al., 2003). Because of this overlapping neural substrate for action execution and observation in both humans and other primates, mirror neurons (MNs) have been defined as implicated with higher-level cognitive functions such as imitation (Carr et al., 2003). As suggested by various studies conducted on human and nonhuman primate newborns (Meltzoff and Moore, 1977; Ferrari et al., 2006; Ferrari et al., 2012), imitative abilities and associated neural patterns seem to be present since birth, and might be crucial in developing social competencies during the first mother-infant interactions.

2 Aim of the thesis

Taking into account the relevance both of own motor experience in understanding and communicating on an emotional level and of the "face-to-face" dyadic interactions between mother and child in structuring and organizing emotional processes of the child himself, this doctoral thesis was focused on emotional processing in children affected by Moebius syndrome. In detail, the aim of the studies shown below was to investigate MBS children abilities to recognize facial expressions of emotions and the extent of their autonomic response to different kinds of emotional stimuli, compared with normal control children. The understanding of these aspects through early years of development, considering that moebius interactions prior to surgery are damaged by facial palsy, could be crucial in order to identify strategies aimed at facilitating and establishing a fully functional interaction between the moebius child and the caregivers.

3 Study 1

Impaired emotion recognition and decreased autonomic activity to emotional stimuli in children with Moebius syndrome

Submitted to Cortex, Under revision

Ylenia Nicolini, Barbara Manini, Elisa De Stefani, Daniela Cardone, Anna Barbot, Chiara Bertolini, Cecilia Zannoni, Mauro Belluardo, Andrea Zangrandi, Arcangelo Merla, Pier Francesco Ferrari

1. Introduction

Moebius syndrome (MBS) is a rare congenital syndrome affecting approximately 1 in 50.000 to 1 in 500.000 live births (Rasmussen et al., 2015) with no gender predominance (Lindsay et al., 2010). The disorder presents with varying phenotype and severity, resulting in unilateral or bilateral facial paralysis and impaired bilateral movement of the eyes, due to mal-development of the VI and VII cranial nerve nuclei early in prenatal life (Abramson et al., 1998; Bianchi et al., 2009; Cattaneo et al., 2006; Terzis and Noah, 2003; Terzis and Anesti, 2011). The VI and VII cranial nerves control, respectively, the abduction of the eyes and the muscles used to generate facial expressions, lip speech and eye closure. V, IX, X, and XII cranial nerves can also be affected (Kulkarni et al., 2012; Shashikiran et al., 2004; Terzis and Noah, 2002). Other congenital abnormalities are sometimes associated with the syndrome, including sensorineural hearing loss, craniofacial malformations, limb anomalies, Poland syndrome (underdevelopment of the pectoralis muscle and hand malformation), hypoglossia (Terzis and Noah, 2002; Verzijl et al., 2003), and poor coordination.

Most of the patients present normal intelligence, but approximately 10% have mental retardation, and another 30–40% may be diagnosed with autism (Bandim et al., 2003; Meyerson, 2001). Although MBS etiology is still unknown, theories of the underlying environmental (Terzis and Noah, 2002; Verzijl et al., 2003) and genetic factors (MacKinnon et al., 2014, Tomas-Roca et al., 2015) have been considered.

One of the most prominent features of patients with MBS is the impairment, if not absence, in the ability to smile, frown, raise an eyebrow. Their incapacity or difficulty to produce any facial movement limits the patients' ability in communicating emotions through facial expressions (Ekman et al., 1969; Ekman and Friesen, 1971, 1986; Matsumoto and Willingham, 2009). Such motor impairment could impact several psychological processes that are linked with emotions such as the capacity to communicate internal affective states or to recognize emotions in others. In relation to this, some recent studies have investigated MBS young patients' (children and adolescents) social aspects and quality of life (Bianchi et al., 2016; 2017; Strobel and Renner, 2016), showing that they have reduced social relations and higher frequency of social rejection, especially during adolescence, indicating potential precursors of social problems later in life.

Literature posits that receiving proprioceptive feedback from facial skeletal muscles while mimicking facial expressions (facial feedback hypothesis) plays a crucial role in regulating emotional experience (Buck, 1980). Many studies demonstrated that the same motor regions involved in the generation of facial expressions of emotion are also implicated in recognizing that emotion in others, when the same emotion is conveyed by facial expression or other mechanisms (Leslie et al., 2004; Keysers and Gazzola, 2009; Gallese et al., 2007). According to current literature, the neuronal bases of facial mimicry are underpinned by a mirror mechanism, constituted by a parietal-premotor cortical network named the "mirror neuron system" (MNS) (Niedenthal, 2007; Iacoboni and Dapretto, 2006). The MNS in humans has been described and explored after the discovery of "Mirror neurons" (MNs), firstly discovered in the monkey's parietal and premotor cortex, and which respond not only when the monkey performs an action, but also when the animal observes another individual performing the same or a similar action (Gallese et al., 1996; Fogassi et al., 2005; Ferrari et al. 2003). Further evidences demonstrated that the same cortical regions are activated during both action or facial gesture generation and observation of similar actions/gestures performed by another individual (Gallese et al., 1996; Grafton et al., 1996; Rizzolatti et al., 1996; Ferrari et al., 2003; Carr et al., 2003; Wicker et al., 2003; Caruana et al., 2016, Rizzolatti and Craighero, 2004). It has been proposed that the MNS, involved not only in movement generation but also in action understanding and language comprehension (Rizzolatti and Craighero, 2004;

Gentilucci et al., 2012; Campione et al., 2014), supports the emotional recognition process through the activation in the observer of a neural motor representation similar to that expressed by the observed individual (Iacoboni, 2005; Carr et al., 2003; Wicker et al., 2003; Ferrari et al., 2003; Ferrari et al. 2017). Thus, emotion recognition in other occurs as unconscious mimicking of the observed expression, which requires the implicit activation of the specific motor programs responsible for the production of a particular facial expression and, crucially, of its associated physiological responses (also named *reverse simulation model*) (Goldman and Sripada, 2005; Oberman et al., 2007; Stel and van Knippenberg, 2008; Gros et al., 2015).

Several neuroimaging studies showed that in addition to the motor regions of the parietal and premotor cortices, the observation and the direct experience of an emotion may activate a more distributed network involving the anterior insula, the anterior cingulate cortex (ACC) and the amygdala (van der Gaag et al., 2007). Such regions are known to be important not only in the control of the motor components of emotions, but also in orchestrating the complex visceromotor responses associated to an emotional state (increase/decrease in heart rate, modification in blood pressure, pupil dilation, piloerection, metabolic changes etc.) (Panksepp, 2005; Jezzini et al., 2012; Caruana et al., 2011; Caruana et al., 2016). Thus, a mirror mechanism for emotions may rely on a more complex network in which some structures, such as the insular cortex and the ACC could coordinate the autonomic responses typical of the limbic system with the motor modifications associated to the expression of an emotion (Ferrari et al. 2017). This tight connection between motor and autonomic responses has several implications for theories on emotions based on simulation processes and is of utmost importance when investigating disturbances involving the motor commands controlling the expression of emotions.

Individuals with deficits in producing or expressing an emotion may therefore have difficulties at different levels: a) on the capacity to activate motor programs similar to those observed, and therefore to recognize facial expressions of emotions based on a simulation neural mechanism, and b) on the capacity to activate the autonomic responses associated to a specific emotional state (Goldman and Sripada, 2005; Adolphs et al., 2003). These two levels are clearly interacting but it is not clear whether the deficits in the activity of the autonomic system might interfere with the recognition process. MBS patients represent an interesting population to be studied in this regard. The evidence of deficits in the emotion recognition process in MBS patients has been tested only in adults and the results, in the few studies available, are ambiguous and rather controversial.

In one of the first studies assessing emotion recognition in MBS, Giannini et al. (1984) examined facial expression processing in a single Moebius patient. The study showed that the Moebius participant, compared with 300 control subjects, was not able to predict the action of an actor depending on facial expressions. This first finding was suggestive of a possible link between perception and production of facial expressions. In another study, Calder and colleagues (2000) examined facial identity processing as well as facial and vocal expressions recognition in three individuals with MBS. Even if the participants did not show any problem in tests of emotional sound, they showed mild, but non-significant deficits in facial expression recognition.

According to these results, Bogart and Matsumoto (2010a) took advantage of an online Internet-based research to test facial expression recognition of 37 MBS adults and 37 age and gender matched controls. In detail, participants were asked to attribute an emotional expression to 42 faces. Subjects were asked to complete a Facial Expression Communication Questionnaire, so as to self evaluate their capacity to communicate the facial expressions of the previous test. MBS subjects did not differ from the control group.

More recently, a study focusing on MBS and facial expressions investigated the capacity of six adult Moebius participants to imagine facial displays and to recognize facial expressions (Bate et al., 2013). The authors reported that only one of the subjects was impaired in the imagery test, that consisted in visualizing facial expressions of emotions and answering questions about them; however, five of six MBS participants were impaired in the expression recognition, although they did not show an absolute inability to complete the tasks.

Overall, these studies show that adult MBS patients show some degree of recognition of others' emotions, but the results are mixed, probably due to some methodological limitations, such as patients' sample size, lack of clinical evaluation, non-objective assessments (i.e. self-evaluation), variations in the tasks used. It is therefore possible that MBS adults can use compensatory strategies (i.e. vocal prosody, body language, hand gestures, specific facial cues of emotion, such as the mouth corners turned up or down) (Krueger and Michael, 2012; Bogart et al., 2012) and take into account information from multiple channels in developing the capacity to recognize emotions. The lack of facial expressivity in MBS patients could therefore interfere with their capacity to recognize emotions, at least in the early phases of development. In this regard, developmental studies focusing on emotional processing indicate that children's explicit recognition of emotional expressions underlies on developmental milestones, with happiness recognized earliest than sadness, anger, surprise and fear (Camras and Allison, 1985). Children's facial expression recognition develops then rapidly with increasing age (De Sonneville et al., 2002) and more complex cognitive strategies emerge later in life, especially in the transition between childhood to adolescence, and between adolescence and adulthood.

Given that no MBS research concerning emotion recognition has been conducted during developmental age, in the current study we aimed at investigating the capacity to recognize facial expressions in MBS children. We were also interested in assessing their autonomic responses to emotional stimuli.

Specifically, we hypothesized that the lack of a motor simulation process during the decoding of emotions could implicate not only a difficulty in recognizing emotions but also an alteration in the autonomic response while children are viewing videos depicting characters experiencing emotions. To this aim, we monitored participants' autonomic responses by using functional infrared thermal imaging (fIRT), a dynamic non-invasive method able to measure skin temperature distribution (Ring and Ammer, 2012). Facial skin thermal patterns depend on subcutaneous vessels transporting blood heat. These vessels regulate blood flow via local vascular resistance (vasodilation and vasoconstriction) and arterial pressure (Anbar, 2002). Therefore, by recording the dynamics of the facial cutaneous temperature it is possible to assess ANS activity and infer the subject's emotional state (Merla and Romani, 2007; Ioannou et al., 2013; Manini et al., 2013; Merla, 2014).

fIRT has shown its effectiveness in detecting several affective states like extreme stress (Pavlidis et al., 2007), startle (Shastri et al., 2009), fear (Levine et al., 2001), arousal (Nozawa et al., 2009), and happiness (Nakanishi and Imai-Matsumura, 2008). For example, fear experienced during a threatening and distressing situation (Nakayama et al., 2005; Kuraoka and Nakamura, 2011, Ebisch et al., 2012; Manini et al., 2013) as well as stress (Pavlidis et al., 2012) or guilt (Ioannou et al., 2013) have shown to be related to a decrease in nose temperature, due to subcutaneous adrenergic vasoconstriction (Kreibig, 2010); on the contrary, social interaction (Ioannou et al., 2014b; Manini et al., 2013) and sexual arousal (Hahn et al., 2012) produce a temperature increase due to the vasodilation effect of parasympathetic nervous system on the autonomic state of the individual. Crucially, due to its low invasiveness and versatility fIRT results particularly suitable on developmental samples as well as on clinical populations (Manini et. al., 2013; Ioannou et al., 2013; 2014a; Aureli et al., 2015).

In detail, in this study we expected to observe a weaker thermal modulation in MBS participants compared with control subjects. Our hypothesis is that any impairment in the motor system could result in defective autonomic response during the observation of characters expressing different emotions.

2. Materials and methods

2.1. Task 1: Test of Emotion Comprehension (TEC-1)

2.1.1. Participants. We recruited 10 children (four males) with MBS; ages ranged from 4 to 8 years old (mean age 5.55, SD = 1.80). MBS subjects had moderate to severe facial unilateral or bilateral paralysis, as well as related neurological symptoms (see Table 1); none were observed to have intellectual disability and all of them were attending mainstream schools at a level appropriate to their age. We also recruited 15 healthy children (control group) (9 males) in the same age range (mean age 5.60, SD = 1.45). Moebius children who took part in both Task 1 and Task 2 were recruited through the clinical center at the University of Parma, which is specialized for diagnosis and therapy of the Moebius syndrome. Based on this diagnosis only the patients with no mental retardation and with no diagnosis of autism were included in the experiments. Written assent and parent or guardian consent were obtained after full explanation of the procedure of the study, in agreement with the Declaration of Helsinki. The study was approved by the Ethical Committee of Parma.

2.1.2. Materials. The *Test of Emotion Comprehension* (TEC-1) (Pons and Harris, 2000) was administered to the child in its Italian standardized version (see Albanese and Molina, 2008). The TEC-1 consists of an A4 book (male and female versions) measuring nine different components of emotion understanding. For the aim of our study, we only focused on the component I, outlining emotions recognition by means of facial expressions. Component I presents on an A4 paper simple drawings, which include four out of five possible emotional outcomes depicted by cartoon facial expressions. The child is asked to recognize which one of the facial expressions is happy, sad, angry, scared or just alright (neutral component). Figure 1 illustrates one of the items used to assess children's emotion recognition. Children were administrated the TEC-1 on an individual basis in a quiet room. During the test, the experimenter simply named an emotion and the child had to point (nonverbal responses) to the appropriate emotion represented as facial expression; five successive test items were used to assess child's recognition of emotions. The experimenter introduced the first item saying: "Let's look at these four pictures. Who is the person who feels sad?" The four possible choices were happy, sad, angry and just alright. For the following items the emotions to be identified were "happy", "angry", "just alright" and "scared" (Figure 1). The session lasted a maximum of 10 minutes.

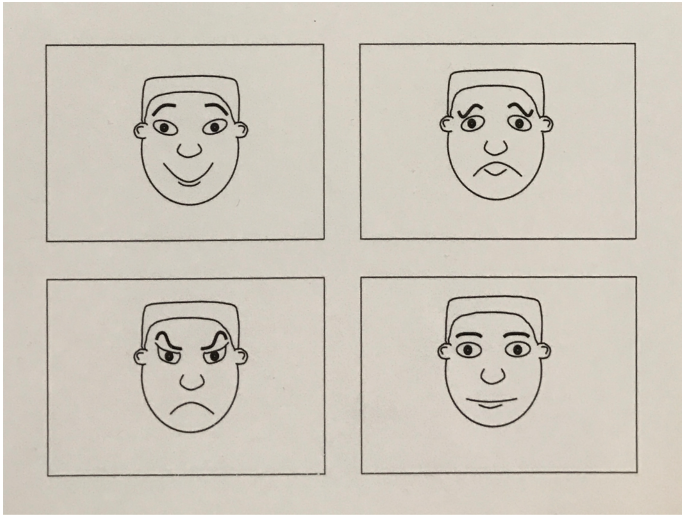

FIGURE 1. Example of cartoon pictures presented during TEC-1 (component I, emotion recognition).

2.1.3. Data analysis: Test of emotion comprehension (TEC-1) scoring. Children's answers were noted on the answer sheet and then coded giving 1 point for each correct answer and 0 for each wrong answer. According to the authors' coding system, children were given one point if they were correct on at least four of the five items. The correct outcomes were, in order, "sad", "happy", "angry", "just alright" and "scared".

2.1.4. Statistical data analysis. A non-parametric Mann-Whitney *U*-test for independent samples was used to compare Moebius (MBS) and control group. Data were analyzed by means of Statistica 8.0 (Stat-Soft, Tulsa, OK, USA).

2.2. Task 2: Thermal Imaging (IR) study

2.2.1. Participants. We recruited 9 children (five males) with MBS aged 4 to 8 years old (mean age 5.66, SD = 1.78); 7 among them participated in both task 1 and task 2 (see Table 1). MBS subjects showed unilateral or bilateral paralysis, as well as related neurological symptoms (see Table 1); they were referred for the study as cognitively able, and all of them were attending mainstream schools at a level appropriate to their age. We also recruited 16 healthy children (control group) (9 males) in the same age range (mean age 6.6, SD = 1.79); 4 among them participated in both task 1 and task 2 (see Table 1). All participants were informed that they would be videotaped by means of a thermal camera and a webcam. Moebius children who took part in both Task 1 and Task 2 were recruited through the clinical center at the University of Parma, which is specialized for diagnosis and therapy of the Moebius syndrome. Based on this diagnosis only the patients with no mental retardation and with no diagnosis of autism were included in the experiments. All parents of the participants gave their informed written consent after full explanation of the procedure of the study, in accordance with the 1964 Declaration of Helsinki. The study was approved by the Ethical Committee of Parma.

TABLE 1. Moebius subjects' medical case.

ID nr	Sex	Task	Laterality	Cranial nerves involved	Additional functional deficits and associated pathologies
1	m	Task 1, Task 2	unilateral left	VI, VII	-
2	m	Task 2	bilateral	VI, VII, III, IV	strabismus, hypotonia, hypoacusia of right ear, speech deficit (articulation-phonetic disorders), right plagiocephaly, psychomotor delay; epileptic seizures, cardiac crisis
8	m	Task 1, Task 2	bilateral	VI, VII, XII left	respiratory difficulties, micrognathia, hypotonia, psychomotor delay, club foot
3	f	Task 1, Task 2	bilateral	VI, VII, XII	feet malformations
4	f	Task 1, Task 2	unilateral left	VI, VII, XII	speech deficit, club feet
5	m	Task 1, Task 2	bilateral	VI, VII, XII	club foot, brain stem atrophy with enlargement of the fourth ventricle, hand deformities
6	f	Task 1, Task 2	unilateral right	VI, VII, XII right	micrognathia, tongue hypoplasia
7	f	Task 1, Task 2	bilateral	VI, VII	bilateral mixed hypoacusia, hypotonia, delayed growth, laryngomalacia, palatal schisis, coloboma of right optic nerve
9	m	Task 2	bilateral	VII	no ocular deficits, speech delay
10	m	Task 1	bilateral	VI, VII, VIII, XII	micrognathia, psychomotor delay, palatal schisis, reduced opening of the jaw, hand malformation; Poland Syndrome
11	f	Task 1	unilateral right	VI, VII	-
12	f	Task 1	bilateral	VII	no ocular deficits

TABLE 1. Moebius subjects' medical case. The term "Laterality" refers to the kind of facial paralysis that can be unilateral or bilateral; the sixth and seventh cranial nerves are usually involved, but other nerves may also be affected; "Associated pathologies" linked to Moebius syndrome can involve possible hands and feet anomalies, muscles hypotonia, hypoacusis, swallowing and speech problems, Poland syndrome.

2.2.2. Materials. Thermal IR imaging was performed 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 the children's behavior so as to record their level of attention while watching video stimuli.

2.2.3. Procedure and stimuli. Previous to testing, each participant was left to acclimatize for 10 minutes to the experimental room, to allow the skin temperature to stabilize. The recording room was set at standardized temperature (23°C), humidity (50–60%) and without direct sunlight, ventilation, airflow. During a first neutral interaction, the experimenter asked the child to answer some questions on personal data (e.g. name, age). Then, the child was invited to watch a series of video stimuli displayed on a computer monitor (32.5 X 22.7 cm) placed 60 cm far from the chair where he/she was sitting. Each child, at the end of each short video-clip, underwent a series of questions concerning the stimuli just visualized and his/her own arousal (see Supplementary Materials). According to other thermal imaging studies (Merla and Romani, 2007; Pavlidis et al., 2001; Ioannou et al., 2016), our sequences included 6 different video-clips that were presented in the same order (neutral baseline-happy-neutral baseline-sad-neutral baseline-scaring), with each emotional video stimulus preceded by a neutral video stimulus. Chosen stimuli were short clips in which the main character of the scene was experiencing a happy, sad, or scaring situation. The video clips had variation in their duration (mean = 81.38 sec; SD = 43.49), while neutral ones (video-clips with no emotional content) lasted about 30 sec (mean = 28.83 sec; SD = 3.69) (Figure 2). Stimuli were chosen in order to create a naturalistic setting and reproduce a context typical for children of this age.

Video-clips were previously validated in order to ensure that they were easily comprehensible and able to represent a specific emotion for the age range of our interest. We presented neutral baseline, happy, sad, scaring videos to a group of 16 children (8 males) with mean age of 7.5 years; participants were asked to categorize the emotion evoked by each of the video clip as “neutral baseline”, “happy”, “sad” and “scaring”. The average percentage of recognition was 95.83%. Based on our validation study, we presented randomly two sequences from a list of six. The choice of presenting two sequences only was due to children’s fatigue, habituation and difficulty in sustained attention for long periods of time.

During the experimental session, thermal and video camera were placed above the monitor one meter away from the participant, were automatically calibrated and manually fixated to allow a frontal recording of the child’s face. Facial thermal images and videotapes were recorded during each video presentation.

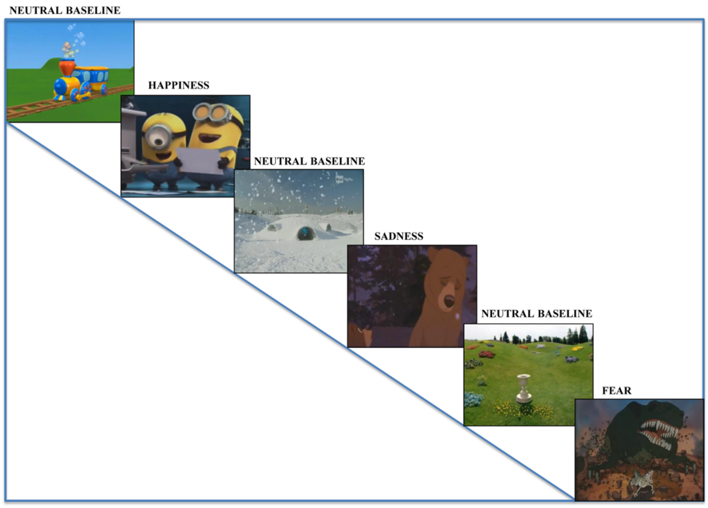

FIGURE 2. Experimental Paradigm. Example of one of the sequences presented to the participants.

2.2.4. Data analysis: Thermal data analysis. A quantitative analysis was carried out to measure temperature variations of the face, in particular of the nasal tip. Thermal signals were extracted through the use of Morphing GUI software, developed with homemade Matlab algorithms (The Mathworks Inc., Natick, MA). This analysis procedure is more extensively described in Paolini et al. (2016). According to previous studies in humans and primates (Kuraoka and Nakamura, 2011; Nhan and Chau, 2010; Shastri et al., 2009), we focused on the nasal tip for two main reasons. The first is that, due to the relatively low incidence of Moebius syndrome on the general population, the specific age sample of our interest and the pioneering nature of the current study, we decided to include both unilateral and bilateral patients. In this regard, nasal tip is a non-lateralized region of interest (ROI), so its temperature it is not expected to be modulated by the lateralization of nerves' impairment. Secondly, nasal tip has shown to be particularly sensitive to emotional states transition (Manini et al., 2013). This area is indeed highly innervated by adrenergic fibers, resulting a privileged window on subject's autonomic state. Specifically, sympathetic nervous responses to emotional and distressing stimulation produce a decrease in nasal tip temperature whereas parasympathetic response results in temperature increase of this ROI (Roddie, 1963; Nakanishi and Imai-Matsumura, 2008; Shastri et al., 2009; Nhan and Chau, 2010; Ebisch et al., 2012, Manini et al., 2013; Aureli et al., 2015). Due to the high computational load associated with the morphing procedure (Paolini et al., 2016), we decided to subsample the dataset collected. Since the slow nature of thermal response, such processing choice did not affect in any way our precision in detecting temperature changes (Paolini et al., 2016; Manini et al., 2013). In this case, for each video stimulus administrated to the infant, three thermal images were extracted (one frame at the beginning, one in the middle and one at the end of each video) and morphed. The frames were specifically selected in order to minimize the effect of the respiratory cycle on the thermal imprinting of the subject (Ebisch et al., 2012). The three frames selected within each condition (emotional or neutral) were averaged. In order to eliminate the inter-individual variability in the subjects' temperature and to minimize the effect of participants' circadian variations on our data, we followed a typical procedure of thermal data analysis (Manini et al., 2013), consisting in the subtraction of the mean thermal value of each neutral condition from the mean thermal value of its following experimental condition (happiness, sadness, fear). In this way, we obtained a dataset of thermal variation for each condition respect to the relative neutral condition. Then, the thermal variations for the two trials belonging to the same condition were averaged between them, in order to obtain a synthetic value for each emotion (happiness, sadness, fear) to be used as variable in the statistical analysis (Figure 3) and to carry out a comparison between the MBS participants and the control group.

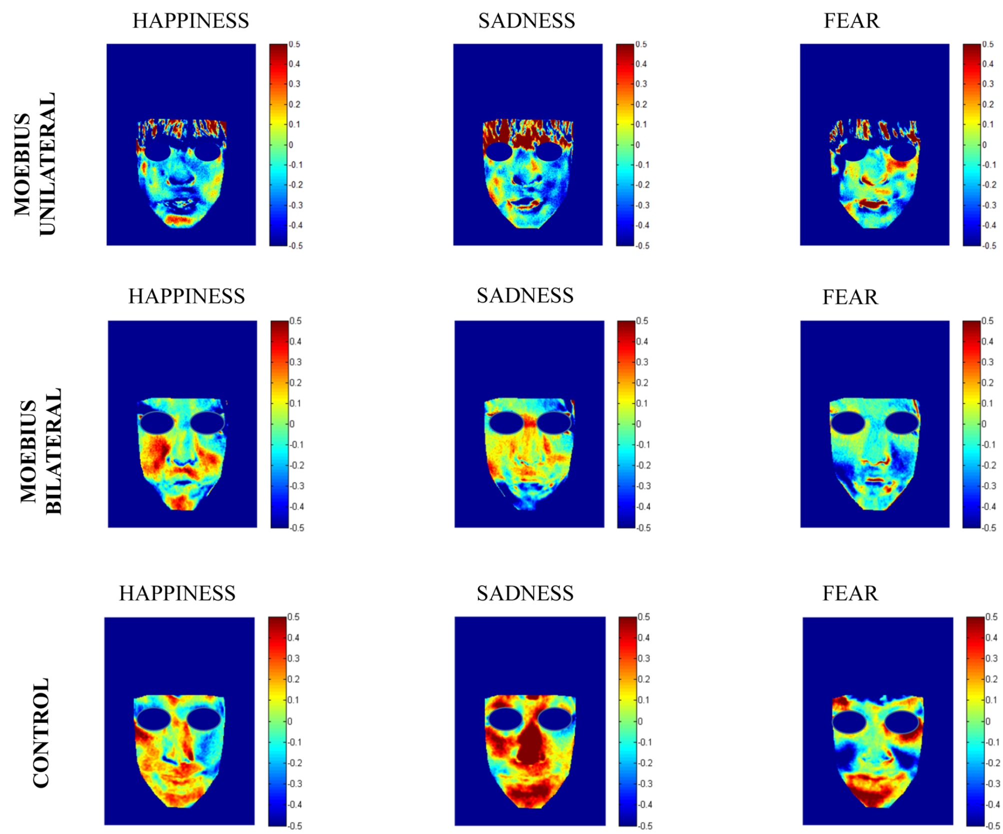

FIGURE 3. Example of thermal modulation in a control and in two Moebius patients (unilateral and bilateral paralysis) during the "happiness", "sadness" and "fear" condition. The control subject shows a stronger thermal variation during the sadness condition than the MBS patients.

2.2.5. Statistical Data Analysis. Repeated measures (3 X 2) ANOVAs were performed on the obtained mean nasal tip variation of the three conditions respect to the baseline for all participants. The conditions (happiness, sadness, fear) were set as within-subject factor, while the 2 groups (MBS children and control subjects) were set as between-subject factor (Ardizzi et al., 2015; 2016). Newmann-Keuls post-hoc followed the two-way ANOVA. If data violated sfericity assumption, we reported Greenhouse-Geisser ($\mathcal{E} < .75$) or Huynh-Feldt ($\mathcal{E} > .75$) corrected values. One control subject was excluded from data analysis because considered as 'outlier'. Data were analyzed by means of Statistica 8.0 (Stat-Soft, Tulsa, OK, USA).

3. Results

3.1. Task 1: Test of Emotion Comprehension (TEC-1)

Mann-Whitney *U*-tests were performed to assess if control and MBS subjects' scores significantly differed during the *Test of Emotion Comprehension (TEC- 1)* administration. The results showed that MBS children had a lower level of emotion recognition (mean = 3.18; SD = 1.51) than control children (mean = 4.80; SD = 0.41) (U = 28.50; p = 0.007; r = 0.51) (Figure 4).

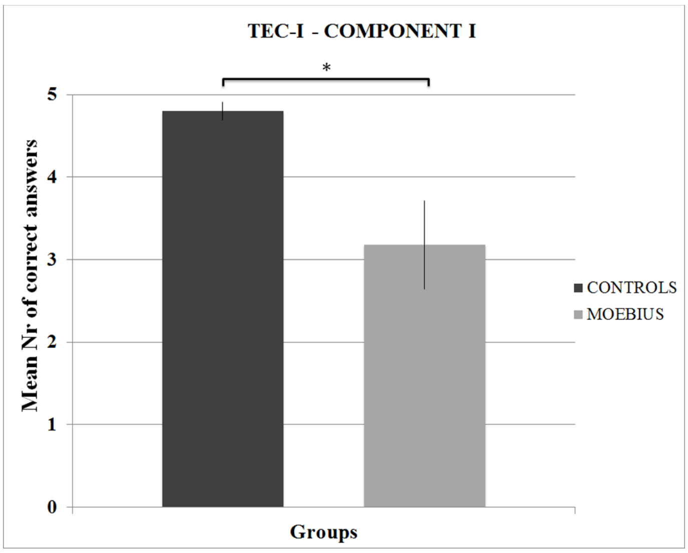

FIGURE 4. Number of correct answers in both control and MBS subjects. During the emotion recognition task *(TEC-1)* control participants performed better than MBS participants.

3.2. Task 2: Thermal Imaging (IR) study

3.2.1. Group temperature variation in relation to conditions

A repeated measures ANOVA (3 X 2) was performed on the re-sampled variations of mean temperatures of the nasal tip. We did not find any difference between the two groups ($p = 0.185$). The results highlighted a significant emotion condition effect ($F_{(1.40,\ 30.91)} = 5.330$; $p = 0.018$; $\eta^2 = 0.195$); post-hoc tests showed that nasal temperature during the sadness condition significantly increased compared with happiness ($p = 0.040$) and fear ($p= 0.003$). No significant difference was observed between fear and happiness ($p = 0.184$) (Figure 5). The interaction group x condition was not significant ($p = 0.729$).

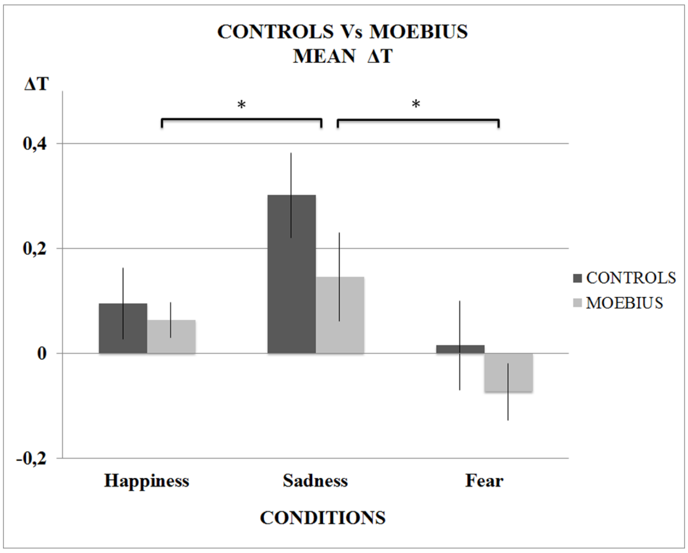

FIGURE 5. Variations of mean temperature values during each of the experimental condition with respect to the neutral condition. Both control and MBS subjects show a significant nasal temperature increase during the “sadness” condition.

During all of the experimental conditions MBS participants, compared to controls, exhibited a less appreciable thermal modulation while watching emotional enhancing stimuli. To measure a possible different intensity of thermal modulation between the two groups we considered the absolute values of temperature variations. As previously suggested, control subjects showed a larger thermal response compared to MBS participants during each experimental phase (Figure 6).

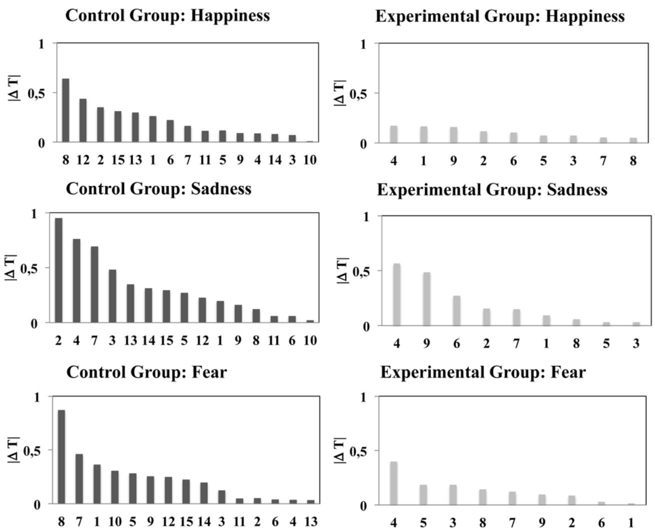

FIGURE 6. Absolute values of temperature variations per subject during each of the experimental condition. MBS subjects show a lower thermal modulation compared with control subjects.

Repeated measures ANOVA (3 X 2) performed on the absolute values of temperature variations revealed a significant main effect for group factor ($F_{(1, 22)} = 4.732$; $p = 0.041$; $\eta^2 = 0.177$). Control subjects showed higher temperature values (0.26 ΔT) than MBS subjects (0.15 ΔT) (Figure 7). No significant difference was observed among conditions ($p = 0.125$). The interaction group x condition was not significant ($p = 0.993$).

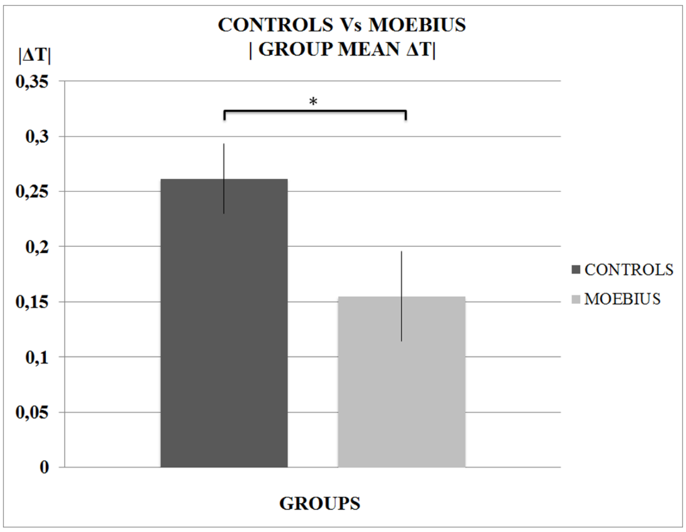

FIGURE 7. Group mean absolute temperature values in control and MBS subjects. Control subjects show a significant more intense thermal modulation compared with MBS participants.

4. Discussion

4.1.Facial emotion recognition ability in children with MBS

We first examined emotion recognition ability in 10 children with MBS by means of a standardized test, TEC-1. Compared with control subjects, MBS participants showed impairments on emotion recognition task, displaying lower scores than healthy children of comparable age. These findings are in line with the hypothesis that motor control of the muscles involved in the emotional display has a role in facial expression recognition (Oberman et al., 2007; Stel and van Knippenberg, 2008; Wood et al., 2016; Niedenthal et al., 2010; Künecke et al., 2014). Seemingly, activation of somatosensory representations and facial musculature movements while observing emotions is fundamental for emotion recognition (Lundqvist and Dimberg, 1995; Blakemore and Frith, 2005; Ponari et al., 2012; Oberman et al., 2007); thus, the understanding of the facial expressions of others may rely on the capacity of activating facial muscles involved in facial expressions of emotions. Such findings are also compatible with the reverse simulation model, which proposes that the preservation of the cortical control of the facial muscles is necessary (Goldman and Sripada, 2005) to achieve a full comprehension of the emotional state of the other. Considering that MBS facial paralysis is present since birth, we can hypothesize mild deficits in the production of a fully functional mirror neuron network during the early stages of life. According to a developmental theoretical account (Ferrari et al., 2013; Tramacere et al., 2016), after birth, facial expressions synchronization with caregivers is critical to create a link between the "self" and the "other" and to ensure the shaping of the mirror mechanism adjusted to support social communicative functions. Indeed, neonates are able to recognize and respond to social signals from birth, engaging in reciprocal and emotional face-to-face interactions with their mothers. These exchanges, including facial and vocal expressions and gestures, immediately present after birth and in the first month of life, can be important for the development and functioning of the mirror neuron system (Meltzoff and Moore, 1977, 1983; Marshall and Meltzoff, 2011). Recent findings have shown that based on such mother-infant face-to-face exchanges, the capacity of neonates to increase social expressions is influenced by their ability to produce appropriate facial expressions and is correlated to mother's skill to mirror or mark such expressions. We do not know how such early experiences could impact on brain development and this requires further investigations related to brain activities in motor cortical regions during such interactions in early development. In this context, a few reports tested the capacity of MBS patients to recognize emotions, and results are contrasting (Giannini et al., 1984; Bate et al., 2013; Calder et al., 2000; Bogart and Matsumoto, 2010a). Most of these studies tested few adult patients, with significant inter-individual variability. In a single study the sample size consisted of a considerable number of adult patients (Bogart and Matsumoto, 2010a), and the authors did not find any evidence of facial emotion recognition deficits. However, it must be noted that this study suffers from some critical methodological limitations. First, it's a completely Internet-based study with all tasks computerized and performed at home; thus, there has not been direct assessment of the subjects' performance and of their neurological deficits. Secondly, a total of 31 participants out of 37 indicated through self-report that they had been diagnosed with MBS, while six subjects stated they had not been formally diagnosed with the syndrome.

Our study is the first to investigate with a relatively large sample of very young patients the effects of facial muscles paralysis on both emotion recognition and autonomic response. The investigation of these issues early in the development is critical for the detection of mechanisms of emotion recognition at a stage where more complex cognitive strategies might not compensate yet their deficits. In this regard, a large amount of literature has focused on how and when children's decoding of emotions develops. In the early stages of postnatal development it has been shown that infants discriminate different facial expressions, and respond appropriately to different emotions displayed by the caregiver (Field et al., 1982). Furthermore, infants are capable of mimic some facial expressions, such as smile, suggesting an early capacity to match own and others' facial expressions (Meltzoff and Moore, 1977). Even if typically developing children start to use verbal labels to identify discrete categories of emotions by four and five years of age (Bretherton et al., 1986; Izard, 1971; Stifter and Fox, 1987; Walden and Field, 1982), children normally begin to understand the basic emotional display rules from eight years of age (Custrini and Feldman, 1989; Van Meel et al., 1993; Hortaçsu and Ekinci, 1992) when their abilities become comparable to adults' ones (Boone and Cunningham, 1998).

Despite our findings show that MBS children have indeed some deficits in recognizing emotions, nevertheless they are still capable to socially interact with others and to appropriately understand the emotional content of complex stimuli as those presented with cartoons. This finding suggests that probably several cognitive processes are used by MBS subjects in order to understand the emotional content of complex stimuli. It is possible that even though subtle aspects of the emotion recognition are impaired as consequence of altered facial mimicry, the plastic changes of the brain occurring during development and the exploitation of other cognitive strategies can be employed by MBS subjects. Indeed, if the absence or reduction of motor representations did generate some deficiencies on their early facial expression recognition mechanism (as a consequence of having limitations in controlling facial muscles), it is admissible that individuals, during development, might have learned to cognitively deduce the emotional states of others by using a number of visual cues related to the face and the contextual stimuli provided by the environment (Gross and Ballif, 1991; Denham and Couchoud, 1990). By exploiting such cues, people can extract regularities and develop a conceptual knowledge of an emotion (Wood et al., 2016).

4.2.Thermal response to emotional video cartoons in children with MBS

The second part of our study was conducted by detecting psychophysiological responses in MBS children by means of a fIRT camera. Thermal imaging is an ecologic non-invasive method, which does not require specific contact sensors. MBS and control participants were asked to observe two sequences of emotional cartoon video stimuli representing three main emotions, namely happiness, sadness, fear. Nasal temperature variations were measured during the observation of the stimuli. Results showed a significant difference among emotion conditions.

Our findings show that MBS participants exhibited a weaker modulation of the ANS response while watching emotional enhancing stimuli, if compared with controls. In particular, both MBS and control subjects showed a nasal temperature increase during the “sadness” condition, but MBS participants were characterized by a less pronounced nasal temperature variation across all of the three experimental conditions. Increased nasal tip temperature during the “sadness” condition is supported by literature (Kreibig, 2010). Actually, recent studies investigating the effects of autonomic nervous system during emotion focused on a dual sympathetic-parasympathetic co-activation in response to a “sadness” condition. While crying sadness has been associated with a sympathetic activation, a parasympathetic activation seems typical of sadness not involving crying. More specifically, an activating sadness response (crying status) appears to be typified by increased cardiovascular sympathetic response and changed respiratory activity, while a deactivating sadness response (non crying status) has been distinguished by a sympathetic withdrawal. Several studies using video clip stimuli to induce sadness, have found a decrease of the heart rate and electro-dermal activity (Kreibig, 2010). These results are in line with our thermal findings. Although temperature increased in both groups during sadness condition, Moebius patients showed a general weaker thermal response even if the difference between groups was not significant. This is probably due to the inter-individual variability of the participants' thermal response to each emotion. For this reason, we considered the absolute values of participants' thermal responses (independently of the direction of thermal variation with respect to neutral condition) in order to highlight the differences between groups. Our data revealed a significant difference between groups, in particular we observed a weaker and non-specific thermal response of Moebius children with respect to controls.

The diminished temperature changes observed in MBS patients could be ascribed to a minor modulation of the ANS parasympathetic component, generally responsible for vasodilation. In fact, the ANS is involved in the cardiovascular (Garbey et al., 2007), metabolic (Pavlidis et al., 2007) and cutaneous thermal responses (Merla et al., 2004; Ebisch et al., 2012).

This differential intensity of thermal change could be interpreted in terms of the strict link existing between the action-perception mechanism, which contributes to sensorimotor simulation and to the process of recognition of others' emotions, and the synchronized changes in the autonomic system (Panksepp, 2005; Iacoboni, 2009; Carr and Winkielman, 2014).

Neuroimaging studies have shown that the observation and production of facial emotional expressions activate similar patterns of brain areas (Wicker et al., 2003; Carr et al., 2003). More specifically, in addition to the temporo-parietal-frontal areas, which are core in the action-observation network, other regions such as the amygdala, the ACC and the anterior insula show an overlapping activation during both imitation and observation of facial emotional expressions (Carr et al., 2003). These regions are involved not only in processing the emotional content of a stimulus, but also in coordinating the physiological responses associated to the emotions (Wicker et al., 2003; Caruana et al., 2011; Jezzini et al., 2012; Caruana et al., 2016). Electrical stimulation of the anterior insula in the monkey has revealed that this region is composed by several sectors and that different autonomic responses and facial motor patterns can be elicited by its stimulation (Caruana et al., 2011). This strengthens the proposal of a tight link between the production of emotional facial expressions and the physiological modifications associated to them. Our findings seem to echo such studies and their theoretical perspective in that MBS children show impaired autonomic responses compared to age-matched controls.

Summing up, Moebius patients' decreased capacity to activate a motor simulation process during the decoding of emotions could interfere also during the observation of complex stimuli of emotional content. These videos depict characters expressing emotions within a context. It is therefore possible that both the context and the dynamics of the interaction in which facial expressions are expressed may contribute to the changes in autonomic activity. Questionnaires, administered after each video-clip, although completed only by a limited number of participants, indicated that Moebius patients correctly understood the emotional content of the scene, but the diminished thermal response might have hampered the amplitude of the subjective experience of the emotional content of the video. Patients' impairments in mimicking seem therefore to affect not only their cognitive processes of emotion recognition, but also the way these latter are related to the autonomic changes associated to emotions. Further studies should address more in depth the relation between the level of deficits in recognizing emotions and the magnitude of the autonomic response in order to better understand the possible cause-effect relationship.

A few methodological limitations of our study have also to be addressed. Cartoon stimuli were different in length; differences in duration between videos are linked to our specific aim to be consistent with an authentic content able to induce a particular emotion. Since emotional content is the actual variable expected to influence thermal values, the differential duration of the stimuli alone wouldn't have affected thermal results, given the slow dynamic of thermal response. This is further confirmed by our main result showing a difference between the experimental and control group that was independent of stimuli duration.

Additionally, concerning the first task, it would have been very interesting to evaluate children's emotion recognition at different ages and/or gender differences; however, the extreme rarity of the syndrome, the limited age-range taken into account and the exclusion of patients with autism or mental retardation let us to include only a limited number of subjects (10 subjects), which did not permit to conduct further analysis. Despite the challenges that studies of gender differences must face, as the necessity of a large sample, we think it will be worthwhile to increase our sample and explore such differences.

Unfortunately, a correlation between task 1 and task 2 could not be run because not all children who took part in the first experiment did complete the second one as well. Indeed, the two tasks required different levels of collaboration by the children; the emotion recognition task required to explicitly respond to the researcher, but some of the participants did not talk because of embarrassment or fatigue; to the contrary, thermal imaging just requested cartoons' watching, but some of the children had to be discarded because of their excessive movement or drops of attention.

Lastly, although IR thermal imaging is an extremely at the forefront technique allowing ANS recording in a naturalistic setting, the thermal signal as a result of perspiration, muscles activity and metabolism is rather sluggish. Nevertheless, the reliability and feasibility of IR thermal imaging have been confirmed by several comparisons with other standard methods of ANS measurement such as electrocardiography (ECG), skin conductance or galvanic skin response (GSR) (Kuraoka and Nakamura, 2011). Being this technology still in development, the need to determine if heat patterns mean discrete emotions (Ekman, 1992) or dimensional responses (Russell, 2003) is still ongoing; according to this, it would be very useful to integrate this method with other techniques so as to compare more ANS measurements within the same paradigm.

An interesting aspect that should be further explored is the link between the higher number of errors performed by Moebius children during emotion recognition (task 1) and the weaker thermal response to emotional stimuli (task 2). Unfortunately, we can not compare the two tasks because not all of the participants took part in the task 1, further studies involving a sample of older children will be very useful to elucidate the link between the number of errors performed during (TEC-I) and the thermal ANS response to emotional stimuli.

Supplementary materials

Task 1: *Test of Emotion Comprehension* (Component I)

Materials and methods

The *Test of Emotion Comprehension* (TEC-1) (Pons and Harris, 2000) was administered to the Moebius and control children. The TEC-1 consists of an A4 book (male and female versions) measuring nine different components of emotion understanding. For the aim of our study, we only focused on the component I, outlining emotions recognition by means of facial expressions. Each child was asked to recognize which one of the facial expressions was happy, sad, angry, scared or just alright (neutral component). Supp. Figure 1 illustrates the 5 items used to assess children's emotion recognition. During the test, one of the experimenters simply named an emotion and the child had to point (nonverbal responses) to the appropriate emotion represented as facial expression; five successive test items were used to assess child's recognition of emotions. The experimenter introduced the first item saying: "Let's look at these four pictures. Who is the person who feels sad?" The four possible choices were "happy", "sad", "angry" and "just alright". For the following items the emotions to be identified were "happy", "angry", "just alright" and "scared" (Supp. Figure 1). Depending on children's own gender, a corresponding version of the table with either female or male drawings was presented.

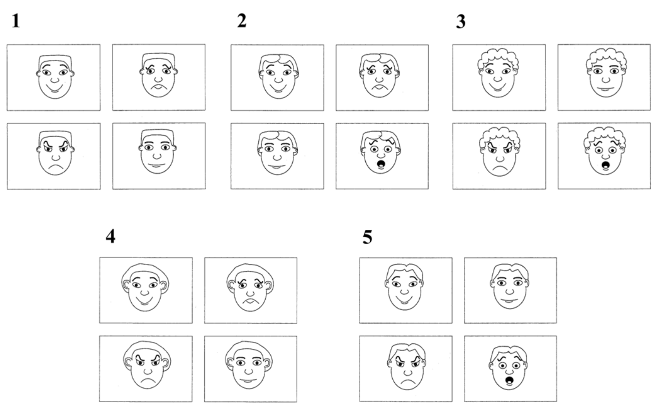

Figure 1. Example of the 5 tables of cartoon pictures presented during TEC-1 (component I, emotion recognition).

Results

We explored, separately, the unilateral and bilateral patients with respect to the TEC scores so as to investigate potential differences between unilateral and bilateral patients. It was not possible to apply any statistical analysis given the low number of participants per group; however, the TEC average score of unilateral and bilateral patients was very similar (mean $_{unilateral}$ = 2.25; SD $_{unilateral}$ = 2.06; mean $_{bilateral}$ = 3.50; SD $_{bilateral}$ = 1.37). This indicates that even if half of the face is preserved, the performances of the unilateral patients are still affected (Supp. Figure 2).

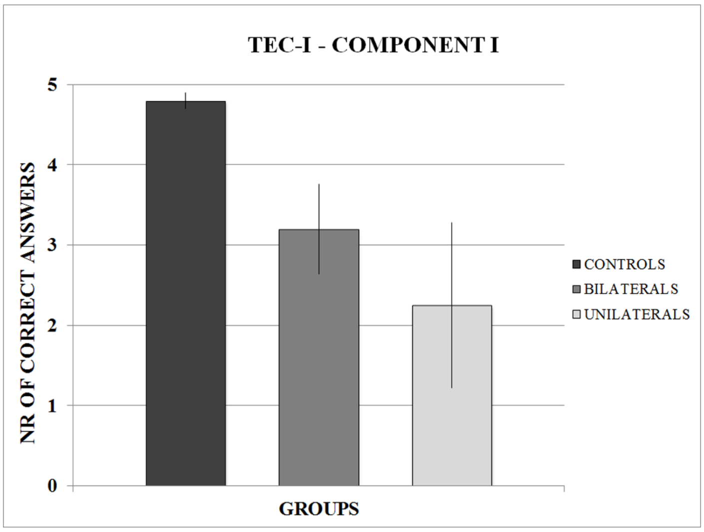

Figure 2. Number of correct answers in controls, bilateral and unilateral MBS subjects. During the emotion recognition task *(TEC- 1)* control participants performed better than MBS participants.

Task 2: Thermal Imaging (IR) study

Materials and methods

During the Task 2 we recruited 9 children with MBS aged 4 to 8 years old (mean age 5.66, SD = 1.78) characterized by unilateral or bilateral facial paralysis, and 16 healthy children (control group) (9 males) in the same age range (mean age 6.6, SD = 1.79). Children were asked a series of questions concerning the emotional state of the main characters depicted in the video cartoon, and children's emotional involvement and arousal (see below). Specifically, each child was first asked to watch 2 sequences of a series of video stimuli (neutral baseline-happy-neutral baseline-sad-neutral baseline-scaring) displayed on a computer monitor; after each short video-clip, he/she was asked to answer a few questions about the stimulus just visualized (see the questions below). Unfortunately, not all the children responded to the questions and therefore it was not possible to apply a statistical analysis.

The questions were asked as follows:
1) How does the main character of the video-clip feel?
The possible 5 outcomes were: “Happy”, “Angry”, “Sad”, “Scared” and “Neutral”.
2) How strong was the main character’s emotion (1 to 5)?
(The numbers 1 to five corresponded to pictures of faces varying in size from the smallest to the biggest one) (Supp. Figure 3).
3) How did you feel while watching the cartoon video?
The possible five outcomes were: “Happy”, “Angry”, “Sad”, “Scared” and “Neutral”.
4) How strong was your emotion (1 to 5)?
(The numbers 1 to five corresponded to pictures of faces varying in size from the smallest to the biggest one).
The child was always asked to point on a small panel (Supp. Figure 3) the size of the face depicting the facial expression his/her answer.

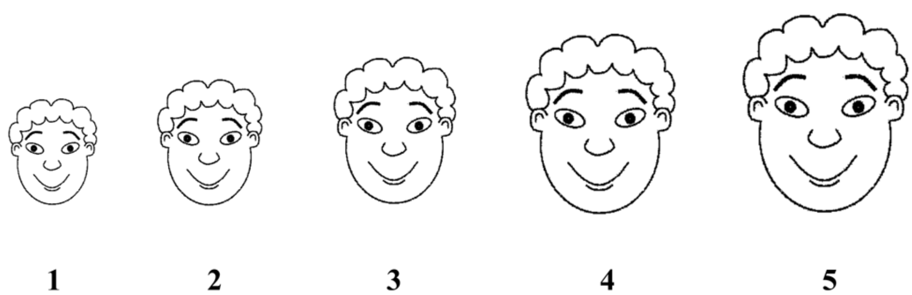

Figure 3. Example of the pictures of faces varying in dimension presented during Task-2.

Results

Given that only 10 children (5 Moebius, 5 controls) were collaborative and answered some of the questions, no statistical analyses have been applied to these data. Below we report the partial results obtained.

Table 1 shows the percentage of correct answers to questions 1) and 3) (listed above). Table 2 represents the median number indicative of the arousal of the character of the video-clip and of the child while watching the video cartoon (see questions 2) and 4)).

	Neutral		Happy		Sad		Scaring	
Questions	Moebius	Controls	Moebius	Controls	Moebius	Controls	Moebius	Controls
1	20%	7%	70%	60%	70%	90%	50%	40%
3	na	20%	70%	40%	40%	na	60%	20%

Table 1. Number of correct answers to questions 1) and 3) in both Moebius and control group. Na numbers correspond to not available responses from the children.

	Neutral		Happy		Sad		Scaring	
Questions	Moebius	Controls	Moebius	Controls	Moebius	Controls	Moebius	Controls
2	5	5	5	5	5	5	4	5
4	2	4	2	4	2	4	2	4

Table 2. The numbers 1 to 5 were used to quantify the arousal of the main character of the video-clip (question 2) and of the child while watching the video cartoon (question 4). Median numbers per each emotional video-clip are reported. It is worth noting that Moebius children always reported to have a lower level of arousal than controls while watching the stimuli.

Acknowledgements

I am really grateful to all the children and their families for their patience and their incredible efforts to help this research through the numerous visits and long trips from all over Italy to reach the lab. I also would like to thank the Associazione Italiana Sindrome di Moebius for the continuous work and support to our research. Lastly, I would like to thank the *Centro Diagnostico Europeo Dalla Rosa Prati,* which supported these studies.

4 Study 2

Impaired recognition of emotional facial expressions in children with facial palsy. The case of Moebius syndrome

1. Introduction

It has been suggested that some emotions are primary and associated with distinct facial expressions (Ekman, 1973) that correspond to subjective emotional experience (e.g., Ekman et al., 1980; for a review see, Matsumoto et al., 2008). The discrimination of other's facial expressions is an integral component of interpersonal communication, and the capacity of infants to respond to the facial expressions of the caregiver indicates a precocious tuning of the human brain to emotional communication (Ekman, 1986; Tickle-Degnen, 2006; Murray et al. 2016).

According to motor theories of perception, observing other’s facial expression activates sensorimotor representations involved in the execution of that expression, facilitating recognition processes (Adolphs, 2006; Niedenthal et al., 2010). In particular, our own emotional information would be achieved through both somatovisceral and motoric re-experiencing of emotions observation (Barsalou, 2008; Iacoboni, 2009). The ‘‘mirror neuron system’’ (MNS), is assumed to be the key mechanism of this shared representation (Gallese, 2001; Rizzolatti and Craighero 2004; Ferrari et al., 2003; Ferrari and Gallese, 2007; Ferrari et al. 2012; Rizzolatti and Sinigaglia, 2008). And it provides a foundation for the idea that knowledge of the others' behaviours is essentially grounded in our own experiences. When individuals observe an action, their mirror neurons become active as if they acted themselves. This mechanism would support action understanding and the close relationship between the external manifestations of facial/body simulation with the inner imitation of the feeling associated with that specific emotion (Gallese, 2001, Gallese, 2006). In addition, further studies demonstrated that viewing of facial expressions recruited similar brain regions concerned with motor as well as somato- and limbic sensory processing (Hennenlotter et al. ,2005; van der Gaag et al., 2007). This process seems to be present since very early in ontogeny as suggested by the capacity of newborns primate infants to imitate facial gestures and to recognize when being imitated (Meltzoff and Moore, 1977; Ferrari et al. 2006; Sclafani et al. 2015).

To recognize facial expressions, important source of information are the facial feedbacks generated when an observer automatically mimics the expressions of others' faces (Goldman and Sripada, 2005; Niedenthal et al., 2010). Previous studies showed that when individuals were exposed to emotional faces, they spontaneously reacted with distinct facial electromyographic (EMG) reactions in emotion-relevant facial muscles, a mechanism named "facial mimicry" (Dimberg, 1997a, 1997b; Dimberg and Thunberg, 1998; Dimberg et al., 2002; Dimberg, 1982; Lundqvist and Dimberg, 1995; Ardizzi et al., 2016). This spontaneous and automatic mechanism enables to predict what the other person is feeling and may contribute to generate an understanding of others' intention (Goldman and Sripada, 2005; Niedenthal et al., 2001).

In fact, the subthreshold muscle contractions in the perceiver's face would generate an afferent muscular feedback signal from the face to the brain. This involuntarily mimicking emotional expressions of others supports the understanding of these emotions by simulating the corresponding physical experience and possibly mental states in the perceiver (Gallese et al., 2004; Gallese, 2005).

Researchers have attempted to artificially inhibit participants' facial movements (Niedenthal et al. 2001; Oberman et al., 2007), and their results demonstrated a reduced capacity to perform facial expressions affected subjects' ability to recognize emotions. Specifically, the authors requested participants to perform a facial expression recognition task holding a pen in their mouth. They found that blocking facial muscles involved in mimicry impaired recognition of the emotions that engaged those muscles. This demonstrated a close relationship between the ability to express facial emotions and the ability to recognize facial expressions expressed by others (Oberman et al., 2007; Wood et al. 2016). Similarly, further studies demonstrated that blocking participants' mimic muscles interfered with the recognition of stimuli representing facial expressions (Hennenlotter et al., 2009; Ponari et al., 2012; Davis et al., 2010; Kim et al., 2014).

Other studies have yielded evidence of a lower face recognition performance in Parkinson's disease (PD) patients than in matched healthy controls (e.g., Alonso-Recio et al., 2014, Ariatti et al., 2008, Clark et al., 2008; Clark et al., 2010, Sprengelmeyer et al., 2003; Suzuki et al., 2006; Gray and Tickle-Degnen, 2010; Argaud et al., 2016). Parkinson's disease classically involves impaired movement initiation, rigidity, tremors, and postural instability but, in these patients, expressivity was reduced not only in the face but also in the body and voice (Tickle-Degnen and Lyons 2004). Even if it is not clear which mechanisms may contribute to face processing deficits in these patients, according to Embodied Simulation Theory, the link between the reduced facial musculature control and deficit in emotion perception may be the result of PD patients' inability to simulate emotional expressions (Marneweck et al., 2014).

Studying facial expression recognition process on patients with facial palsy is another empirical strategy to assess the effect of the muscle impairment on facial expression recognition. Among facial palsies, Moebius syndrome (MBS) is the most interesting condition because it is a neurological condition present from birth and it mainly affects children of normal intelligence and cognitive development (Bianchi et al., 2009, 2010). It is characterised by weakening or paralysis of the muscles in the face, which directly control expression and lateral eye movements, and it is often accompanied by limb, orofacial, muscular and oculomotor deficits (Briegel, 2006; Möbius, 1888; Verzijl et al., 2003; Verzijl et al., 2005). MBS patients are unable to smile or frown, blink their eyes, or suck. Predominantly the sixth and seventh cranial nerves are involved even if other nerves may also be affected causing problems with feeding, speech difficulties and dental problems. These nerves are either absent or underdeveloped, resulting in bilateral or unilateral facial palsy when only one side is affected.

Despite the relevance of these patients in demonstrating the importance of the simulation mechanism in emotion processing, only few studies on MBS patients' facial expression recognition ability are currently present in literature, and their results are not conclusive.

Giannini and colleagues' study (1984) examined facial expression processing in a 36 years old female with MBS and compared the patients' results with approximately 300 control participants. Participants were presented with a series of gambling task video-clips and they had to determine the amount of money at risk after viewing the faces of slot machine players. The authors found the inability of the patient to perform the task. Authors suggested that the deficit in facial expression recognition was probably related to patient's inability to succeed in the task.

Calder and colleagues (2000) provided evidence that three adult people with bilateral MBS (mean age: 28,7 years) were considerably better at recognising facial expressions than the person tested by Giannini et al. (1984). They were able to recognize basic facial expressions, despite some difficulties in a more complex task. In particular, they showed a relatively mild deficit affecting the recognition of morphed facial expressions.

Bogart and Matsumoto (2010a) carried out an online assessment of facial expression recognition in 37 adult Moebius participants. The majority of them had produced a self-report about the diagnosis of MBS. The authors used a facial expression recognition task selecting a set of 42 validated photos representing seven emotions (anger, contempt, disgust, fear, happiness, sadness, and surprise). Participants observed the images as long as necessary. Then, they identified the stimulus by selecting the correct response from a list of possible response choices. Results showed that facial expression recognition accuracy did not differ from the matched control group and, according to these authors. Results did not support the hypothesis that embodied simulation mechanisms with facial mimicry are necessary for facial expression recognition.

In a more recent study, Bate and colleagues (2013) examined the ability of six Moebius participants to recognize and imagine facial expressions. Their results fit with those of Calder et al. (2000), suggesting that MBS patients present difficulties in expression recognition but their deficits are not absolute, in fact, only some facial expressions were impaired. Their conclusions did not support the embodied simulation theory of the expression recognition.

These results highlight three aspects that must be taken into consideration: the existence of compensatory strategies, the dynamic character of facial expression and the necessity to disentangle recognition and labelling. First, adults with MBS have never mimicked facial expressions in their lives. It is possible that they have developed compensatory cognitive strategies for facial expression recognition reducing the relevance of facial feedback in emotion recognition. For example, through associative connections between concepts, contexts and somatic states (associative learning), they would acquire the ability to recognize others' emotions and normally perform. In fact, although facial expression plays an important role in facial expression recognition, people also use other expressive channels to communicate (e.g., gestures, posture, proximity) (Calbi, 2017; de Gelder et al., 2015) and the voice (e.g., prosody, language) (Liebenthal et al., 2016) and these channels play a supporting role to social interaction (De Stefani et al., 2016; De Stefani et al., 2013; Innocenti et al., 2012). Over the years, these patients may likely have compensated for their facial paralysis by increasing their use of these channels.

In the present research, we aimed to study the ability of young children with MBS to recognize basic facial expressions. If compensatory skills are developed over time, children with MBS will perform worse in facial expressions recognition task than an age matched control group.

Secondly, previous studies tested MBS people's ability to recognize facial expressions by means of static photos displaying emotions (Bogart and Matsumoto, 2010a). Nevertheless, dynamic rather than static stimuli are more ecologically valid than photographs or drawings of faces. In fact, some behavioural studies, indicate that dynamic facial expressions improve the recognition (Ambadar, et al., 2005; Fujimura and Suzuki, 2010), elicit more widespread patterns of brain activation than static faces (Sato et al., 2004; Yoshikawa and Sato 2006; Trautmann, et al., 2009) and cause more intense responses of facial mimic than static stimuli (Sato, et al., 2008; Rymarczyk et al., 2011). For these purposes, in this study, we used the morphing technique to create dynamic facial expression video-clips presented for a limited time. We expected that, if facial muscles are fundamental for facial expression recognition, MBS participants' performance would be more impaired than a healthy control group.

Lastly, in this study, we explored two aspects of facial expressions recognition process: speed of facial information processing and accuracy in emotion labelling. We used a facial morphing approach to examine if children with MBS required longer time to recognize emotional face than controls. The morphed faces were presented for a limited time and participants had to press a "stop" button as soon as they recognized the stimuli. Then, we requested participants to select the stylized image that best described the emotional face shown. If facial mimicry is causally involved in facial expression recognition, the lack of facial feedback would negatively impact speed and accuracy of facial expressions recognition.

2. Materials and Methods

2.1 Participants

The study involved 28 subjects. Ten children with MBS took part in the study but two were discarded for making a significant number of errors (> 20% of total tests). The remaining 8 patients (experimental group: 5 males and 3 females, mean age of 9 +/- 2,27 years, see table 1) were recruited at the Operative Unit of Maxillofacial Surgery, Head and Neck Department, University of Parma (Italy). In table 1, demographic data of all the study participants with MBS were collected; clinical information such as unilateral or bilateral facial paralysis were included. MBS patients' inclusion criteria were: absence of significant cognitive deficits; a certified diagnosis of unilateral or bilateral facial paralysis; absence of congenital malformations of the hands and feet; absence of any psychiatric or physical illness at the time of participation. The healthy developing control group consisted of 18 (15 males and 3 females) with a mean age of 8,94 +/- 1,39 years who did not meet criteria for a clinical diagnosis of MBS and had an estimated IQ within the normal range.

Participants with Moebius Syndrome				
Group	**Age**	**Gender**	**Paralisis**	**Cranial nerves involved**
MBS1	11	Female	Bilateral	VI, VII, XII
MBS2	11	Female	Unilateral left	VI,VII
MBS3	11	Male	Bilateral	VII, X, XII
MBS4	8	Male	Bilateral	VI, VII,XII
MBS5	6	Male	Bilateral	VII
MBS6	8	Male	Bilateral	VI, VII, XII
MBS7	11	Male	Unilateral left	VI, VII
MBS8	6	Female	Unilateral right	VI, VII, XII

Table 1. Participants with Moebius Syndrome. Demographic and clinical characteristics of participants with Moebius Syndrome.

In order to evaluate participants' cognitive level and their ability on facial expression recognition skills, Colored Progressive Matrices (CPM, Raven, 1984) and Test of Emotion Comprehension (TEC-1, Albanese and Molina, 2008) were performed. All participants included in this study had CPM scores >70. For the aim of our study, we only focused on the component I of TEC, outlining emotions recognition by means of facial expressions. None of the participants experienced any problems in these two tests.

All participants' legal tutor gave written informed consent for the experimental procedure, which was approved by the Ethics committee of Parma. Participation in the study was voluntary and the participants were not paid.

2.2 Stimuli

Pictures of 4 actors' faces expressing five different emotions were selected from a set of validated pictures Nim Stim Face Stimulus Set (Tottenham et al., 2009). These consisted of four Caucasian actor faces (2 males and 2 females) expressing a positive (happiness or surprise), negative (disgust or anger), or a neutral facial expression (Figure 1).

Figure 1. The image depicts the highest intensity of the emotional facial expressions selected from the NimStim Emotional Face Stimuli Database (Tottenham et al., 2009). Short video-clips lasting 3000ms (*25 fps; 800* × 560 pixels) were created from these photographs using computer-morphing software (Abrosoft FantaMorph software package, Figure 2).

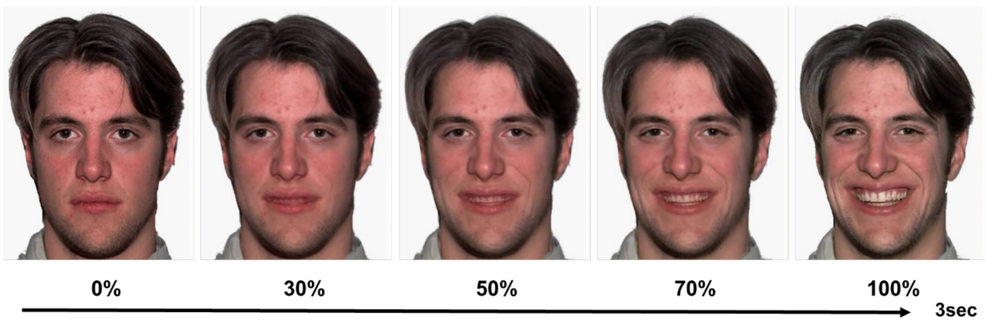

Figure 2. Dynamic video-clip. Example of dynamic sequence elaborated for the happiness condition.

Each video-clip showed the transition from a neutral facial expression to an emotional one (12 neutral stimuli, 12 happiness, 12 surprise, 12 disgust and 12 anger; 60 stimuli in total). We created neutral stimuli using two neutral facial expressions so that neutral stimuli were the result of the transition from a neutral face to another. E-Prime 2.0 software (Psychology Software Tools, Inc.) was used for stimuli presentation.

2.3 Procedure

The experimental procedure was conducted in a soft-lighted, sound-attenuated room. Once informed consent was obtained, participants seated in a comfortable chair after being introduced to the experiment. The viewing distance was 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. In order to allow the participants to familiarize with the task, they first completed ten practice trials during which the experimenter explained the procedure.

Video-clips were randomly presented one at a time. Each trial started with a fixation cross, presented for 500ms. Each video-clip lasted for 3000ms on a white background, with a dynamic morph starting from neutral and going to full facial expression. Following this 3000ms of (morphing) stimulus, a still photo of the full expression was presented for another 1000ms.

Participants were asked to press the space bar as soon as they recognized the emotional expression presented in the video-clip. They were instructed to maximize speed and accuracy. When participants pressed the "stop" button (space bar), the stimulus disappeared and the response time was recorded as an indicator of the time necessary to recognize the emotion. If participants did not press the space bar the stimulus disappeared after 4000ms. When the video-clip disappeared, participants were asked to identify the facial expression from a forced-choice list of five options (stylized emotional faces, Figure 3).

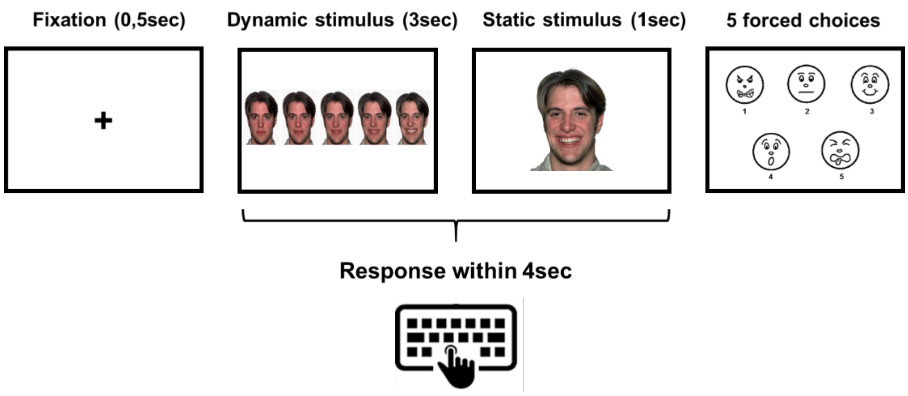

Figure 3. Experimental procedure. Schematic illustration of the task.

Participants were instructed either to name the emotion or to select the corresponding number or to point the stylized emotional face that best described the facial expression shown. To prevent errors in selecting the corresponding number associated with each emotion, the experimenter itself pressed the key corresponding to the emotion that participants selected. One practice trial was given prior to the 10 test trials (two trials for emotion).

2.4 Statistical data analyses

We considered two dependent variables: responses times (RTs) and accuracy rates. Response timewas the time elapsed between the onset of the stimulus and the onset of the response. We considered RTs correct responses only. RTs of less than 920ms (less than 30% of morphing) and any RTs more than 2.5 standard deviations from the overall sample average were excluded from analyses. Accuracy rate was the proportion of correct responses on the total answer given. Accuracy 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), the value obtained under a perfect performance (Wagner, 1993).

RTs and accuracy were included as dependent variables into two mixed-design analysis of variance (ANOVA). “EMOTION CATEGORY” (6 levels: disgust, neutral, surprise, anger and happiness) was used as within-subjects factor and “GROUP” (two levels: EG, CG) as between-subjects factor. For ANOVAs, if the sphericity assumption was violated, then Greenhouse–Geisser degrees of freedom corrections were applied. The probability value was set at $p < .05$ for all analyses. Newmann-Keuls post-hoc followed the two-way ANOVA. Data were analysed by means of Statistica 8.0 (Stat-Soft, Tulsa, OK, USA).

3. Results

3.1 Speed of facial expression processing

Table 2 contains means and standard deviations of participants’ response times in emotional expressions recognition of the experimental group (EG) and control group (CG), respectively. Overall, disgust was the emotion that required the longest time period (2349ms) to be recognized, while happiness was the most rapidly recognized (1931ms).

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

Table 2. Response Time. Mean and standard deviation (SD) of response time (in milliseconds) for disgust, anger, happiness surprise and neutral stimuli for the control (CG) and experimental group (CG).

The sphericity assumption was not violated (Mauchly's Test of Sphericity: $p>0.05$) and the data were normally distributed for both groups (Shapiro-Wilk: $p>0,05$). A mixed ANOVA on RTs with the within factors EMOTION CATEGORY and between factor GROUP revealed a main effect of EMOTION CATEGORY only [$F(4,96) = 9,9$; $p < 0.001$; $\eta 2=0,29$].

As shown in figure 4, post-hoc t-tests revealed that participants responded significantly faster to happy video-clip (1931ms) with respect to neutral (2149ms, p=0,004), disgust (2349ms, p= 0,001), surprise (2182ms, p= 0,002) and anger (2083ms, p= 0,024).

On the other hand, disgust was the emotion that took the longest time period to be recognized (disgust vs. neutral, p= 0,009; disgust vs. surprise, p=0,013; anger, p=0,001). Neutral, surprise and anger did not differ from each other, with EG and CG being not significantly different in their RTs (no significant main effect of the factor group. No significant interaction was observed.

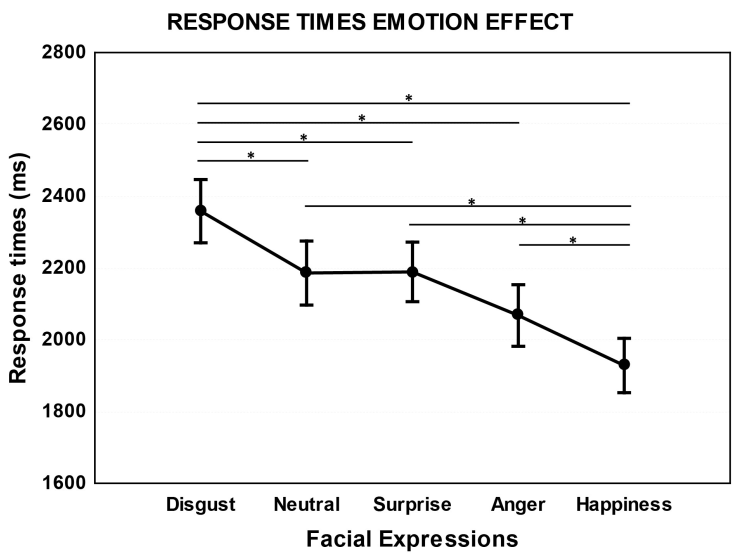

Figure 4. Response times results. Mean response time for all subjects on recognition of five facial expressions. Error bars represent standard errors of the means.

3.2 Response accuracy

Table 3 contains means and standard deviations of participants' accuracy rates for the recognition of emotional expressions from EG and CG. In general, as shown in Table 3, the judgments of facial stimuli were overall highly accurate (mean = 96%) with the CG more accurate than EG (mean=97,4% versus mean=92,2% respectively). Surprise, Anger, and Neutral faces received the highest accuracy scores in both CG and EC whereas Disgust received the lowest in particular in EG.

	Accuracy (%)									
	Neutral		**Disgust**		**Surprise**		**Anger**		**Happiness**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
CG	100	2	91	9	100	0	99	3	98	4
EG	96	9	79	24	96	6	96	9	95	15

Table 3. Accuracy rates. Means and standard deviations (SD) of participants' accuracy rate for the recognition of each emotional expression from the control group (CG) and the experimental group (EG), respectively.

Mauchly's test was conducted on accuracy rate (arcsine of the percentage of correct responses). Sphericity violation was identified ($\chi2(9) = 43.4$, $p < 0.001$) and Greenhouse correction was used. Results revealed a main effect of GROUP [$F(1,24) = 7,55$; $p < 0.011$; $\eta2=0,24$, figure 5]. Specifically, accuracy scores were significantly lower for EG than CG indicating that, in general, MBS participants demonstrated a lower performance in emotions recognition than controls.

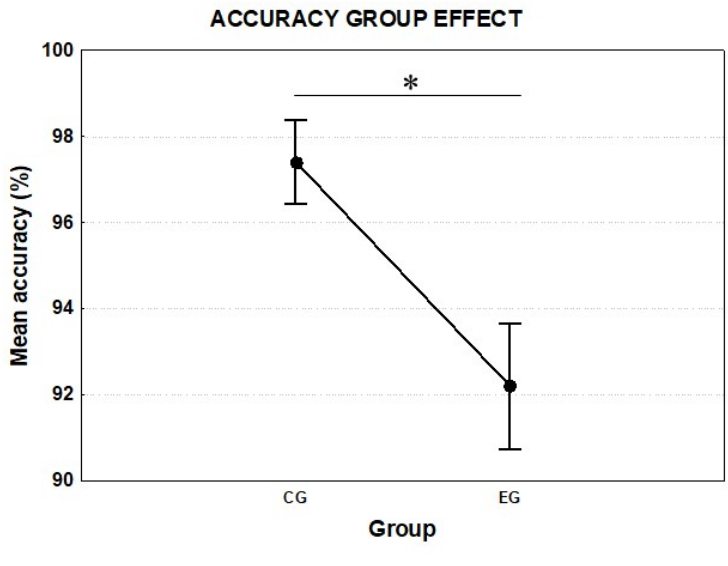

Figure 5. Accuracy group effect. Mean accuracy scores in percentages for control (CG) and experimental (EG) groups. Error bars represent standard errors of the means.

A main effect of EMOTION CATEGORY [F(1,958,96) = 14.56; p < 0. 001; η2=0,38, figure 6] was also observed. Post hoc comparisons revealed that accuracy rate was significantly lower for disgust (1,26) than for any other emotions (neutral:1,53, p=0,001; surprise: 1,53, p=0,001; anger: 1,52, p=0,001; happiness: 1,49, p=0,001). No significant interaction (GROUP vs EMOTION CATEGORY) was found.

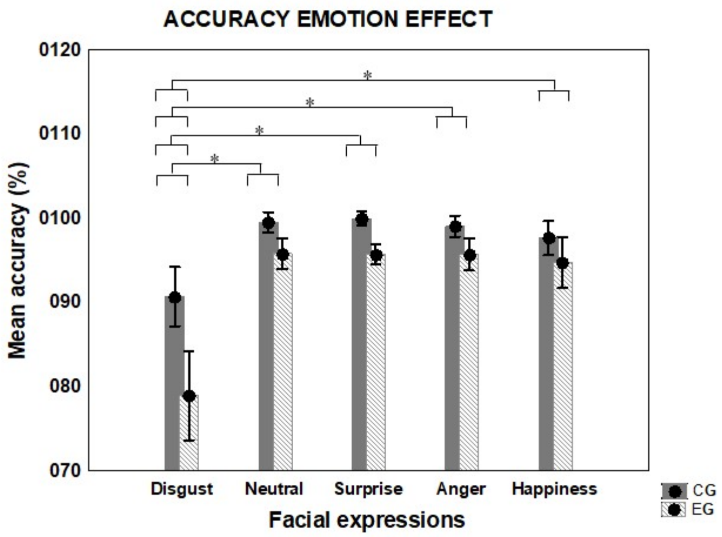

Figure 6. Accuracy emotion effect. Mean response time for control (CG) and experimental (EG) groups on recognition of five facial expressions. Error bars represent standard errors of the means.

Summing up, all participants produced more errors in recognizing disgust than other emotions. More importantly, MBS participants yield a higher error rate than controls in all emotions tested.

4. Discussion

According to embodied theory, individuals recognize facial expressions by implicitly mimicking observed expressions and, in turn, generating the corresponding emotional experience in the observer (Dimberg, 1997a, 1997b; Dimberg and Thunberg, 1998; Dimberg et al., 2002; Dimberg, 1982; Lundqvist and Dimberg, 1995). Many authors have demonstrated a link between emotional facial expression recognition, spontaneous activation of facial motor movements (Dimberg, 1997a, 1997b; Dimberg and Thunberg, 1998; Dimberg et al., 2002; Dimberg, 1982; Lundqvist and

Dimberg, 1995), and activity in motor and somatosensory areas of the brain (Carr et al., 2003; Wicker et al. 2003), as well as in regions that integrate visceral information.

The aim of the present study was to test facial expression recognition in individuals with MBS, a condition characterized by congenital unilateral or bilateral facial paralysis. In fact, we hypothesized that MBS children, who are unable to mimic expressions and experience facial feedback, might be impaired in recognizing emotions.

This study tested both the speed of facial information processing (response times) and the accuracy in emotional faces recognition (accuracy scores, see figure 3) in children with MBS and a control group.

Our results showed that no overall group differences in speed of facial information processing were identified. This suggests that participants with MBS did not show slower response times in identifying the stimuli.

In both experimental and control groups, the speed of responding varied with emotion, being faster for `happiness' (1931ms) and slower for `disgust' (2349ms). These results are in line with previous consistent evidence showing that emotionally positive facial expressions are recognized substantially faster than emotionally negative facial expressions (Feyereisen et al., 1986; Stannerset al., 1985; Ducci, 1981; Stalans and Wedding, 1985; Kestenbaum and Nelson, 1992; De Sonneville et al., 2002).

Findings on accuracy scores showed that MBS participants performed overall higher error rate than the control group (97% and 92% respectively). These results suggest that young patients with MBS recognized emotional faces as quickly as controls but their accuracy in emotion labelling was impaired.

A possible explanation of these results is that participants with MBS, in the absence of a peripheral feedback, probably presented greater difficulties than controls in matching the emotional faces with the stylized images used to label the emotions, committing a greater number of errors in the forced choice task.

These findings are in contrast with a previous study, where individuals with MBS did not differ from the control group in facial expressions recognition accuracy (Bogart and Matsumoto 2010a). One potential explanation for the different findings between Bogart and Matsumoto’s study and our own is that in their study the authors used static images that were presented without a time limit, possibly allowing cognitive strategies which facilitate the association between facial expression and the corresponding verbal labeling. In addition, our study explored this issue in a group of children, in which the frequency of errors in similar tasks is higher than in adult population (De Sonneville et al., 2002; Leime et al., 2013; Mondloch et al., 2003; Kolb et al., 1992).

In contrast with Bogart and Matsumoto's study, our results partially fit with those of Calder et al. (2000) suggesting that MBS individuals present difficulties in the morphed facial expression task but they maintained some residual ability to recognize expressions. We agree with the conclusion of Calder et al. (2000) and Bate et al. (2013) that difficulties in emotional faces recognition are prevalent in individuals with MBS but are not absolute. Specifically, in this study, we have further clarified that individuals with MBS have specific difficulties in associating facial expressions to a stylized emotional face. Without the benefit of fast, spontaneous embodied mechanism, children with MBS might engage compensatory and less automatic strategies when processing facial expressions. The higher number of errors we found in participants with MBS might be related to the use of compensative strategies not yet fully developed. In fact, previous studies focused on adults, while we included children who probably may not have developed adequate compensatory strategies to recognize other's emotions.

For example, they may have used strict rule-based strategies in which memorized lists of characteristics defined emotional expressions, leading them to look for the presence of these specific characteristics when performing emotional perception tasks. Our results demonstrated that, in young individuals with MBS, these strategies were not as efficient as peripheral facial feedback (facial mimicry). For example, a "rule" for disgust could be "corners of the actor's mouth turned down". This characteristic was present both in disgust and anger so that individuals with MBS could have confused them. In such a case the response times would have been similar to those of controls, but they could have been less accurate in labelling emotion.

A further explanation is that mimicking a facial expression affects the subjective experience of emotions and induces emotional contagion through feedback from the facial muscles (Gallese, 2006; Gallese and Goldman, 1998; Keysers and Gazzola, 2006). According to embodied simulation theory, the production of facial expressions triggers the somatosensory system in order to simulate the embodied experience of how the body feels an emotion and this process contributes to representing its meaning (Barsalou, 1999; Atkinson, 2007; Decety and Chaminade, 2003; 2004; Gallese, 2003; 2005; Goldman and Sripada, 2005; Keysers and Gazzola, 2007; Niedenthal, 2007; Niedenthal et al., 2005; Winkielman et al., 2009). Mimicking facial expressions would initiate feedback processes activating the corresponding emotional experience in the observer. This process permits to understand the emotional faces and emotional state of others. Thus, interfering with

facial mimicry (as in the case of individuals with MBS) would impair facial expression recognition performance (Neal and Chartrand 2011; Oberman et al. 2007; Rychlowska et al. 2014).
In a recent unpublished experiment with MBS children at younger ages (mean: 5 years) we found that the autonomic activity, assessed through thermal imaging, which records the changes of temperature of the face, in response to cartoon videos with different emotional content is somehow impaired compared to same age controls. This finding further supports the hypothesis that the impaired motor programs that are normally involved in the production of facial expressions interfere with the associated bodily responses and, ultimately, with the process of facial expression recognition.
Interestingly, feedback from the face has been regarded as critical to emotional face recognition in patients with Parkinson's disease that showed deficit both in producing and discriminating emotional faces (Jacobs et al., 1995; Narme et al., 2011; Alonso-Recio et al., 2014; Aiello et al., 2014). It is not clear which mechanism may contribute to face processing deficits in these patients. Nevertheless, it is interesting to note that patients experienced difficulties producing voluntary facial expressions are also impaired in the discrimination of emotional faces (Alonso-Recio et al., 2014, Ariatti et al., 2008, Clark et al., 2008, Clark et al., 2010, Suzuki et al., 2006; Gray and Tickle-Degnen, 2010; Argaud et al., 2016).
To sum up, our results show a worse performance of children with MBS than controls in emotional faces labelling, where individuals with MBS had to choose from a set of images the one that best described the emotion displayed by the actors' faces. Specifically, they showed difficulties in the stimulus categorization process needed to decide on the label that best describes the observed expression. Their poorer performance does not seem to be related to a slower response, as their response times were similar to those of the control group. These findings point to the possibility that simulation did become a fundamental aspect in recognition tasks when participants were required fine distinctions among emotions.

5. Conclusion

Our results clearly demonstrate that impaired facial expression labelling is a characteristic of children with MBS. According to embodied simulation theories, the observation of an emotional expression leads to the simulation of a corresponding affective state in the perceiver, which in turn facilitates the access to the emotional concept. This simulation may facilitate the recognition of emotional faces through the activation of corresponding emotional concepts. When this motor simulation cannot be activated, as in the case of patients with MBS, individuals show less accuracy than healthy controls in explicit recognition of others' facial expressions. This impairment is particularly evident in young people who probably have not yet developed adequate compensation mechanisms, which, instead, may help adults to recognize facial expressions of others with an adequate level of accuracy.

Acknowledgements

I am really grateful to all the children and their families for their patience and their incredible efforts to help this research through the numerous visits and long trips from all over Italy to reach the lab. I also would like to thank the Associazione Italiana Sindrome di Moebius for the continuous work and support to our research. Lastly, I would like to thank the *Centro Diagnostico Europeo Dalla Rosa Prati,* which supported these studies.

5 Study 3

Thermal response to facial emotional stimuli in children affected by Moebius syndrome

1. Introduction

Most mammalian species can produce facial movements, a meaningful and adaptive component of animals' communication (Diogo et al., 2009). Faces constitute a basic trait, providing a source of diverse social information such as identity, gender, attentional and emotional state of another individual (Burrows, 2008; Darwin, 1872); humans are experts at catching these types of information and able to process facial expressions during interpersonal communication (Adolphs, 2002; Adolphs, 2003a; Gliga and Csibra, 2007).

During face-to-face interactions individuals produce, without awareness, facial signals that change and whose dynamics might depend on the receiver's response and/or emotional/attentional states. Such interactions take different forms and are often characterized by attunement in the intensity of the expressions and also in the form the facial movements are expressed.

"Facial mimicry", considered as the automatic, reflex-like process with the observer's facial movements matching the facial expression of the observed individual, plays a key role in understanding of other's affective states during social interaction (Iacoboni, 2005; Stel and van Knippenberg, 2008). This is the cornerstone of embodied cognition theories suggesting that humans decode each other's expressions partly by simulating the perceived expression in their own facial musculature (Goldman and Sripada, 2005; Niedenthal, Mermillod, Maringer, and Hess, 2010).

Specifically, in agreement with the facial feedback hypothesis (Buck, 1980), individuals may detect changes in the facial expression of another person through the feedback, and change in subjective state, caused by their own facial mimicry (Dimberg, 1982; Dimberg, Thunberg, and Elmehed, 2000; Stel and van Knippenberg, 2008). This theoretical approach was further confirmed by the discovery of "mirror neurons" (MNs) (Gallese et al., 1996; Fogassi et al., 2005) and of a mirror neuron system (MNS) in humans (Rizzolatti and Craighero, 2004) showing that the same neural substrates are activated when hand actions or facial gestures are both executed and perceived (Gallese et al., 1996; Rizzolatti et al., 1996; Ferrari et al., 2003). Therefore, the observed expression and simultaneous activation of underpinning motor programs that produce the same facial gesture would guarantee emotion recognition in the observer (Oberman et al., 2007; Stel and van Knippenberg, 2008; Gros et al., 2015).

A number of neuroimaging studies have further underlined that in addition to motor regions, a more widespread network of structures consisting of the anterior insula, the anterior cingulate cortex (ACC) and the amygdala (van der Gaag et al., 2007) is crucial in processing social information from the face, in generating an affective response, and regulating affective states (Philips et al., 2003; Adolphs, 2003b; Carr et al. 2003). These brain areas are activated when subjects perceive certain basic facial emotions, but may also affect visceromotor responses associated to emotional states (Jezzini et al., 2012; Caruana et al., 2011; Caruana et al., 2016). A bond between motor and autonomic responses (e.g. changes in blood pressure, heart rate, breathing rate, pupil dilation, hormonal and gastric functions) could therefore reflect simulation processes involvement when studying physiological responses linked to the expression of emotions.

Individuals with congenital facial paralysis who present deficits in producing facial expressions could presumably exhibit more difficulties in recognizing facial expressions or, in line with embodied simulation theories, in activating motor programs and associated visceromotor responses when observing facial expressions of emotion (Goldman and Sripada, 2005; Niedenthal et al., 2001; Oberman et al., 2007). In this regard, Moebius syndrome (MBS) patients represent an optimal model to test this hypothesis. MBS is a rare congenital neurological disorder with an incidence of 1 in 50.000 to 1 in 500.000 live births (Rasmussen et al., 2015) and no gender predominance (Lindsay et al., 2010). The syndrome affects the VI and VII cranial nerves, resulting in unilateral or bilateral facial palsy and impaired bilateral movement of the eyes (Bianchi et al., 2009; Cattaneo et al., 2006; Briegel, 2006). Due to musculature paralysis, MBS patients are thus unable to mimic or form any sort of facial expression.

A number of studies conducted on MBS patients explored their ability to recognize facial expressions of emotions. A very first investigation (Giannini et al., 1984) examined facial expression processing in one individual with MBS. During the task, a single MBS patient had to watch a series of videotapes and to evaluate facial expressions of a person playing a slot machine; authors compared MBS patient's ability to understand which of the different "jackpot" prices the person in the video was playing for, with 300 healthy subjects' performance. MBS participant was totally unable to correctly complete the task.

Subsequently, Calder and colleagues (2000), unlike Giannini et al. (1984), demonstrated mild, but non-significant deficits in facial identity processing of three adult people with bilateral MBS; however, compared to controls, MBS subjects showed more impairments in the morphed facial expression task characterized by a greater degree of complexity.

Conducting a study on internet, Bogart and Matsumoto (2010a) presented a total of 42 pictures from Matsumoto and Ekman's (2006) Multi-Ethnic Facial Expression set to 37 people with MBS and an equal number of age and gender matched controls; subjects had to select from a list including the seven universally-produced emotions which one was being expressed. MBS patients did not differ from control group in their capacity to distinguish facial expressions.

In a more recent study, Bate and colleagues (2013) investigated six MBS subjects' expression recognition and imagery; during the experiment, five out of six participants presented deficits in at least one of the three tasks of emotion recognition, even if they did not show absolute inability to perform the tasks; only one was impaired when he was asked to imagine facial expressions and answer questions about them.

As a whole, these studies offer evidence that MBS patients succeed in basic facial emotion recognition, although they show higher impairments in more complex tasks. Even if part of these investigations did not support the hypothesis that embodied simulation mechanisms and facial mimicry are necessary for facial expression recognition, we should take into account that these experiments mainly focused on adult samples; these conflicting and mixed results could thus probably be ascribed to patients' ability to acquire compensatory strategies over the years; namely, they can learn to use specific facial cues of emotion and related contexts to identify facial expressions. Furthermore, despite previous researches investigated MBS performance in emotion recognition tasks, no studies have focused on physiological responses associated to emotional facial expressions yet.

In previous experiments, we observed that emotion recognition abilities in MBS children (mean age of 5 and 9) are somehow defective in comparison with age-matched controls (Nicolini et al. under revision; De Stefani et al., submitted). Furthermore, our first findings on autonomic activity, registered using thermal imaging, showed a weaker thermal response in MBS children compared with controls, highlighting that impaired motor programs could affect body responses (Nicolini et al., under revision). However, the stimuli used in this first assessment were emotional enhancing animated cartoons with different emotional content, instead of facial stimuli.

In the current study, we carried out a test to examine the extent of autonomic nervous system (ANS) response to neutral, happy, surprised, angry and disgusted facial emotional stimuli in children with MBS. In detail, we investigated ANS response to emotional facial expressions by means of a functional infrared thermal imaging (fIRT) camera. To this aim, we used morphed facial emotional stimuli. Previous researches investigating the processing of facial expressions of emotion mostly employed static images (Bogart and Matsumoto, 2010a; Nicolini et al. under revision) rather than dynamic stimuli. However, recent studies highlighted a stronger impact of dynamically presented expressions on emotion recognition (Ambadar et al., 2005; Cunningham and Wallraven, 2009), arousal (Sato and Yoshikawa, 2007) and activation patterns in the brain (for a review see Arsalidou et al., 2011).

Thirteen MBS subjects took part in the current study and they were required to recognize dynamic facial expressions of emotion after having observed these emotional cues. No previous investigations examined the effect induced by different types of facial stimuli in MBS children; we thus compared their ability to recognize facial expressions of emotion and their modulation of the ANS during the task with an age and gender matched control group. We hypothesized that MBS patients' impaired capacity to produce facial signals since birth could have influenced their modulation of the autonomic response to facial emotional stimuli; we therefore expected to observe a lower ability to recognize facial expressions in MBS participants and a differential autonomic variation in MBS patients than in control subjects.

2. Materials and Methods

2.1 Participants

MBS participants were recruited via the Italian Moebius Syndrome Association and the University Hospital of Parma. A total of 13 children (6 males, 7 females) with MBS (mean age 8.65; SD = 2.78) participated in the study. They all were observed to have moderate to severe unilateral or bilateral facial paralysis; none was observed to have cognitive disability. All parents indicated that their children had been formally diagnosed with MBS. Demographic and clinical data of all MBS

participants were included (see Table 1). Additionally, we recruited 16 healthy children (control group) (10 males, 6 females) in the same age range (mean age 9.25, SD = 1.69). All participants were informed that they would be videotaped by means of a thermal camera and a webcam.
In order to evaluate participants' cognitive level and their ability on emotion recognition skills, Colored Progressive Matrices (CPM, Raven JC. Coloured Progressive Matrices. Oxford Psy. Oxford; 1995) were administered. All participants had CPM scores >70. None of the subjects experienced any problems in this test. Informed written consent was obtained from parents of the children involved in the study, and all procedures were in accordance with the 1964 Declaration of Helsinki. Ethical approval for the reported study was obtained from the Ethical Committee of the University of Parma. Participants did not receive any payment for their participation in the study.

Subject ID	**Sex**	**Age**	**Laterality**	**Cranial nerves involved**
12	f	11	bilateral	VI, VII, XII
15	f	5,5	unilateral right	VI, VII
16	f	5,5	unilateral right	VI, VII, XII
17	m	10	bilateral	VI, VII, XII
18	f	9,5	bilateral	VI, VII, XII
20	m	13	unilateral left	VI, VII
21	f	6	unilateral left	VI, VII, XII
22	m	7	bilateral	VI, VII, VIII, XII
24	m	8	bilateral	VI, VII, XII
25	m	8	bilateral	VI, VII, XII
27	m	12	unilateral left	VI, VII
28	f	5	bilateral	VI, VII, XII
30	f	12	unilateral left	VI, VII

Table 1. Moebius subjects' demographic and clinical data.

2.2 Stimuli

We selected coloured pictures of four models' faces expressing five different emotions from a validated set (NimStim Face Stimulus Set, Tottenham et al., 2009). In detail, we chose four Caucasian models' faces (two women, two men) expressing a neutral, happy, surprised, angry and disgusted facial expressions (Figure 1).

Figure 1. Images selected from the NimStim Face Stimulus Set (Tottenham et al., 2009)

Stimuli consisted of short video-clips of three seconds (25 fps; 800X560 pixels) created from static photographs by means of morphing software (Abrosoft FantaMorph software package). Neutral expressions of models (expressive intensity of zero) were morphed with their full emotional expressions (expressive intensity of 100%) to create a graded transition of expression for each emotion. One second of a static full-blown emotional face was added at the end of each video-clip to create a four seconds video-clip. Figure 2 shows an example of the morphed stimuli from 0% to 100% expressivity of happiness.

Figure 2. Example of an emotional continuum elaborated for the expression of happiness.

Neutral video-clips were created through two neutral facial expressions so that neutral stimuli were the result of the transition from a neutral face to another one. E-Prime 2.0 software (Psychology Software Tools, Inc.) was used for stimuli presentation.

2.3 Materials

Thermal IR imaging was performed 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 the children's behavior so as to record their level of attention while watching video stimuli.

2.4 Procedure

Previous to testing, each subject was accompanied in a soft-lighted, sound-attenuated, climate-controlled room (room temperature: 23 ± 1 °C; relative humidity: 50–55%; no direct sunlight or ventilation), sat easily on a chair and underwent a familiarization period to get used to the experimenters. Prior to commencing data collection, each participant was left to acclimatize for 10 minutes to allow the skin temperature to stabilize (Ring and Ammer, 2012). During the first neutral interaction, the child was asked to answer some questions on personal data (e.g. name, age). Then, visual stimuli were presented to each participant on a 17-inch computer monitor (1024X768@75Hz) placed at a distance of 60 cm from the chair. Each subject was asked to maintain from excessive movement during the experiment. Written instructions were presented on the screen before the beginning of the task and were read by one of the experimenters. Five different blocks of morphed emotional faces (i.e. neutral, happiness, surprise, anger, disgust) were randomly shown to the subject. Each randomized block was composed by three repetitions of four (two males, two females) randomized morphed emotional faces video-clips preceded by a fixation cross of one-second duration. Each video-clip was presented on a white background and lasted for three seconds, with a dynamic morph starting from neutral and going to the full emotional expression. The full emotional expression lasted additional one second, for a total duration of four seconds for each video-clip. Each block lasted for a period of 60 seconds and was preceded by a neutral screensaver baseline lasting 30 seconds (Figure 3).

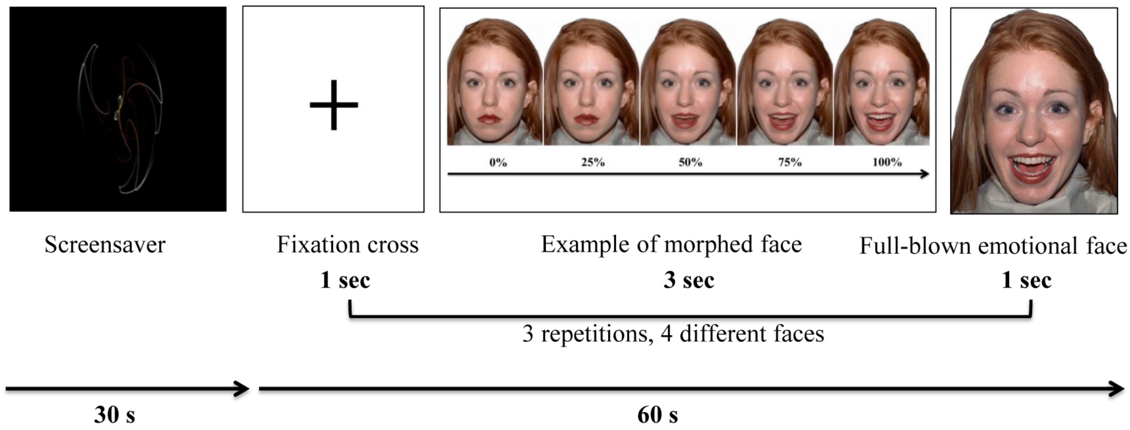

Figure 3. Example of stimuli presentation during the experimental procedure.

During video-clips presentation, the participant was just requested to observe the stimuli. Thermal camera was placed above the screen of the monitor one meter away from the participant, was automatically calibrated and manually fixated to allow a frontal recording of the child's face. Behavioral recordings took place by means of a webcam. At the end of each block, an image with five-forced choice picture options and corresponding number label appeared on the screen (Figure 4) and the experimenter asked the subject which of the 5 emotions the block was representing. Participants were instructed either to name the emotion or to point the corresponding number or the selected image that best described the emotion just seen. Child's answer was then noted on the answer sheet.

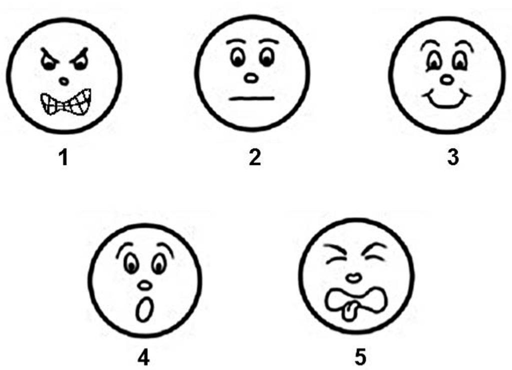

Figure 4. Image with five-forced choice picture options and corresponding number associated.

2.5 Thermal data analysis

A visual observation of the changes in facial thermal imprints was carried out to qualitatively examine children's autonomic responses throughout the experiment (Figure 5). A quantitative analysis followed, in order to measure temperature variations of the nasal tip. According to previous studies (Kuraoka and Nakamura, 2011; Nhan and Chau, 2010; Shastri et al., 2009, Ioannou et al., 2013), the nose has been shown to be the most reliable region for the detection of psycho-physiological arousal. Therefore, in the present study, we focused on the nasal tip, whose temperature variation is highly associated with autonomic nervous system responses (Roddie, 1963; Nakanishi and Imai-Matsumura, 2008; Ebisch et al., 2012, Manini et al., 2013). Thermal signals were extracted and processed 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). Specifically, to define the participants' nasal temperature variations, a circular marker was made on the region of interest (ROI, nasal tip) and an automatic tracking algorithm moved this marker to follow the same region across the task. Circular markers did not vary in size across frames and thermal values were extracted only when the face was in direct angle (Ioannou et al., 2013). Thermal data were extrapolated in a consistent manner across frames since participants' movements were minimal because of the nature of the experiment. To avoid any possible noise or artifacts, thermal data have been subsequently elaborated with PostTracking software. On average, we extracted 150 frames (30 secs) for each pre-block baseline and 300 frames (60 secs) for each emotional block (neutral, happiness, surprise, anger, disgust).

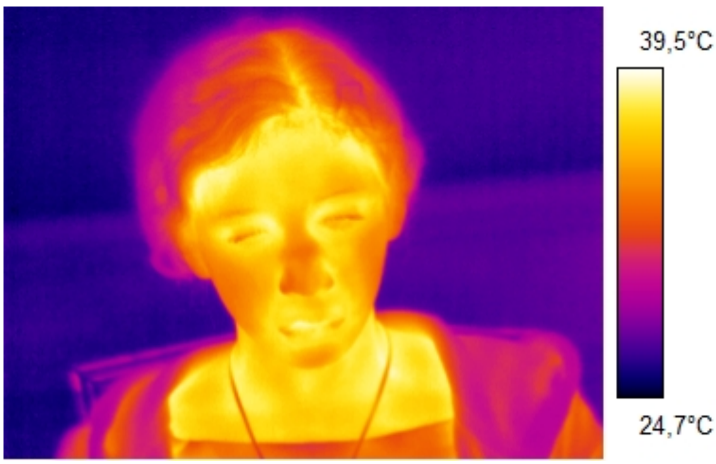

Figure 5. Thermal image of a MBS child while watching facial emotional stimuli.

2.6 Statistical data analysis

During the five-forced choice picture matching, children's answers were noted on the answer sheet and the proportion of wrong responses (% of errors) was calculated by dividing the number of errors by the total number of blocks (i.e. 5 blocks) per each participant. A non-parametric Mann-Whitney *U*-test for independent samples was used to compare Moebius (MBS) and control group.

Concerning thermal data, we first qualitatively assessed whether the facial skin thermal signal did present any visual appreciable variation during stimuli presentation. The thermal signals of the nasal tip for all of the subjects were then processed subtracting the mean thermal value of each pre-block baseline condition from the mean thermal value of its following experimental block (neutral, happiness, surprise, anger, disgust). Absolute values of temperature variations were then calculated in order to measure the magnitude of thermal modulation independently of the direction of thermal variation; indeed, in our previous study (Nicolini et al., under revision) we found that children did not present specific directions in their autonomic responses, rather they were characterized by remarkable inter-individual variability in responding to emotions. For this reason, we took into account the absolute values of participants' thermal changes in order to evaluate their responses independently of the direction of thermal variation. The mean thermal value of each neutral block was subtracted from the other conditions (happiness, surprise, anger, disgust) thus obtaining the thermal variation of the signal with respect to the neutral condition. These values were used as dependent variable in the statistical analysis. Repeated measures (4 X 2) ANOVA was performed on the happiness, surprise, anger and disgust condition for all participants. The conditions (happiness, surprise, anger, disgust) were set as within-subject factor, while the 2 groups (MBS children and control subjects) were set as between-subject factor.

The probability value was set at $p < 0.05$ for all analyses. If data violated sfericity assumption, we reported Greenhouse-Geisser ($\varepsilon < 0.75$) or Huynh-Feldt ($\varepsilon > 0.75$) corrected values. Data were analyzed by means of Statistica 8.0 (Stat-Soft, Tulsa, OK, USA).

3. Results

3.1 Five-forced choice picture matching

Mann-Whitney *U*-test was performed to assess if controls and MBS subjects' proportion of errors significantly differed during the five-forced choice picture matching. The results showed that MBS children performed higher number of errors (mean = 0.11; SD = 0.13) than control children (mean = 0.025; SD = 0.068) ($U = 68.00$; $p = 0.042$) (Figure 6). The low number of trials per subject in this task (each subject saw 3 repetitions of 4 faces per emotion for once) did not allow us to apply statistical analysis to compare different emotions. Despite this, percentages % of errors in MBS and control children per emotion category were illustrated in Table 2.

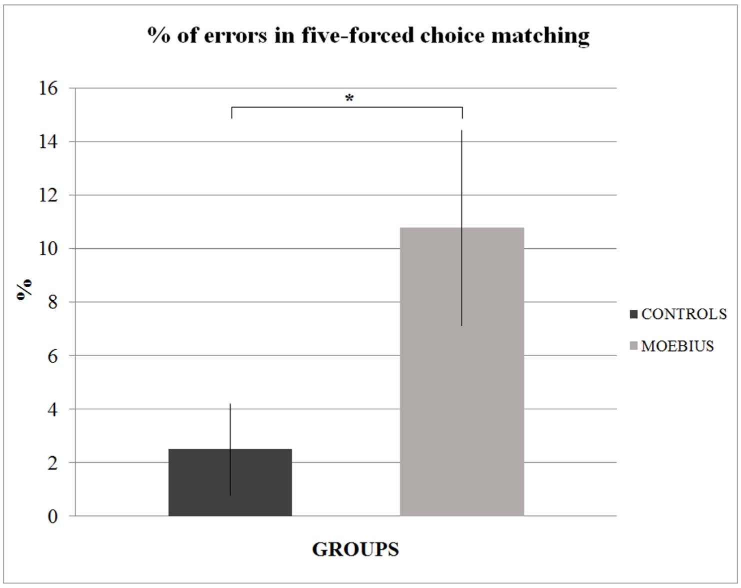

Figure 6. Percentage (%) of errors performed by MBS and control children in five-forced choice picture matching. MBS subjects performed higher number of errors than controls.

% Errors	**Happiness**	**Surprise**	**Anger**	**Disgust**	**Neutral**
Controls	6,25	0,00	0,00	6,25	0,00
Moebius	7,69	0,00	0,00	15,38	30,77

Table 2. Percentage (%) of errors per each emotion performed by MBS and control children in five-forced choice picture matching.

3.2 Thermal modulation across conditions

Concerning the qualitative visual inspection of thermal imprints, no appreciable variation of skin temperature distribution was detected during stimuli presentation. A visual exploration of thermal responses in both control and experimental group for each emotion suggested a lower thermal variation in Moebius compared to controls while watching emotional faces, independently of the emotion observed (Figure 7).

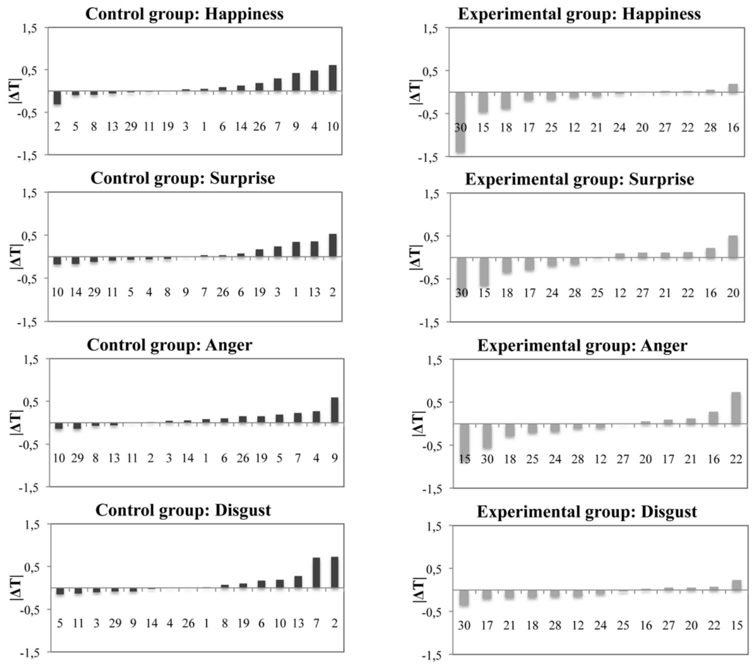

Figure 7. Absolute values of temperature variations per subject during each of the emotional conditions.

To quantitatively measure the thermal variation, a (4 X 2) ANOVA was performed on the obtained absolute values of temperature variations of the nasal tip during the happy, surprised, angry and disgusted stimuli presentation. A significant difference between the two groups was highlighted ($F_{(1, 27)} = 6.933$; $p = 0.014$); in this regard, MBS subjects (MOEBIUS) presented a significantly lower thermal variation than controls (CONTROLS) while watching emotional stimuli; this effect was not emotion-specific (Figure 8; Figure 9). No significant difference was observed among conditions ($p > 0.05$). No significant interaction group x condition was found ($p > 0.05$).

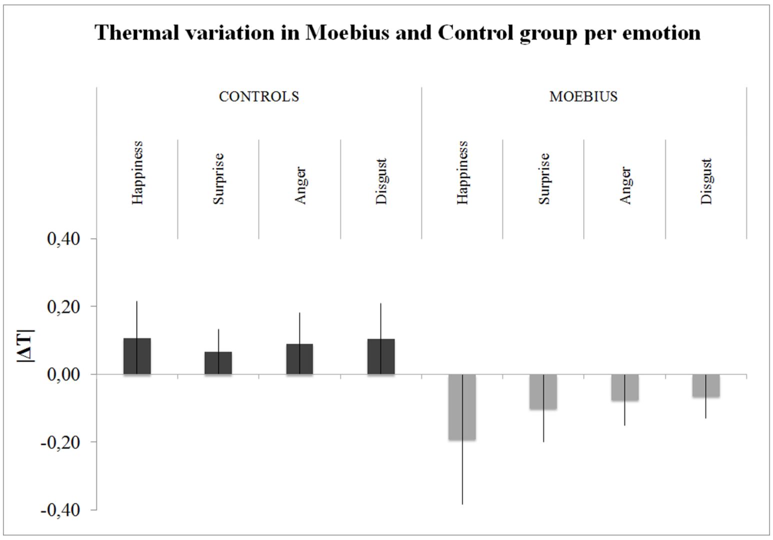

Figure 8. Group mean absolute values of temperature variations in controls and MBS subjects per each emotion. Control participants showed a positive thermal variation compared to neutral condition, while MBS subjects showed a negative thermal variation compared to neutral condition. The figure highlights that MBS subjects presented a lower thermal variation than controls while watching facial emotional stimuli.

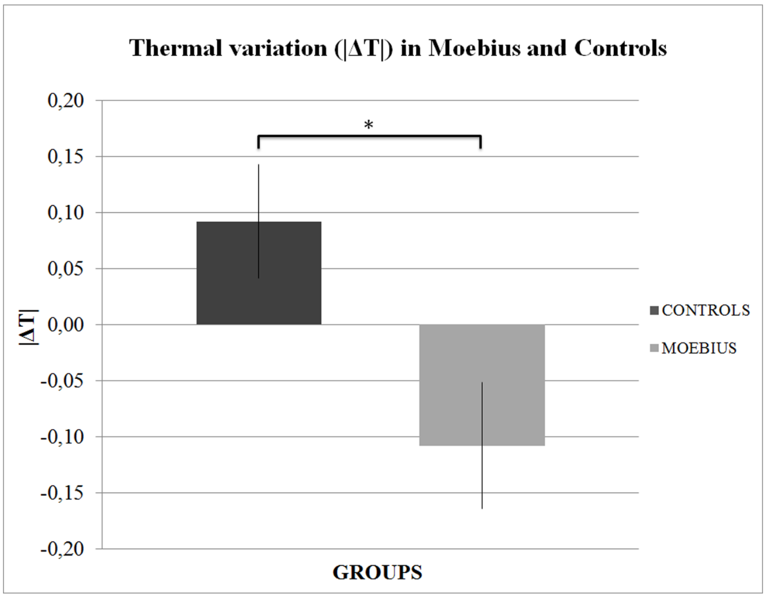

Figure 9. Group mean absolute values of temperature variations in controls and MBS subjects. Control participants showed a positive thermal variation compared to neutral condition, while MBS subjects showed a negative thermal variation compared to neutral condition. The figure highlights that MBS subjects presented a lower thermal variation than controls while watching facial emotional stimuli.

4. Discussion

In this study, we examined emotion recognition and the autonomic response to happy, surprised, angry and disgusted morphed facial stimuli in 13 children with MBS, a rare congenital disorder characterized by lack of facial expressions. According to our hypothesis, and in line with embodied simulation theories (Niedenthal et al., 2010), Moebius patients, who are unable to mimic expressions and experience facial feedback, could have difficulties in recognizing and processing emotions.

In support of our hypothesis, children with MBS reported lower scores in the five-forced choice picture matching than age and gender matched control subjects. This first result is in conflict or partially contrasts with previous findings (Bogart and Matsumoto, 2010a; Calder et al., 2000; Bate et al., 2013) highlighting no (or just mild) differences between MBS and control subjects in recognizing emotional expressions. However, our sample was just composed by children in their developmental age; that is, 8-year old children, unlike adults, may not have completely developed compensatory strategies, which could be helpful in discriminating facial expressions in everyday life. It is indeed possible that MBS patients, while developing, construct compensatory mechanisms, such as the use of special facial displays, which could allow them to more easily distinguish different kinds of emotions. These specific features consisting in, as an example, the corners of the mouth turned up for happiness or down for sadness, could provide them an additional cognitive instrument to deal with others in emotional contexts. Additionally, since judging complex emotions may require the integration of multiple information, including prosody and nonverbal visual cues such as body postures in addition to facial expressions, it is possible that growing up, MBS patients learn to discriminate specific facial features by associating them to further information coming from other perceptual channels (Herba and Phillips, 2004). In this experiment, children were not asked to identify as soon as possible the emotion displayed in the monitor, but they were requested to observe a video sequence of 60 secs duration presenting a number of morphed faces expressing a particular emotion. Then, children had to match the emotion exemplified in the sequence with one of the simplified faces shown at the end of the block. As a consequence, the task did not only demand children to recognize a particular emotion, but also to memorize the emotion expressed by multiple faces and then to associate it to a stylized face. Multiple cognitive processes were therefore involved in this first experimental part, but the procedure employed does not permit us to discard those errors that could be ascribed to lack of memory or concentration.

Previous studies provided behavioural evidence for the role of facial mimicry in perception and processing of emotional facial expressions. Niedenthal and colleagues (2001) compared control participants who were free to move their faces with other participants who were prevented from moving their facial muscles since they were holding a pen in their mouth; all subjects were asked to identify the point where a dynamic face changed from happy to sad and viceversa, but experimental participants detected the change in facial expression later than controls. Similar findings were then obtained by Oberman and colleagues (2007) and replicated by Ponari et al. (2012) who blocked mimicry on the lower half of receivers' faces and observed a minor recognition of happiness and disgust expressions. Our first finding is therefore in support of the hypothesis that facial mimicry involved in emotional expression is also crucial for others' expression recognition (Oberman et al., 2007; Stel and van Knippenberg, 2008; Wood et al., 2016; Niedenthal et al., 2010). This result was further confirmed by our previous studies (Nicolini et al., under revision; De Stefani et al., submitted) showing that impaired facial mimicry can affect the process of emotion recognition in MBS children.

Concerning thermal findings, our analyses highlighted a difference between MBS and control group. Controls showed a stronger nasal-skin thermal modulation observing emotional facial stimuli than neutral faces, while MBS children did present a lower thermal modulation to emotional stimuli than to neutral ones. Growing literature is now focusing on the use of facial thermography to classify emotions in human communication and interactions. A number of studies have used thermal imaging to observe affective-state specific thermal variations of the face (Khan et al., 2005; Pavlidis, 2004; Sugimoto, 2000), suggesting that fluctuations in facial skin temperature could provide a noninvasive measure of emotional responses and ANS activity (Pavlidis et al., 2007; Shastri et al., 2009). In detail, a few studies demonstrated that thermal imaging can distinguish not only between baseline and affective states, but even between self-reported high and low arousal (Nhan and Chau, 2010). In our study, we focused on absolute values to measure the magnitude of thermal response or, in other words, of the arousal response of participants across different emotional conditions. Our findings indicate that MBS and control subjects presented a differential non-specific thermal modulation during the emotional conditions. While controls presented a stronger extent of thermal variation to emotional stimuli compared to neutral facial stimuli, MBS children did present a less pronounced thermal response to emotional faces compared to neutral ones. The lower thermal variation of MBS patients in emotional conditions could be ascribed to a lower thermal modulation during emotional stimuli (compared with neutral - not emotional ones), which in contrast, triggered a more intense thermal variation in controls. This finding could reveal that MBS subjects are still responsive to facial stimuli, in particular to neutral (not emotional) faces, but are less aroused by faces expressing emotions.

A differential response to emotional stimuli between experimental and control participants could be attributed to a sensorimotor simulation network that is crucial not only for emotion recognition, but also for triggering visceromotor patterns (Iacoboni, 2009; Panksepp, 2005). According to functional MRI studies (Carr et al., 2003; Wicker et al., 2003), facial expression observation and production are supported by the same neural mechanisms. In addition to the superior temporal and inferior frontal cortices that are critical for action representation (Iacoboni, 2001), the observation and execution of facial expressions activates other brain regions such as the insula and the anterior cingulate cortex (Carr et al., 2003). Consistent with previous reports using static (Carr et al., 2003; Phillips et al., 1997; Vuilleumier et al., 2001) and dynamic (Leslie et al., 2004; Wicker et al., 2003) face stimuli, increased activity in the amygdala in response to affective facial expressions compared to the neutral conditions has been highlighted (Hariri et al., 2002).

It is possible that the impossibility, in MBS patients, to perform facial expressions could have triggered a non-specific and stronger response to neutral stimuli with no facial movements compared with emotional ones. Caruana and colleagues (2011) demonstrated that direct stimulation of the insula in macaque monkey produces visceromotor responses and facial motor displays. In detail, in their experiment, direct stimulation of the ventral insular cortex in monkeys did elicit disgusting and socially affiliative facial expressions as well as autonomic responses consisting in a decrease in heart rate, which is typically associated with the emotion of disgust. Heart rate is innervated by sympathetic and parasympathetic systems, and prior research has suggested a complex association with affective responses; it has indeed been reported that the ease with which a subject regulate its own emotional responding and switch between high and low arousal depends on the ability of the ANS to regulate heart rate (Cuthbert et al., 1990; Vrana et al., 1989; Gross, 1998).

A link between facial emotional expressions and autonomic responses (Jezzini et al., 2012) could explain a differential response between MBS and control children while watching facial stimuli. The absence, in MBS patients, of facial mimicry, could have influenced their emotional and arousal responses to morphed facial stimuli; in this sense, compared to MBS subjects, only control participants exhibited to be able to adjust with respect to emotional rather than neutral stimuli.

Despite the innovative nature of this kind of studies investigating emotional responses in very rare patients affected by facial palsy since birth, we are aware that a few limitations should be addressed. Firstly, given that our focus was to investigate emotional processing in MBS children during developmental age, we could only involve a very limited number of subjects. In these terms, the necessity to include only patients in a specific age-range with no autism or mental retardation limited our sample. In addition, although IR thermal imaging has been confirmed as a reliable technique by comparisons with other standard methods of ANS measurement (i.e. GSR), it is a currently developing technology, which still requires further investigations on the matching between thermal response and emotional meaning.

In conclusion, our results sustain the hypothesis that facial paralysis, and therefore the absence of facial mimicry, arouses impairments in MBS children's capacity to label and categorize emotions. Furthermore, a differential thermal response to emotional stimuli between control subjects and MBS patients is probably ascribed to MBS children's inability to control facial muscles, which could result in a lower activation of the physiological responses associated to the observation of visual facial emotional stimuli compared to neutral ones. This could represent a lower predisposition in MBS children to rapidly self-regulate in response to social stimuli.

Acknowledgements

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6 General conclusion

The core of this dissertation was the analysis and interpretation of MBS children behavioral and physiological responses to emotional stimuli. In detail, this doctoral work was included within a framework where congenital facial palsy in children with MBS could have implicated impairments on an emotional level. Since the absence of facial mimicry and potentially of embodied simulation processes (Niedenthal et al., 2010) could result in a defective emotional processing, Moebius children represent a very good model to explore this hypothesis through the developmental age.

In agreement with our predictions, all three studies highlighted greater difficulties in MBS patients to recognize facial expressions of emotions compared with healthy children of the same age. In this regard, our first findings investigating emotion recognition abilities by means of the *Test of Emotion Comprehension (TEC-1)* (Pons and Harris, 2000) showed impairments in 5 years old MBS participants, who displayed lower scores in decoding emotions than control children of comparable age; in line with this result, study 2 showed that 9 years of age Moebius children were as fast as controls in the timing requested to identify and label the photographs of facial expressions, but they made higher number of errors in the task than controls, revealing that their accuracy in emotion labeling was impaired. In support of this finding, Moebius children who participated in study 3 (mean age of 9) confirmed previous outcomes, reporting higher deficits in recognizing and labeling facial expressions of emotions in the five-forced choice picture matching than control subjects. These data are in accordance with embodied simulation theories sustaining that others' emotion recognition is based on the subtle mimicking of their own facial expressions. When perceiving individuals expressing basic emotions the observer's facial muscles activate in a congruent way (Dimberg et al., 2000; Lundqvist and Dimberg, 1995). Such phenomenon has been named 'facial mimicry'. The activation of motor programs congruent with those executed by the observed agent are probably linked to neural mechanisms that are capable to match observed actions with internal motor representations of those same actions. One of the most studied networks that is likely involved in such psychological/behavioral processes is the “mirror neuron system” (MNS). The property of this system is that the observation of a particular action activates regions involved in the execution of the same action (Rizzolatti and Craighero, 2004; Rizzolatti, Fogassi, and Gallese, 2001). Non-invasive neuroimaging techniques like fMRI have been applied to study the involvement of the MNS in the understanding of facial expressions. In support of this idea, it was demonstrated that the perception and production of the emotion-related facial expressions activate the same brain structures both in the observer and in the one being observed (Carr et al., 2003), in particular the motor and premotor cortices, the anterior insula and the anterior cingulate cortex. The impossibility, in MBS patients, to produce facial expressions could have affected their abilities in accurately recognizing them, at least during the developmental age. Previous studies conducted on MBS adults (Calder et al., 2000; Bate et al., 2013) have found only mild deficits in Moebius patients compared with controls, that is, MBS subjects were not totally unable to perform the tasks, rather their performance was considered below-control-level. In this regard, it has to be taken into account that the difference between MBS children and adults’ abilities in recognizing facial expressions of emotions could be attributed to compensatory strategies (such as the learning of specific facial cues and display rules that could be linked to particular emotions) across the lifespan. Conversely, compared to controls, children with MBS may not have developed yet any substitute strategy, that is, they may not cognitively employ yet specific facial cues of emotion, which could help them in recognizing facial expressions at the same level as control subjects. At various times, research has investigated how and when children start to identify and discern different emotions. It has been shown that typically developing infants can discriminate static displays of happy, sad, and surprised facial expressions since the age of three or four months (Young-Browne, Rosenfeld, and Horowitz, 1977) and dynamic happy and angry faces by the age of seven months (Soken and Pick, 1992). By the age of four years, they are able to label displays of happiness, sadness and anger with good levels of accuracy, and they start to recognize fear and surprise (Widen and Russell, 2003). Emotion cognition, which is based upon the knowledge of display rules to discriminate emotions, is thought to develop later, as the child gains more sophisticated cognitive skills, has more experience in social interactions, and begins to acquire culture-specific display rules. Some researches suggest that the ability to recognize most emotional expressions is comparable to adults’ one since 8 (Boone and Cunningham, 1998) or 10 years of age (Durand et al., 2007; Mondloch et al., 2003). In our experiments, it is possible that MBS children may not have learned yet compensatory mechanisms to discriminate emotions and therefore performed worse than controls at recognizing basic facial expressions.

Our findings result particularly relevant in relation to numerous developmental psychology studies that underlined the importance of face-to-face interactions between the caregiver and the child after birth. It has been shown that human infants at birth are attracted to specific kinds of stimuli, including faces (Turati et al. 2006; Valenza et al. 1996) and hands (Van Der Meer 1997; Von Hofsten 2004). In the neonatal period, infants develop visuomotor coordination based on both observing their own moving hands and synchronizing facial expressions with caregivers, processes that are relevant for mirror neurons (MNs) development (Del Giudice, 2009; Ferrari et al. 2013). According to the "Evo-devo" perspective (Ferrari et al., 2013), in fact, facial expressions synchronization with caregivers after birth could be crucial to create a link between the "self" and the "other" and to ensure the shaping of the mirror neurons social function, especially during face-to-face interactions. Indeed, neonates are able to recognize and respond to social signals, engaging in reciprocal complex, and emotional interactions with their mothers; these active exchanges, including facial and vocal expressions and gestures, are immediately present after birth and in the first month of life (Meltzoff and Moore, 1977; 1983; Marshall and Meltzoff, 2011; Murray et al. 2007; 2016; Rayson et al., 2016). Concerning this theoretical account, it will be important for future research to explore more in depth the communication channels preferentially used by MBS neonates from birth through the early postpartum months while interacting with their main caregivers. In fact, neonates are able to recognize and prefer their mothers' faces (Burnham, 1993; Bushnell, Sai, and Mullin, 1989), and also to recognize and prefer their mothers' voices (DeCasper and Fifer, 1980; Mehler, Bertoncini, and Barriere, 1978), as well as to distinguish the smell of their mothers' milk from that of a stranger (Cernoch and Porter, 1985; Marlier, Schall, and Soussignan, 1998). It is therefore possible that MBS neonates prefer other forms of communication based on the vocal-auditory channel since their capacity to express facial gestures is strongly impaired. This kind of studies would be helpful for both parents and children, supporting parents in their attempts to interpret infants' signals.

In relation to life satisfaction, a few studies investigated the quality of life in MBS adults and adolescents. One study by Briegel (2007) found that people with MBS had higher incidence of inhibition, introversion, and interpersonal sensitivity (feelings of inadequacy), a lower satisfaction with life, greater tendency toward psychological distress. In contrast, Bogart and Matsumoto (2010b) observed no differences between MBS and control groups in depression or anxiety, even if Moebius patients reported lower social functioning. In a more recent study, Strobel and Renner

(2016) focused on adolescents' quality of life and their self-reports indicated specific reductions in social quality of life, but not in other domains. Overall, these studies suggest mild or some degree of difficulties in MBS patients, probably ascribed to their impossibility to perform facial movements. With this regard, Meyerson (2001) conducted a qualitative interview to 18 MBS adults who noted that social interactions can be very difficult without facial expression, but they reported using eye contact to display confidence and prosody, body language, and verbal disclosure to express emotion. Given that no studies have been conducted on very young patients' social counterpart in everyday lives, future studies aimed at investigating social functioning and interactions in MBS infants and children through their developmental ages will be very helpful to better support their psychological needs since birth to adulthood.

The previously described behavioral findings on MBS children facial emotion recognition were in accordance with our thermal results. In study 1, a weaker and non-specific thermal response of Moebius children with respect to controls while watching emotional stimuli (animated cartoons) was observed. In line with this finding, study 3 highlighted that MBS children, compared to controls, present a lower nasal-skin thermal modulation while observing emotional facial stimuli than neutral faces. Specifically, these physiological outcomes showed that MBS children were featured by a lowered non-specific autonomic thermal modulation while viewing different kinds of emotional stimuli, compared with age and gender matched controls. MBS and control group thermal differences in responding to emotional stimuli could be interpreted within the perspective of action-perception mechanisms. Indeed, it has been shown that the observation and production of facial expressions of emotions, in addition to activate the same brain structures in the observer and observed individual, also trigger a shared neural network involving brain structures which are associated with emotional processing and visceromotor responses (Carr et al., 2003; Wicker et al., 2003). In fact, these findings showed that in addition to the classical mirror network, both facial observation and execution of facial expressions activate the amygdala and the anterior insula. In line with this, a study by Wicker et al. (2003) showed that the anterior insula and the anterior cingulate gyrus, whose functions are linked to autonomic responses such as changes in heart rate, blood pressure, electrodermal responses (Critchley et al., 2003; Critchley, 2005; Fredrickson et al., 1998), were activated both when participants observed and experienced the emotion "disgust". The evidence of the pregenual anterior cingulate cortex (pACC) involvement in a mirror mechanism for smiling has been further suggested by recent findings. In fact, it has been shown that the same pACC region from which laughter is evoked by electrical stimulation is also activated by the presentation of video-clips depicting laughing actors. On the contrary, individuals producing a neutral expression or crying did not elicit any response (Caruana et al, 2017). These data are in support of the crucial role of the pACC in transforming a sensory representation of others' laughter into a motor representation.

The different thermal responses, reflecting the magnitude of the autonomic change, between MBS and control group could therefore be associated to a defective sensorimotor simulation mechanism in MBS patients due to facial palsy, which in turn could have influenced their reduced autonomic body changes. In fact, it has to be considered that in study 1 both Moebius and controls showed a nasal temperature increase during the sadness condition, which triggered a stronger thermal response than happiness and fear. MBS children, as controls, are still responsive to emotional animated cartoons, but that their physiological response is somewhat less intense compared to the controls' one. Considering that no neuroimaging studies to date have been conducted on Moebius patients, future work using brain imaging methodologies will be extremely useful to address how MBS subjects' brain responds to emotional stimuli as well as to associate the measured brain activity to the cognitive deficits resulting from the damage in the VI and VII cranial nerves.

Thermal data were collected by means of functional infrared thermal imaging (fITI) method, which is a novel technology still under development. Further studies of psychophysiology, particularly concerning emotional functioning, will be therefore necessary to investigate more in depth the matching between thermal changes and specific autonomic responses to emotions. The aim of this thesis was to highlight possible differences between a control and a MBS sample of very young patients. However, MBS is a very rare syndrome with an incidence of approximately 1 in 50.000 to 1 in 500.000 live births (Rasmussen et al., 2015). Given the specific age range object of these studies and the rarity of the syndrome, one of the main limitations in carrying out the research was the limited number of participants involved in the studies. Despite the methodological and sample-size related limitations noted above, the current research has indeed a remarkable strength; in fact, it is worth mentioning that no previous studies in the literature tested emotional responses in MBS children and, rather, these are the largest samples of children with MBS investigating emotional functioning in MBS research to date. Further possibly longitudinal studies planned to track patients' changes through development would certainly be critical in order to add a dowel to our knowledge of this very rare syndrome and to support MBS patients in facing the challenges of everyday life.

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