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Coordinatore: prof. Flavio Nobili

Curriculum in scienze delle attività motorie e sportive

Coordinatore: prof. Piero Ruggeri

CICLO XXXIV

(e)motion

The interplay between emotional processing and the sensorimotor system

TUTOR

Prof.ssa Laura Avanzino

CANDIDATO

Alessandro Botta

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1. Emotions: from neurophysiology to behavior

"Everyone knows what an emotion is, until asked to give a definition. Then, it seems, no one knows."

(Fehr and Russel, 1984)

1.1 The nature of emotions

"There is little hope that there ever will be agreement on a common definition of emotion, given the sacred traditions of the disciplines involved and the egos of the scholars working in these disciplines.". This is how Mulligan and Scherer begin their paper whose aim was to suggest a broader (and modest) working definition of 'emotion' (Mulligan & Scherer, 2012). At the very end of their work, where the authors 'dissected' most of the main emotion-related philosophical and psychological issues, they agreed on one thing: it is extremely difficult to give a complete and univocal definition of emotions (Mulligan & Scherer, 2012). But this reported struggle in framing these three syllables has a reason, or better, has several reasons. Emotions are conceived as a composite and integrated construct that relies on the synchronized response to a stimulus by the hand of different and widespread systems which range from physiology and psychology to behavior (Hamann et al., 2020; Mulligan & Scherer, 2012). Already from this brief description, it appears clear why being able to define such a complex phenomenon leads inevitably to conceptual frictions between theorists. Emotions imply the most diverse, although intertwined, disciplines and consequently the most disparate methodologies that range from Freud's

psychodynamic to the psychophysiological tradition defined by William James, from the cognitive approach until the most recent neurobiological method (Plutchik, 2001).

If little consensus is retrievable among scientists on the terminological definition of the word ‘emotion’ and on the methods of investigation, it is also true that, starting from the pioneering work of Charles Darwin ‘The Expression of the emotions in man and animal’, the evolutionary theory of emotions has become an almost universally accepted cornerstone in affective and cognitive neuroscience (Darwin, 1872; Plutchik, 2001). According to the evolutionary approach, emotions have been selected across species because of their adaptive role which made it possible to adjust behavior in response to the environment and in relation to survival and social functions (Caruana et al., 2017). More specifically, living beings show all more or less the same kind of basic behaviors that are strictly linked to the environment they live in or that they create for themselves; eating, mating, caregiving, investigation ,and fight or flight response are common behaviors among species at mostly all phylogenetic levels (Scott, 1980).

But to a closer look, it appears clear that these behaviors are also inevitably linked to another general aspect that binds almost all sentient organisms which might be referred to as ‘inner state’ or more simply, emotions. A fight or flight response relies essentially on one emotion: fear. Fear is considered to be the most primitive emotion ever experienced by a human being, it is thought to be essential in surviving and evolution (Stanley, 1894) and is one of the so-called ‘basic emotions’ together with joy, sadness, anger, disgust ,and surprise (see fig. 1)

(Dailey et al., 2002; Ekman, 1992, 1999; Hamann et al., 2020). The concept of basic emotion is not trivial and is nowadays one of the most debated issues in the field of affective neurosciences (as an example see Cohen, 2005). These emotions have to be 'pure', meaning that a basic emotion should not contain another 'spurious' (in a mathematical sense) emotion (e.g., guilt is a non-basic emotion that is an ensemble of fear and joy) (Caruana et al., 2017). Moreover, emotions are considered to be basic, or primary, because they all share some features: they are innate, universal (i.e., the emotion shows similar manifestation across species and cultures), they are linked to distinctive memories and subjective experiences, they are automatic and show a rapid onset and a short duration and, finally, they show a peculiar set of physiological responses (Caruana et al., 2017; Ekman, 1999).

Without analyzing thoroughly all the different aspects of basic emotions, there are two that are functional in the framework of this work: universality and physiological responses. At the very basis of the universality of basic emotions there might be the evolutionary role of emotions themselves. One of the biggest experts in the field is the psychologist Paul Ekman which, in a series of works, he profusely described and studied the distinctive universal signs of emotional facial expressions (Ekman, 1992, 1999; Ekman & Davidson, 1995; Ekman & Heider, 1988; Ekman & Rosenberg, 2005). Ekman claims that basic emotions have to be universally recognized because of their evolutionary and social contributions; if we see someone expressing disgust, we know that he is probably reacting to a disturbing smell or taste by inferring his emotional state through the stereotypical visual cues characterizing disgust as a universal and basic emotions (Ekman, 1999).

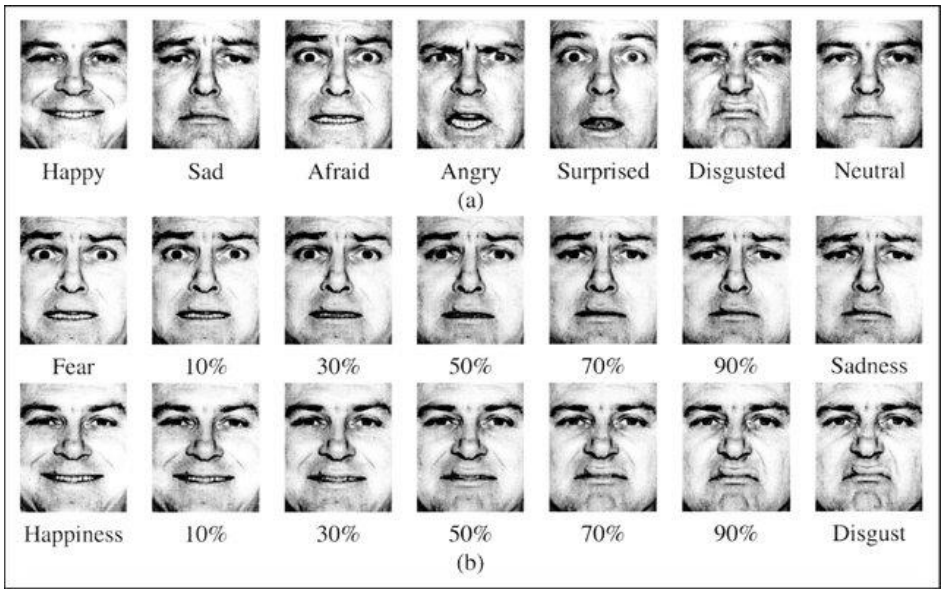

Figure 1: First row. Examples of prototypical facial expressions for the six basic emotions and a neutral face for actor "J. J." in Ekman and Friesen's Pictures of Facial Affect (POFA) (Ekman & Friesen, 1976). Second and third rows. Morphs from fear to sadness and happiness to disgust, generated from the corresponding prototypes (Dailey et al., 2002).

Anyway, differently from Ekman's view, this kind of shared universal emotional patterns are a peculiarity not only of facial expressions, but also of other emotional behaviors like body language. Emotional body language has been shown to be able to carry as much information as facial expressions (Aviezer et al., 2012; De Gelder, 2009; Meeren et al., 2005; Ross & Flack, 2020) and to be able to induce similar physiological response (Nummenmaa et al., 2014). Hence, it appears that basic emotional information expressed through the whole human body, with little-to-no distinctions between facial expressions or body language, are recognized as a universal, ontological, language crucial for survival, empathy and sociality (Gallese et al., 2004).

But, as previously stated, this universal 'language' expressed via basic emotions has also to express specific physiological adaptations (Ekman, 1999). Physiological responses to emotions are thought to be fundamental in order to prepare for an adequate behavior. As suggested by Stemmler et al., the autonomic nervous system (ANS) adaptations are not only necessary for priming the subject for a proper response, but are also specific for every emotion so that the behavioral output might be differentiated (Stemmler, 2004). It seems pretty obvious that the reaction that we have in a situation of danger is different from the one that we might experience when we are in a comfortable environment with our partner, but actually the ANS adaptations are not always so dissimilar. As an example, if we take the physiological modifications derived from experiencing fear or joy, in both we will observe increased heart and respiratory rates but the sympathetic activity will show different regional effects in inducing vasoconstriction or vasodilation (Kreibig, 2010). In front of a threat, hence experiencing fear, the ANS leads to an increased sympathetic tone which in turn will induce vasoconstriction on the skin vessels and vasodilation on the big muscles of the legs in order to prime the body for an explosive and rapid motor response (Montoya et al., 2005), while the opposite will happen in case of joy, where the most typical physiological reaction is blushing (Kreibig, 2010). Anyway, reducing the behavioral modification induced by emotions solely to the activity of the ANS might result simplistic, so for a better understanding of the neurophysiological aspects of emotional processing it is necessary to dig deeper into the central nervous system.

1.2 Emotions and the nervous system

In the previous paragraph we described how the emotions modulate the ANS. If we consider that autonomic reflexes are coordinated at the spinal cord level, functions such has blood pressure, pupillary dimensions and respiration derive from a brain stem level, which in turn is affected by the activity of subcortical structures. More specifically, the autonomous system is influenced by the hypothalamus, a subcortical structure central for ANS control, and in turn the hypothalamus is affected by the activity of other cortical and subcortical structures crucial in emotional processing such as the insula, the anterior cingulate cortex (ACC), the ventromedial prefrontal cortex and, most importantly, by the amygdala (AMG) (Kreibig, 2010). To a closer look, the ACC, the AMG, and the same hypothalamus, together with the hippocampus and the thalamus, are all parts of the limbic system. The AMG is a subcortical structures that plays a pivotal role in decoding emotional information, and it shows a widespread influence on cortical and subcortical structures (Davis & Whalen, 2001). More specifically, the AMG receives peripheral sensory information which in turn are elaborated by its basolateral (BLA) nucleus, and sends at first 'internal' projections to the central nucleus (CeA) and consequently to a whole set of structures involved in motor and non-motor functions (see fig. 2) (Davis & Whalen, 2001; Grèzes et al., 2014; Roelofs, 2017).

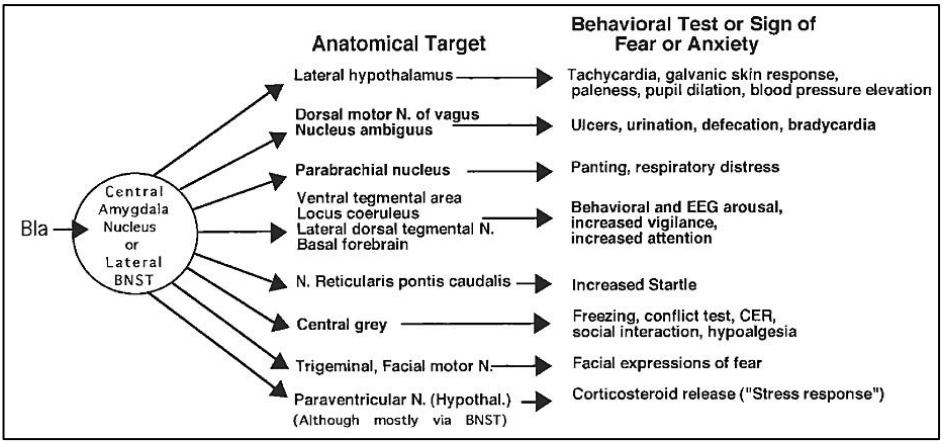

Figure 2: Schematic diagram of the outputs of the central nucleus or the lateral division of the bed nucleus of the stria terminalis (BNST) to various target structures and possible functions of these connections (Davis & Whalen, 2001).

Furthermore, studies have shown that the AMG does not have only a role in emotional processing, but also in orienting attention over salient external stimuli; in fact, in response to aversive emotional stimuli, the CeA is able to project to the basal forebrain (BF) and the locus coeruleus, two subcortical structures strictly implicated in attentional control (Davis & Whalen, 2001; Vuilleumier, 2005). The term basal forebrain refers to an extended continuum of subcortical neurons that provides projections to a variety of neocortical fields and limbic structures implicated in various aspects of cognitive function (Baxter & Chiba, 1999). The BF, and in particular one of its main regions called the nucleus basalis of Meynert which receives direct projections from the CeA (see fig. 2), has been shown to be involved in non-conscious attentional control to fearful emotional stimuli (Baxter & Chiba, 1999; Tamietto & De Gelder, 2010). More in details, the activation of cholinergic neurons by the hand of the AMG in the BF

has shown to be able to lower the response threshold of sensory cortical areas through the release of acetylcholine. This mechanism is particularly relevant when in presence of an ambiguous stimulus that might, or might not, represent a threat for the self. If we assume that an ambiguous stimulus requires an increased amount of information to be acquired by the brain in order to decide for an appropriate behavior (e.g., approaching or avoidance), it seems more than plausible to expect an augmented activity of the brain networks involved in attentional control (Davis & Whalen, 2001). Moreover, as shown by Kapp et al., it appears also that the AMG is able to trigger a behavioral response that induces a transient decrease of the heart rate which might reflect an initial increase of attentional control over a threatening stimulus, made possible through the activation of a cholinergic network that projects to the cortex, hence through the BF (Kapp et al., 1992). This last feature related to the BF and the attentional control exerted over aversive emotional stimuli is linked also to another important structures that is responsible for behavioral response. We already saw that, when confronted with a potential threat, the primary and most investigated response is the fight or flight, hence a mechanism related to motion. But it has also been observed another type of reaction mainly linked to subcortical structures responsible for autonomic response and attentional orientation that is called 'freezing'. We know that the ANS consists of two branches, the sympathetic and the parasympathetic nervous systems; while the first one is responsible for the autonomic responses we previously listed (e.g., increased heart rate, vasoconstriction, etc.), the parasympathetic branch of the ANS is responsible, among others, for bradycardia (Roelofs, 2017). Freezing response to threatening stimuli has been shown to be characterized by a parasympathetic dominance,

which in turn is apparently mediated by another subcortical structure strictly related to the CeA and that shows an enhanced activity during fear processing: the periaqueductal grey (PAG).

The PAG is a midbrain region implicated in several homoeostatic processes including fear, pain, and analgesia. In particular, one region of the PAG, the ventrolateral (vlPAG), is implicated in the freezing response (Walker & Carrive, 2003). It has been shown that the vlPAG-AMG connectivity is augmented in the first phases of threat response, inducing bradycardia and postural freezing, while is reduced when subjects decide to act in response to the menace, where a switch from the vlPAG to the dorsolateral PAG (dlPAG) is observable (Hashemi et al., 2019; Libby Jr. et al., 1973; Roelofs, 2017). It appears then that the vlPAG functions as a 'brake' on the fight or flight response, making it possible for the subject to enter in a freezing state which enables increased attentional allocation facilitated by increased cholinergic tone and action preparation.

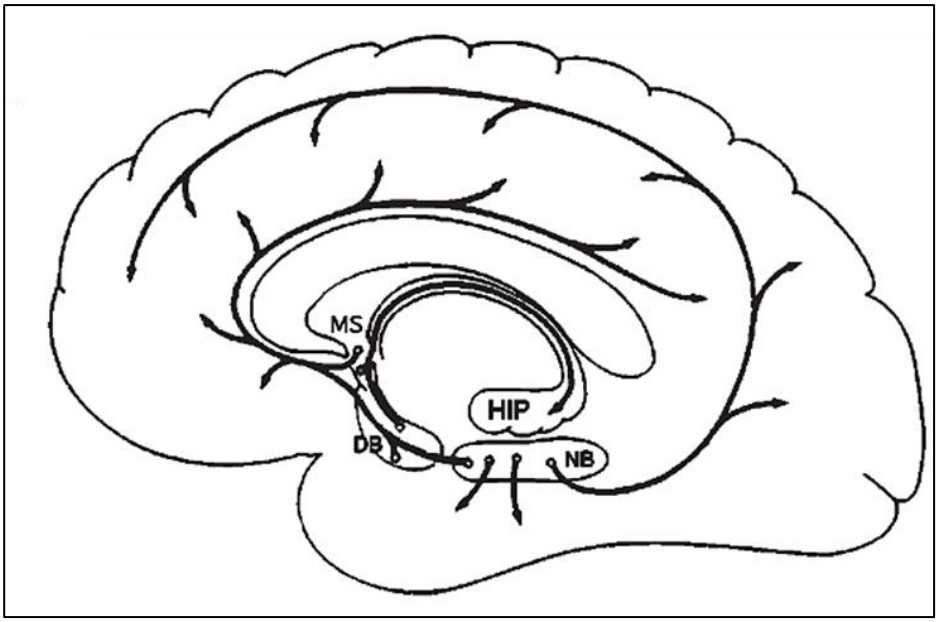

Figure 3: The basal forebrain can be divided roughly into three major divisions (rostral to caudal): the medial septum (MS), projecting primarily to the hippocampus (HIP); the diagonal band (DB) nuclei, consisting of the VDB, projecting to the hippocampus and cingulate cortex, and the horizontal limb of the diagonal band of Broca, projecting to the olfactory bulb and entorhinal cortex; and the nucleus basalis (NB), projecting to neocortex and amygdala (Baxter & Chiba, 1999).

Subsequently, by the switching from the ventral to the dorsal portion of the PAG and through the contextual activation of the perigenual ACC, subjects are able to shift from freezing to action and, it has also been shown that in subjects where the increase of vlPAG-AMG connectivity is stronger, accuracy of the motor output results improved (Gladwin et al., 2016; Hashemi et al., 2019; Roelofs, 2017). Summing up, although freezing might be seen as a defensive mechanism involving the total absence of motion, actually it appears to be a reaction involved in the active preparation of an appropriate motor response.

Anyway, the freezing reaction also shows another peculiar aspect: its duration is related to the proximity of the threat. As far as environmental factors are concerned, the distance from the predator and the presence of escape routes play an important role in determining whether species freeze or not. A distal threat evokes longer freezing reactions than proximal threat, while with escape routes available, freezing is shorter and more likely followed by fleeing compared with a situation were no escape routes are available (Roelofs, 2017). It comes without saying that a crucial role on freezing duration is also played by the AMG. A brilliant example in order to make this statement more understandable was given by Joseph Ledoux in his 'The emotional brain' (Ledoux, 1998). Imagine ourselves walking in a forest and suddenly see a slender curved shape on our path; if the distance between us and the unknown shape is relatively long, we will probably linger a little bit more to make it possible to recognize whether we are observing a simple piece of wood or a snake and then act consequentially. If, on the other hand, we notice the curved shape at a closer distance, our freezing response will probably be abruptly truncated by a motor reaction that firstly will put a reasonable distance between us and the possible threat. This different reactions are explicable only if we take into account the AMG and the fact that the firsts, unfiltered information arriving to this structure are provided by the sensory thalamus via a preferential low road that is responsible for the first response to a potential danger (Ledoux, 1998). In fact, amygdalar response to known aversive emotional stimuli has been shown to appear already at 70 ms after stimulus presentation, leading to the abovementioned subcortical activations which result in a cascade of events leading to an appropriate response (Méndez-Bértolo et al., 2016).

In conclusion, the behavioral response to a salient emotional stimulus shows to be a multifaceted interaction between adaptive evolutionary tracts and autonomic responses mediated by cortical and subcortical widespread networks, nowadays not entirely unveiled in their complexity. We saw that the response to a threat is dynamically modulated by the interplay of attentional control, decision making, freezing response and motor reaction, hence the question: how does emotions interact with the sensorimotor system?

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2. The relationship between emotions and the sensorimotor system

"We organisms are sensorimotor systems"

(Harnad, 2017)

2.1 The sensorimotor system

The sensorimotor system, or sensorimotor network (SMN), a subcomponent of the comprehensive motor control system of the body, is extremely complex. Such complexity comes from the fact that the brain areas which take part in the sensorimotor system have the hard duty to elaborate the most disparate information from the periphery and to integrate them in order to produce an efficient motor output. From an anatomical point of view, the sensorimotor network comprises the sensorimotor cortex (M1/S1), the premotor cortex, the supplementary motor area (SMA), the ACC, the occipital cortex, the temporal cortex, and the insula (see fig. 1A). This circuit also includes: the lentiform and caudate nuclei, the ventral thalami, the rostral part of the left red nucleus and the cerebellum. More specifically, the sensorimotor network shows a bilateral cerebellar involvement located within the hemispheric portion of lobules V and VI (see fig. 1B) and within what is likely the dorsal portion of the dentate nuclei (see fig. 1C) (Habas et al., 2009).

The structure of the sensorimotor system already shows how integrated this system should be, despite its vastity and the heterogeneity of structures involved. This integration results even more evident when we observe how

attentional, cognitive, and emotional loads are able to modulate the motor cortex. The phenomenon called afferent inhibition is one of the main features that characterizes this modulatory effect, and it is referred to the inhibition induced by an afferent sensory volley which reaches S1 and, in turn, inhibits M1. Even though there are several methods investigating sensorimotor inhibition, the underlying mechanisms are still not completely understood, especially when it comes to the neural pathways involved. Among the different protocols able to assess afferent inhibition, one of the most used is the short-latency afferent inhibition (SAI) protocol, which involves transcranial magnetic stimulation (TMS), defined by Tokimura and colleagues in 2000 (Tokimura et al., 2000). Without going through the technical details which will be discussed in chapter 5, in this section we will explore the neurophysiological aspects of such methods and the factors influencing SAI.

As previously mentioned, the neural pathways underpinning sensorimotor inhibition, and hence SAI, remains unclear. Some studies suggest that SAI may result from direct thalamocortical projections to M1 through cholinergic thalamic nuclei. Supporting this hypothesis there are a series of studies (a full review can be found in Turco et al., 2018) where a total suppression of SAI was observed in patients with thalamic stroke ipsilateral to the hemisphere where sensorimotor inhibition was tested, while the N20 component of the somatosensory evoked potentials remained untouched, hence suggesting that thalamic projections to M1 are essential in order to evocate SAI (Oliviero et al., 2005).

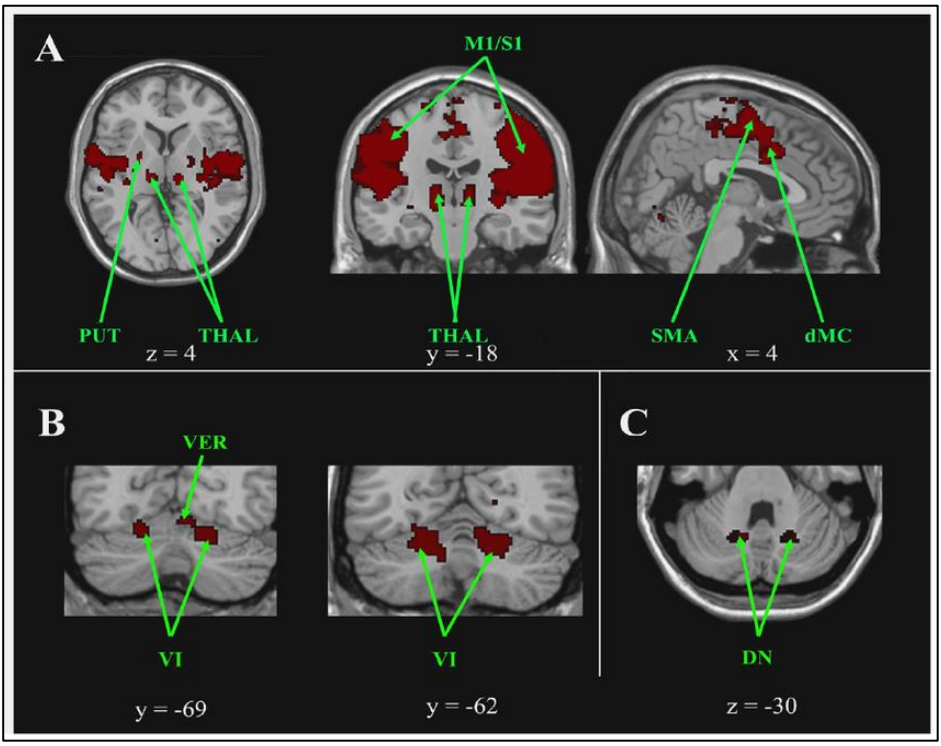

Figure 1: Cortical, subcortical, and cerebellar regions of the sensorimotor network (A) Cortical and subcortical regions of the sensorimotor network are shown on axial, coronal and sagittal slices. (B) Cerebellar regions are shown on coronal slices and in (C) on an axial slice. The left side of the image corresponds to the right side of the brain (radiologic convention). Abbreviations: CN caudate nucleus; dMC dorsal motor cingulate cortex; DN dentate nucleus; PUT putamen; RN red nucleus; SMA supplementary motor cortex; THAL thalamus (Habas et al., 2009).

A working model for this last hypothesis was given by Turco et al. in their review about the mechanisms implied in SAI generation (Turco et al., 2018). In this model, the modulatory pathway for sensorimotor inhibition at short latency involving intracortical modulation exerted by S1 on M1 shows two possible alternatives, the first mainly mediated by GABAergic activity and the second involving cholinergic inputs (see fig. 2). Specifically, both the proposed pathways involve thalamocortical projections carrying afferent inputs from the periphery, but in the first scenario these projections are directed to spiny stellate cells in the fourth layer of S1 which exert an excitatory activity on pyramidal cells located in layer II/III always of S1. Consequentially, the excited pyramidal cells in S1 project onto I3-wave generating interneurons in layer II/III of M1 which exert their activity on the corticospinal neurons. Of notice is the fact that the activity of the interneurons in M1 might be twofold depending on GABAergic tone in the sensorimotor cortex: elevated GABA results in a reduced sensorimotor inhibition, while reduced GABA shows the opposite effect, hence increased inhibition (see the upper pathway in fig.2).

Regarding the second scenario described in the model, it has been proposed that the pyramidal neurons in S1, together with the thalamocortical pathway, also project in layer V/VI of M1 onto basket cells, a type of GABAergic cells in the perisomatic region of the pyramidal neurons in M1 which exert a hyperpolarizing effect on the pyramidal cells themselves. The peculiar feature of these basket cells, which were shown to be associated with behavior-dependent cortical activity patterns throughout their inhibitory functions on pyramidal cells, is the fact that they are activated by cholinergic inputs and not only by GABA (Freund & Katona, 2007; Szabó et al., 2010; Turco et al., 2018). This

distinguishing tract was furtherly confirmed by pharmacological studies, where participants which were administered with acetylcholinesterase inhibitors showed increased SAI (Vincenzo Di Lazzaro et al., 2005).

If we think about the BF structures involved in emotional processing and their widespread cholinergic network projected all over the cerebral cortex, the fact that SAI depends on cholinergic activity indicates that cognitive functions and emotional processing might be able to modulate sensorimotor inhibition and, in turn, our upcoming behavior.

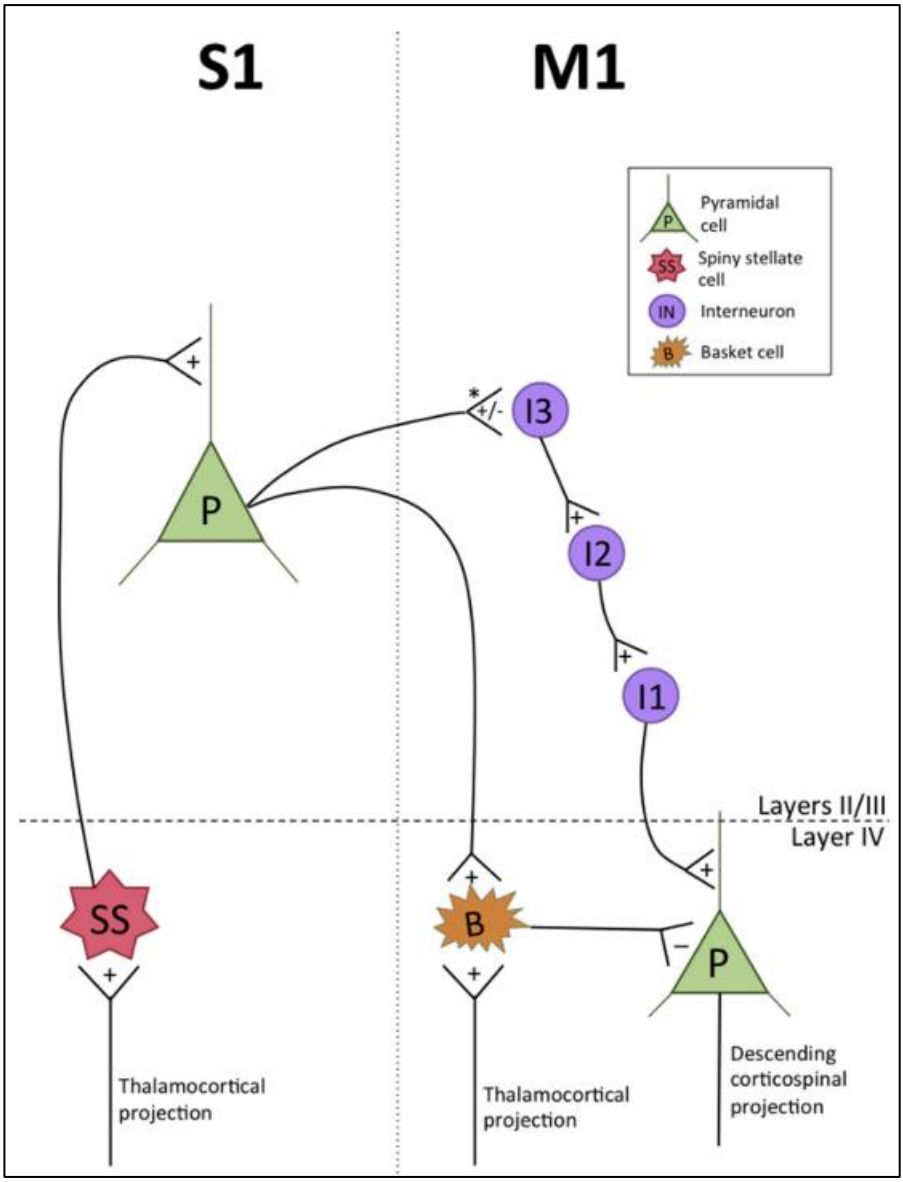

Figure 2: Working model of SAI neural pathways. The picture shows the two proposed models for the neuronal mechanisms underlying SAI by Turco et al. (Turco et al., 2008).

2.2 From emotion to motion

In the previous paragraphs we provided evidence on how emotional processing calls to action a variety of brain structures involved in behavioral control. Emotions can increase sensory vigilance by rapidly involving subcortical structures like the AMG which in turn is able to induce an augmented cholinergic tone throughout the cerebral cortex mediated by the interaction of the BF. This neural circuitry has shown to be able to modulate motor response under a variety of emotional stimuli and in particular when it comes to threatening stimuli. Anyway, considering the vastity of the topics faced so far, it is important to restrict this broad scientific field to the one that will be explored in more details in this thesis, that is the processing of emotional body language (EBL).

In the first part of this manuscript, we discussed how basic emotions are characterized by a universal motor pattern that is easily recognized at an early phase of somatosensory processing, but research in the last decades has mainly focused on the understanding of the effects of the observation of basic emotions representation in facial expressions, to the detriment of other body areas. If it is true that universal patterns are retrievable in faces, consequentially it seems plausible to expect the same also when it comes to other emotional cues such as in EBL, as already briefly previously mentioned. Before discussing the neural correlates of emotional processing in terms of motion, a first analysis on the brain areas activating during the observation of facial and body movements, embedded or not with emotional information, is required so to understand if and how these behaviors interact with motor areas. Digging deeper in this topic, the first step to analyze should be regarding the areas involved in faces and body perception to

clarify whether the amount of information extracted from such behaviors is comparable and to understand the temporal dynamics of such a process.

In a series of studies by the group of Beatrice de Gelder, it has been shown that the areas activated during the observation of facial expressions mainly overlap with the ones activated during processing of EBL. Specifically, the observation of fearful EBL activated two areas firmly attributed to facial recognition, the AMG and the fusiform gyrus (FG) (Beatrice De Gelder, 2009; Peelen & Downing, 2005). If the activation of the AMG while observing aversive emotional stimuli might sound not particularly striking considering what already discussed, the activation of the FG deserves a little bit more of attention. The FG shows activity in two different, although partially superimposing, loci selectively triggered by facial and bodily information; facial expressions showed to activate the so called fusiform facial area (FFA), a well-known portion of the FG specialized in facial discrimination (see fig. 3A, left panel) (Kanwisher et al., 1997) while, on the other hand, body expressions showed an higher activation of the fusiform body area (FBA) which in turn is specific for bodies (see fig. 3A, right panel) (Peelen & Downing, 2005). Interestingly, even though at first these areas seemed to be completely coincident, further studies showed that they are well defined and segregated areas encoding for different bodily information processing (see figure 3B) (Peelen et al., 2006).

EBL has also been shown to be rapidly encoded by another cortical area belonging to the occipitotemporal cortex that, by virtue of its capacity to being active when processing bodily information, has been called the extrastriate body area (EBA) (see fig. 3A, left panel).

As for the FFA and the FBA, also the EBA shows different functional and structural analogies with another cortical area renown for processing facial expressions (i.e., the occipital face area – OFA) (B. de Gelder et al., 2015). These areas respond specifically for faces or bodies, independently whether they are enriched with any emotional information, but the most interesting aspect that shows the similarity in the elaboration of such stimuli is the fact that from a temporal point of view they all show an early processing peaking at 170 ms after stimulus onset (Beatrice De Gelder, 2006). This early elaboration is even faster when it comes to EBL, where the first response to fearful stimuli was observed already at 80-100 ms after stimulus onset mainly in the intraparietal sulcus and intraparietal lobule, indicating that the visual stream is triggered earlier by aversive information (B. de Gelder et al., 2015; van Heijnsbergen et al., 2007).

Based on the evidence shown so far, it appears clear that EBL offers the same amount of information as facial expressions and that the elaboration of whole-body emotion expressions is as rapid as for faces.

Anyway, this early processing is not related only to occipitotemporal areas, but EBL shows an effect also on other associative areas. More specifically, it has been observed that aversive EBL (e.g., fearful or angry EBL) is able to activate a cortical area, namely the superior temporal sulcus (STS), and especially in its posterior part (pSTS) responsible for complex motion recognition (B. de Gelder et al., 2015; Pelphrey et al., 2004; Ross et al., 2019). The pSTS shows functional links ranging from the occipital area V5 to the FBA and the EBA, indicating an integrated system whose aims go from the recognition of body

shapes to the understanding of human movements and which, in turn, is able to shape social interactions (Ross & Atkinson, 2020).

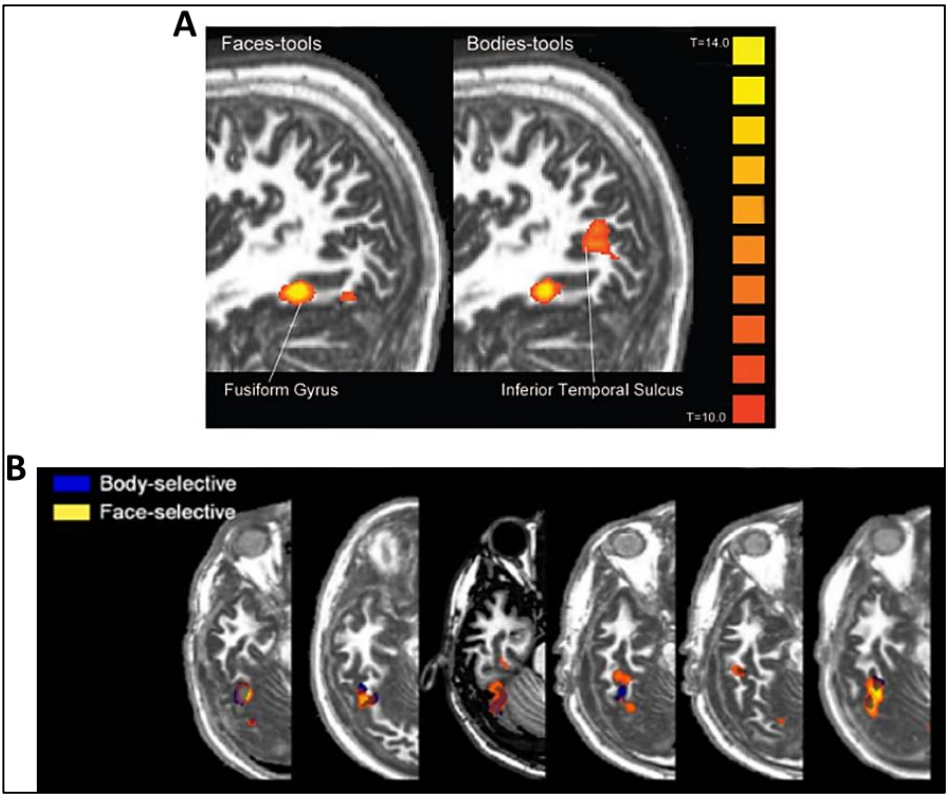

Figure 3: Fusiform gyrus activation during observation of emotional facial expressions and body language. A: significant activation in fusiform gyrus for faces and bodies. The picture shows the difference between activations in the experimental condition (Faces or Bodies) minus the control condition (Tools). It is also observable the activity recorded for bodies in the extrastriate body area (corresponding to the MNI coordinates for the inferior temporal gyrus in Peelen et al., 2005). B: Body-selective (blue) and face-selective (yellow) significant activations in posterior fusiform gyrus (Peelen et al., 2006).

This last statement is related to the fact that the STS is implicated, together with some frontal areas as the medial prefrontal cortex, in mental-state attribution and theory of mind, making it possible for humans to infer emotional state of other subjects by the mere observation of their motor behavior (Allison et al., 2000).

The ability of humans to understand the emotional state of other through their behavior step through also another peculiar aspect which is simulation. The activation of a huge brain network involving not only visual but also temporal and post-central areas reflects the fact that understanding of human's behavior necessarily needs the integration of different systems involving visual, spatial and contextual attention, motion detection and understanding and, finally, embodiment, hence interoceptive and proprioceptive sensation associated to a specific emotion (Ross & Atkinson, 2020). Anyway, EBL has also the ability to induce not only mental simulation of the perceived emotional state, but also to trigger motor activity. From a behavioral point of view, the perception of emotions showed to be able to evoke the activation of muscles commonly involved in the emotional behavior observed within a timeframe compatible with cognitive processing and behavioral responses (i.e., more than 3 seconds) (Huis in 't Veld et al., 2014). Further evidence came also from behavioral studies, where EBL perception showed to influence reaction times (RTs), hence motor readiness. In a forced-choice discrimination task performed on healthy participants by Van den Stock et al. it was shown that emotional stimuli had an influence in accelerating (or decelerating) response times, where shorter RTs were retrieved for happiness and sadness compared to anger and fear, but no clear relations were found for RTs and valence (Van den Stock et al., 2007). To be honest, it must be said that behavioral studies in emotions discrimination, and in particular for EBL

stimuli, are uncommon even though they might provide evidence for later motor modulation during emotional processing.

But the effects of emotions perceived in EBL, as already partially shown, are extremely rapid and this velocity has also a cortical counterpart which in turn is able to modulate motor output. In a series of TMS experiments by Borgomaneri and colleagues where participants were asked to observe emotional stimuli involving pictures from the International Affective Picture System (IAPS) database (Lang Bradley, M.M., & Cuthbert, B.N., 2008) and EBL pictures from an independent database elaborated by the group itself (see fig. 4), they showed that there is an early modulation of M1 already at 120 ms after stimulus onset (Borgomaneri et al., 2012, 2014, 2015).

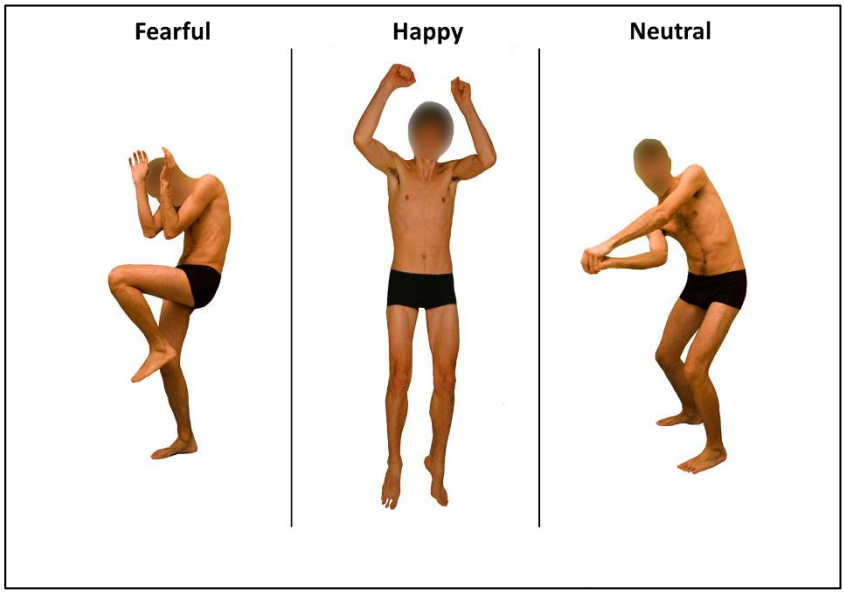

Figure 4: Examples of emotional body language visual stimuli by Borgomaneri et al. All pictures depicted male actors with blurred faces, performing several emotional and non-emotional postures (Borgomaneri et al., 2012).

Specifically, they tested several aspects of M1 modulation, from corticospinal excitability (CSE) to inhibitory (i.e., short intracortical inhibition - SICI) and facilitatory (i.e., intracortical facilitation - ICF) cortical mechanisms. In the first study they found an increased CSE related to the observation of negative IAPS stimuli at 150 ms after stimulus onset, indicating that aversive emotional stimuli can prime the motor system. This was the first neurophysiological evidence recorded with TMS of the so called 'negativity bias' and how it is able to modulate CSE in an early timeframe in participants at rest (Borgomaneri et al., 2014). Beforehand, they already assessed that both IAPS and EBL were able to induce modifications in CSE at longer latencies (i.e., 300 ms after stimulus onset) (Borgomaneri et al., 2012), but after observing the early modulations induced by emotional IAPS, they aimed to assess whether EBL was capable to modulate intracortical networks such as SICI and ICF. Starting from their previous observations and from other neurophysiological studies where activity in motor and premotor areas were reputed to be involved in emotional processing and motor control (Beatrice de Gelder et al., 2010; Beatrice De Gelder et al., 2004; Vuilleumier, 2005), their main aim was to study intracortical modulation of M1 at intervals (i.e., 100-125 ms after stimulus onset) coinciding with the event-related potential component P100 (a positive deflection retrieved in electroencephalography recordings in the occipital areas peaking at ~100 ms after stimulus onset) during emotional processing (as an example see van Heijnsbergen et al., 2007). They observed that the observation of fearful EBL is able to transiently reduce ICF in M1 at 120 ms after stimulus onset, hence inducing what they called a 'freezing-like' phenomenon of the motor cortex, while no effects was observed for the other stimuli (i.e., happy and neutral) and

for SICI (see fig. 5) (Borgomaneri et al., 2015). They explained the fact that fearful EBL had a stronger effect on ICF but not on SICI by suggesting that processing of emotional bodies appears to be mainly associated with a reduction in the input to excitatory glutamatergic interneuronal networks in M1 while it does not conspicuously modulate GABAergic cortical circuits presumably operated by cortical-subcortical networks. Interestingly enough, the effects on ICF, hence the reduced intracortical facilitation retrieved for fearful EBL at short latencies, was observed also in the comparison between positive and neutral EBL, showing that emotional language in general is able to modulate intracortical networks but that the effect is more evident for fear (Borgomaneri et al., 2015).

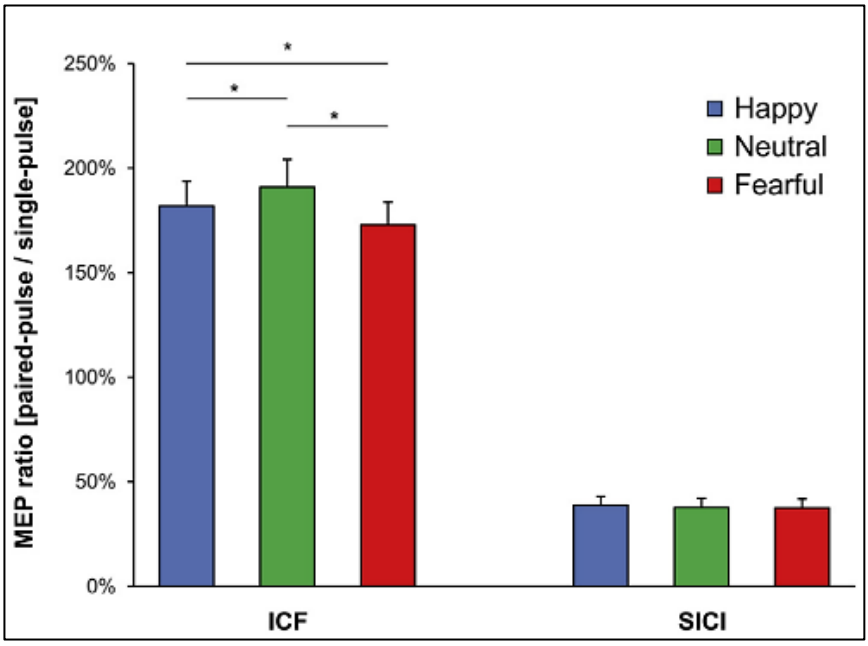

Figure 5: Results from the study by Borgomaneri et al., 2015. In the picture it is possible to observe the significant differences in ICF modulation by the hand of emotional and non-emotional EBL stimuli at 120 ms after stimulus onset, where fearful EBL showed the higher reduction compared to the other conditions. No differences were retrieved for SICI (Borgomaneri et al., 2015).

Beside the investigation of early time windows, they also studied the same phenomena at 300 ms after stimulus onset in order to observe if, by observing emotional stimuli embedded with motor information (as EBL pictures are) the modulation of intracortical network was present or not; what they saw was that no modulation for ICF nor for SICI was present, hence indicating that at longer latencies, where cognitive interference might be at stake, modulation of neural circuitry apparently depends on motor resonance and recognition of body postures.

Summing up, it appears clear that emotional processing influences activity in sensorimotor areas starting from an early modulation of the sensorimotor network and arriving to the motor output, although evidence in the interaction between EBL and the sensorimotor system is still scarce. From neurophysiological studies, the early elaboration of emotions results in modifications of behavioral responses derived from cortico-subcortical pathways able to rapidly integrate peripheral information and to induce autonomic and instinctive responses to potential threats, while at longer latencies sensorimotor system shows the capability to infer the emotional state from other's behavior and to simulate it to orchestrate an appropriate response.

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3. Aim of the work

In the first two chapters of this thesis, it has been shown that the processing of emotional information is a convoluted issue, ranging from macroscopical phenomena expressed by individuals' behavior to microscopical events occurring between widespread cerebral networks. If a large literature upon emotional facial expressions is retrievable among the numerous studies conducted in the last decades, the same cannot be said for emotional body language, where there is a lack of behavioral and neurophysiological evidence.

For this reason, on the verge of a renewed interest in the field grown in the last years, we developed four experiments to address the relationship between emotions and the sensorimotor system in healthy young individuals (experiments 1, 2 and 3) and in patients with Parkinson's disease (experiment 4).

In the first experiment, we aimed to explore the ability to process, discriminate and recognize emotional information carried by body language and to test whether motor response to such stimuli is mainly driven by the emotional content of the pictures or if it reflects the influence of motor resonance. Response times, as a measure of motor readiness, were recorded. Results showed a behavioral advantage with faster motor response for fearful EBL stimuli.

The second and third experiments were aiming to disentangle the underpinning processes relating emotional body language processing to the sensorimotor system, also taking into consideration the behavioral advantage that we observed in the first study.

In the second experiment we tested sensorimotor integration during the passive observation of emotional postures. We used a transcranial magnetic stimulation (TMS) protocol named short-latency afferent inhibition (SAI), capturing sensorimotor interactions, while healthy participants were observing emotional body language (EBL) and International Affective Picture System (IAPS) stimuli. SAI was tested at an early (120 ms) and a late (300 ms) time points from picture's presentation. The most striking finding was that at the earlier time point (120 ms), fear-related EBL and IAPS stimuli selectively enhanced SAI as indexed by the greater inhibitory effect of somatosensory afferents on motor excitability.

The results of the second study drove the third experiment whose aim was to explore whether processing of EBL was able to modulate cortical activity in sensorimotor areas, hence ERPs and cortical oscillations in the μ-alpha and β frequency bands, at short latencies (around 100 ms after the onset of the EBL stimulus). Our hypothesis was that modulation of sensorimotor integration (Botta et al., 2022) was driven by increased activity in the primary sensory cortex, exerting an inhibitory effect on primary motor cortex excitability. By using high-density electroencephalography (hdEEG) coupled to source activity reconstruction we had the chance to obtain information on spatial distribution and temporal dynamics of neural oscillations during EBL processing at early latencies.

Lastly, in the fourth experiments we partly replicated the experimental design of the first experiment but in patients with Parkinson's disease and using not only emotional body language stimuli and emotional scenes, but also

emotional facial expressions. By doing so, we aimed to explore if and how emotional information processing might be impaired in patients affected by Parkinson's disease, a neurodegenerative disease characterized by basal ganglia dysfunction, subcortical structures largely implicated in emotional processing.

4. Experiment 1: Modulation of response times during processing of emotional body language

Alessandro Botta, Giovanna Lagravinese, Marco Bove, Alessio Avenanti and Laura Avanzino

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4.1 Techniques: E-Prime 3.0

E-Prime is a software designed for the management of cognitive neuroscience experiments developed by the company Psychology Software Tool (PST). It is one of the most used software for psychological experiments and it allows the presentation of visual, audio or video stimuli, to collect behavioral responses (e.g., response times) and to store data in pre-determined suites integrated in the main software. E-Prime is based on Object Oriented Programming (OOP) and the programming of the experiment is possible through the main app 'E-Studio'. Specifically, 'E-Studio' is an integrated work environment that operates by graphical interface through which is possible to compile a program without requiring an online code development. The programming suite is based on a 'drag-and drop' system where all the usable tools are shown in the 'E-Objects' toolbox area on the left of the workspace (see fig. 1, red box). The whole experiment, which will include all the temporized stimuli, the fixation screen and the instruction for data recording, will be presented with a tree structure where all ramifications (i.e, SessionProc, BlockProc, TrialProc) will specify a different level of instruction (see fig. 1, light blue box).

Interestingly enough, apart from the classic tools which allow the implementation of a limited number of type of stimuli (i.e., text, image, video and sound), the software makes possible the integration of functions by using a specific tool named ‘InLine’ which makes it possible to write parts of codes in C++ language in order to specify some features of the stimuli used in the experiment (e.g., randomization of stimulus duration in between a specified timeframe, etc.). Moreover, beyond the user-friendly interface and the relative easiness of use, E-Prime 3.0 can be interfaced with several external systems (e.g., electroencephalography systems, transcranial magnetic stimulation devices, gait analysis devices) via the ‘Chronos’, a device which serves as a trigger box for triggering external devices. Actually, the original purpose of the ‘Chronos’ device was not to trigger external events, but to record behavioral responses. In fact, through the device it is possible to acquire reaction times from button press or verbal responses with a millisecond accuracy. Last but not least, considering the fact that the E-Prime suite is used for cognitive neuroscience experiments where usually visual stimuli are presented, the ‘Chronos’ device is equipped with a photosensor in order to assess some monitor’s features such as the refresh rate or the display latency (i.e., the time between the sending of information by E-Prime and the actual appearance of the stimulus on the screen) so to make the response times as accurate as possible.

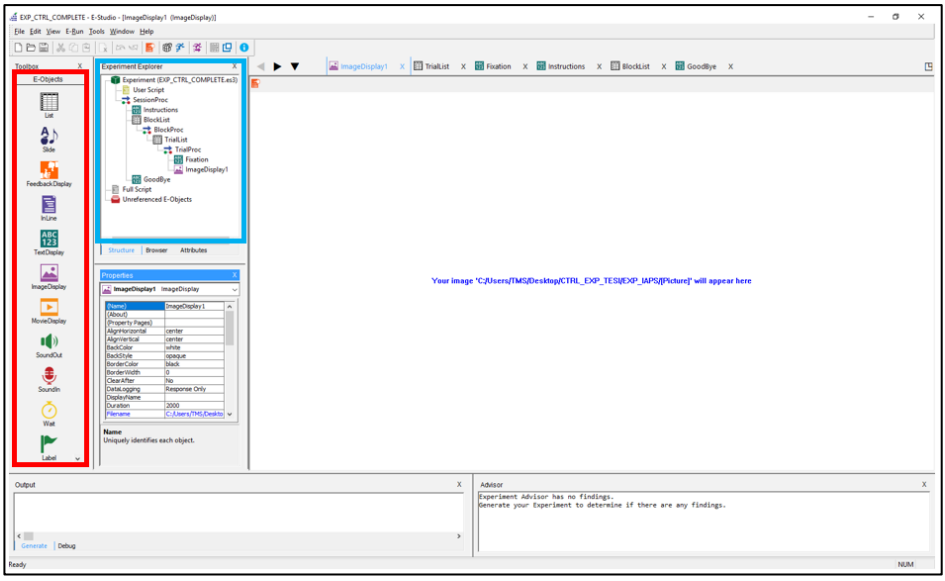

Figure 1: E-Prime workspace. The picture shows the classical workspace where to program experiments in the E-Studio suite. In the light blue square, it is possible to observe the pipeline of the experiments in its making, while in the red square there are the E-Objects usable in order to make the experiment.

4.2. Rationale

The investigation of how humans perceive and respond to emotional signals conveyed by body expressions has been for a long time secondary compared to research addressing the recognition of emotional faces or emotional scenes (Beatrice De Gelder, 2009; Beatrice de Gelder et al., 2010). Only in the last decades, an increased interest in whole-body expressions and their emotional correlates has started to emerge (Borgomaneri et al., 2012; B. de Gelder et al., 2015; Beatrice de Gelder et al., 2010; van Heijnsbergen et al., 2007).

As for facial expressions, processing of emotional body postures activates brain regions involved in perceptual and affective processes such as the superior temporal sulcus, fusiform and post-central gyrus, the amygdala and medial prefrontal cortex (Beatrice De Gelder, 2009; Downing & Kanwisher, 2001; Peelen et al., 2010; Peelen & Downing, 2005; Ross et al., 2020) as well as the mirror neuron system involved in action understanding and imitation (Bachmann et al., 2018; Beatrice De Gelder et al., 2004). Furthermore, processing facial and bodily emotional expressions spontaneously induces motor mimicry in the observer (Huis in 't Veld et al., 2014; Ross & Atkinson, 2020) a mechanism that can contribute to accurate emotion recognition (Borgomaneri, Bolloni, et al., 2020; Oberman et al., 2007; Wood et al., 2016). These studies suggest that perceiving others' emotional expressions involves a simulation of motor plans and associated sensory representations engaged when making the same expressions (Adolphs et al., 2000; Huis in 't Veld et al., 2014; Niedenthal et al., 2010; Paracampo et al., 2017; Ross & Atkinson, 2020), reflecting a simulation of whole-body state associated with the emotion (Ross & Atkinson, 2020).

Additionally, emotional bodily expressions strongly activate subcortical motor areas such as the caudate nucleus and putamen and several regions of the cortical motor system, with stronger (Borgomaneri et al., 2017; Beatrice de Gelder et al., 2010; Beatrice De Gelder et al., 2004) and faster (Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, Gazzola, et al., 2015) response to threating expressions. Such motor activations may reflect sensorimotor simulation and/or the activation of motivational tendencies which facilitate emotionally congruent behavior, with positive stimuli activating approach tendencies and negative

stimuli activating avoidance tendencies (Ekman & Davidson, 1995; Lang et al., 1990; Lang & Bradley, 2010).

Starting from all these considerations, one could speculate that readiness of the motor system may be modulated by the presence of emotional content in body posture and by the valence of the emotion. However, behavioral data in the literature are controversial, showing increased response times (RTs) in recognizing fearful body expressions (Van den Stock et al., 2007) or anger as the most difficult emotion to categorize (Atkinson et al., 2004). Noteworthy, there are methodological issues that could explain these results, as differences in the set of images used and in the behavioral task or the level of uncertainty in categorizing the emotional stimuli.

Readiness of the motor system can be studied by means of neurophysiological techniques in addition to behavioral paradigms. Recently, Borgomaneri and co-workers developed a novel set of visual stimuli to test the activity in the motor cortex during processing of emotional body postures and trying to address the aforementioned methodological issues (Borgomaneri et al., 2012). Results showed that only fearful body expressions were able to modulate cortical excitability at a very early stage of emotional processing (between 70 and 150ms after stimulus onset) (Borgomaneri et al., 2017; Borgomaneri, Gazzola, et al., 2015; Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, et al., 2020; Borgomaneri, Vitale, Gazzola, et al., 2015). However, whether this corresponds to a modulation of motor behavior has not been addressed so far.

4.3. Aim of study

The first aim of the present study was to investigate if there is a specific modulation of motor response during processing of emotional body postures by assessing response times (RTs) in a three-alternative forced choice task using the set of visual stimuli adopted by Borgomaneri and colleagues. Based on the work of Borgomaneri et al. and the notion that threating stimuli are particularly effective in priming the body for action, we expected that response to fearful body expressions would be different relative to happy and neutral body expressions.

The second aim was to compare RTs during processing of emotional body postures with RTs during processing of another class of emotional stimuli, namely emotional scenes, in order to test whether reactivity to fear-related signals are specific to the observation of human bodies. Therefore, we presented participants with emotional pictures taken from the International Affective Picture System (IAPS), and asked them to categorize as fast as possible the pictures they were viewing in one of three domains: negative (fear), positive (happy) and neutral (Lang Bradley, M.M., & Cuthbert, B.N., 2008). The use of IAPS pictures compared to other sets of stimuli gives the opportunity to manipulate the arrays of images used in the experiment, matching the values of valence and arousal of the two groups of pictures that are being used and controlling the new set of images (e.g. emotional postures) in order to accurately investigate whether the effects of the exposure may be driven by the stimulus features or by their intrinsic emotional properties (Calvo & Avero, 2009). Moreover, IAPS pictures selection might be addressed in order to minimize implicit motor information by excluding scenes with full or partial human body representation, making it possible to

compare pure emotional stimuli such as IAPS with faces or body language that intrinsically possess a high level of motor information (for an example on this issue see Borgomaneri et al., 2012b).

4.4. Experimental procedure

4.4.1 Participants

In order to estimate an appropriate sample size, a power analysis was run based on the data retrievable in the work by Van den Stock and colleagues (Van den Stock et al., 2007). Analysis run on G*Power 3.1 for comparisons of means from dependent groups with $1-\beta = 0.80$, $\alpha = 0.05$ and an effect size of 0.62, resulted in an ideal sample size of 23 subjects. Twenty-five healthy subjects (13 males, 12 females, mean age ± S.D.: 22.3 y ± 1.8) were enrolled in the study. All participants were self-reportedly right-handed and participated to both trials (Postures and IAPS) in the same experimental session.

4.4.2 Visual stimuli

A total of 90 emotional visual stimuli were used in the experimental session, 45 for the emotional body language condition and 45 taken from the IAPS database as control (Lang Bradley, M.M., & Cuthbert, B.N., 2008). The emotional posture pictures were selected from a validated database (Borgomaneri et al., 2012; Borgomaneri, Vitale, Gazzola, et al., 2015). Body language pictures depict four actors in different postures with emotional and non-emotional valence, thirty portraying negative (Fear) and positive (Happiness) movements and fifteen with no emotional significance (Neutral). The actors were not handling objects

and their face was blanked out. Luminance and refresh rate of pictures were controlled and matched for all images via a photosensor (data processed via E-Prime 3.0).

Regarding the IAPS pictures, 45 stimuli were taken from the IAPS database (Lang Bradley, M.M., & Cuthbert, B.N., 2008), fifteen with negative emotional valence (Fear), fifteen with positive valence (Happiness) and fifteen neutral pictures (Neutral). All the pictures were mirrored alongside the vertical axis in order to obtain 90 stimuli per trial, implementing the data pool while avoiding the repetition of the same stimulus.

Some issues emerged during the selection of the stimuli. Firstly, fearful emotional postures were emotion-specific; in other words, the body expression depicted in the pictures was unequivocally related to the pure emotion 'fear' (Borgomaneri et al., 2012; Ekman, 1999; Huis in 't Veld et al., 2014). On the other, 'negative' pictures in the IAPS database often show several aversive emotions combined (e.g., fear and disgust or fear and sadness). In order to avoid this possible bias, we selected the IAPS stimuli for the Fear condition from a restricted sample of pictures that have been reported to mainly evoke fear (e.g. human attacks and accident depicting pictures) (Barke et al., 2012). Secondly, to exclude most of the body movement information, we decided to exclude all IAPS pictures that depicted whole human bodies involved in some kind of actions.

Regarding the other two conditions, Happiness and Neutral, we did not find particular differences or risk of bias in the recognition of the intrinsic emotional valence of pictures. We decided to include only families and babies' and 'adventures' pictures in the Happiness condition with partial or no human

body representation. In order to strengthen the aforementioned assumptions, after the experiment we also submitted a questionnaire to each subject for both postures and IAPS, so to evaluate emotional content, valence and arousal of each stimulus.

4.4.3 Task

Visual stimuli were presented on a 22-inches computer screen (resolution: 1680x1050, refresh rate: 60.0Hz; 16.67ms ± 12.37ms) located at 80cm away from the subjects. Refresh rate was assessed via a photosensor connected to the response box and corresponded to normative values (Garaizar et al., 2014). Stimuli were presented using the E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA). The order of presentation was randomized, and each stimulus had a maximal duration of 2000ms, with an inter-stimulus interval fixation-screen of 1500ms (see Figure 1). Participants were asked to keep the right hand on a USB-based data collection device named Chronos (Psychology Software Tools, Pittsburgh, PA), with the 2^{nd}, 3^{rd} and 4^{th} finger on the first, second and third key of the response box, respectively. They were asked to categorize each visual stimulus as negative, positive, or neutral by pressing one of the 3 buttons, with different stimulus-response combinations across participants. RTs were taken as the difference in milliseconds between the onset of the visual stimulus and the pressing of the key on the response box.

4.4.4 Stimuli evaluation

Participants were presented with all the 90 stimuli (45 body postures and 45 IAPS stimuli) and asked to evaluate them using a questionnaire with no time pressure. Participants were first asked to recognize the emotion depicted in the picture by choosing between seven options: fear, sadness, disgust, anger, surprise, happiness and neutral. We considered as correct only three out of the seven options, which are fear, happiness and neutral. The other choices were not taken into consideration because outside of the study main interest. We then asked the participants to rate valence on a Likert scale ranging from 1 to 9, where 1 indicated 'absolutely unpleasant' and 9 indicated 'absolutely pleasant'; they used a similar scale to evaluate arousal, where 1 indicated 'no arousal, and 9 indicated 'high arousal.

4.4.5 Experimental design and procedure

Subjects were comfortably sitting on a chair at approximately 80 cm from the computer screen where the visual emotional stimuli were presented. Participants were asked to keep the right hand on the Chronos device. After a brief explanation of the task and the presentation of a first, fixed screen with the instructions to follow during the trial, participants were asked to press a key in order to start with a five stimuli test-trial to familiarize with the task before starting the complete, 90 stimuli experimental trial.

The experiment was divided in two sessions, one with the emotional postures and one with the IAPS pictures, in which the order was randomized in order to exclude the familiarization with the task in one particular trial and ended with questionnaires' filling.

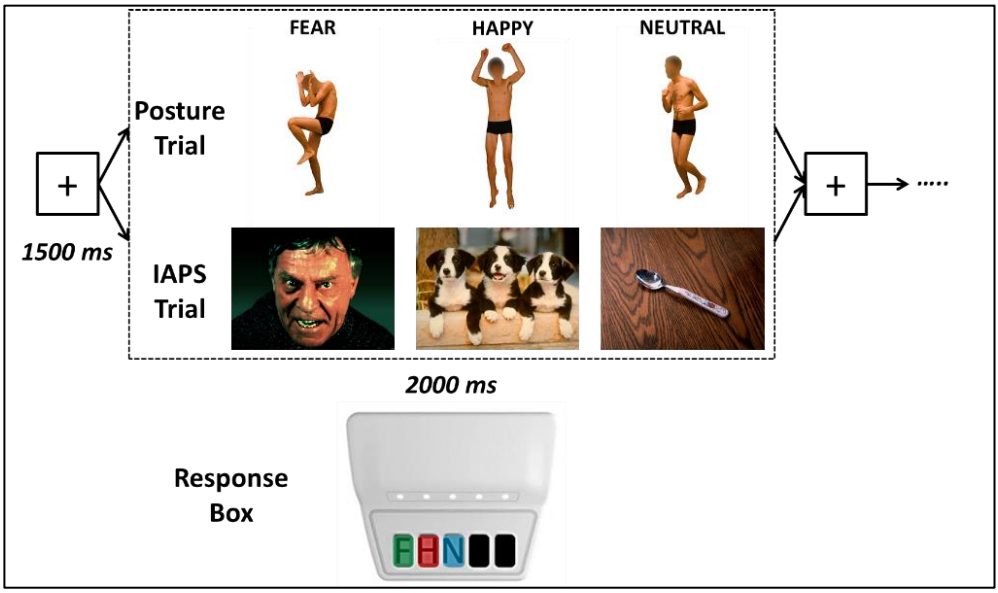

Figure 4: Experimental design. Each visual emotional stimulus had a maximum duration of 2,000 ms [the stimulus disappeared as soon as the participants pressed one of the keys of the response box, recording the response and the response time (RT)], interspersed by a fixation cross screen of 1,500 ms, for a total of 90 stimuli for each trial [postures or International Affective Picture System (IAPS)]. Response times were recorded by pressing one of three keys on the Chronos response box with: key 1 = negative (F), key 2 = positive (H), key 3 = non-emotional (N).

4.4.6 Data analysis

For each body posture and IAPS category, we computed an accuracy index as the percentage of correct responses in the forced three-choice task. Pictures with an accuracy lower than 80% were excluded from further analyses (three happiness postures and three neutral postures for body stimuli and three neutral pictures for IAPS stimuli).

Accuracy

A 2x3 repeated-measure analysis of variance (rmANOVA) with PICTURE (Posture and IAPS) and CATEGORY (Negative, Positive and Neutral) as main effects was performed on the accuracy in the recognition of the stimuli during the forced-choice RT task.

In the categorization task included in the questionnaires a 2x3 rmANOVA with PICTURE (Posture and IAPS) and EMOTION (Fear, Happiness and Neutral) as within-subject factors was performed on accuracy data for both postures and IAPS conditions.

In order to be analyzed via an rmANOVA, accuracy data were transformed in arcsine values.

Valence and Arousal

On valence and arousal data, a logarithmic transformation was performed in order to normalize the data distributions. Post-hoc analysis was performed on significant effects via Bonferroni correction of significance. Valence and arousal data were analyzed performing a 2x3 rmANOVA with PICTURE and EMOTION as within-subject factors.

Response Times and their Coefficient of Variation

RTs were analyzed performing a 2x3 rmANOVA with PICTURE and CATEGORY as within-subject factors. Only trials in which categorization in the forced-choice RT task was correct were considered for the analysis of RTs data and only if they fell in between two standard deviations from their respective

mean. Coefficient of variation (CV) of RTs was computed as the standard deviation of RTs divided by the mean of RTs for each emotion in both postures and IAPS.

Correlations and Reliability analysis

Correlations between valence and arousal were analyzed by means of Pearson's correlation coefficient for normally distributed data whereas non-parametric Kendall's tau correlation method was used in conditions of non-normality. Bonferroni correction was then performed for multiple comparisons. Correction on significance was calculated considering multiple comparisons for valence and arousal, meaning that the correction on α was 0.025.

Cronbach's Alpha coefficient was then studied to assess the reliability of the visual stimuli used in the experiment.

Statistical analysis was performed via SPSS Statistics 23.0 (IBM, Somers, USA). Significant level was set at $\alpha = 0.05$. Normality was tested via Shapiro-Wilk test and violations of sphericity were corrected through the Greenhouse-Geisser method.

4.5. Results

4.5.1 Accuracy

Accuracy in the three-alternative forced choice RT task was high (~94%). The rmANOVA showed a significant main effect of PICTURE ($F_{1,24}$ = 7.907; p = 0.01; $p\eta^2$= 0.248), with a higher accuracy observable for IAPS compared to Posture (94.8% ± 1.4% and 92.6% ± 1.6% respectively), but no main effect of CATEGORY or PICTURE*CATEGORY interaction (F < 1 and p > 0.05). Descriptive statistics are reported in Table 1.

	Postures			IAPS		
	Negative	**Positive**	**Neutral**	**Negative**	**Positive**	**Neutral**
Accuracy (RT task) (Mean ± S.D.)	93.47± 5.57	92.53± 8.18	91.73± 10.05	95.60± 5.16	93.20± 8.02	95.73± 8.25
Reaction Times (Mean ± S.D.)	759.04± 144.38	868.71± 114.24	897.34± 168.49	716.31± 102.48	696.94± 112.96	712.64± 100.11

Table 1: Descriptive statistics. In the table are reported all response times (RTs) recorded during the three-alternative forced choice task and the respective accuracy for all subjects. All values are reported as Mean ± S.D.

Questionnaire data showed lower accuracy, particularly with Fear IAPS pictures (~82% of correct answers). The rmANOVA showed a significance a main effect of EMOTION ($F_{2,48}$ = 14.282; p < 0.01; $p\eta^2$ = 0.373) indicating that

accuracy was lower with Fear (85.9% ± 3.0%) compared to Happiness (91.3% ± 2.5%; $p = 0.05$) and Neutral stimuli (96.2% ± 1.6%; $p < 0.01$), and lower with Happiness compared to Neutral stimuli ($p = 0.05$). Moreover, the PICTURE*EMOTION interaction was significant ($F_{2,48} = 4.870$; $p = 0.01$; $p\eta^2 = 0.169$) accounted for by reduced accuracy in the Fear IAPS stimuli condition. Post-hoc analysis showed that accuracy for Fear IAPS stimuli (81.6% ± 3.0%) was significantly lower compared to Happiness IAPS (93.3% ± 1.5%; $p < 0.01$) and Neutral IAPS stimuli (97.1% ± 1.6%; $p < 0.01$) and lower than Fear Posture stimuli (90.1% ± 3.0%; $p = 0.02$); no other significant differences were observed (all $p > 0.05$). For details see Table 2.

4.5.2 Valence and Arousal

Mean values for valence and arousal are reported in Table 2. The rmANOVA on valence data showed a significant main effect for EMOTION ($F_{2,48} = 298.278$; $p < 0.01$; $p\eta^2 = 0.926$), while no significance was found for the effect of PICTURE and PICTURE*EMOTION (all $F < 1$ and $p > 0.05$). As expected, post-hoc analysis showed lower valence values for Fear stimuli compared to Happiness and Neutral stimuli (all $p < 0.01$) and higher valence values for Happiness compared to Neutral stimuli ($p < 0.01$). The rmANOVA on arousal data showed a significant main effect of EMOTION ($F_{2,48} = 258.971$; $p < 0.01$; $p\eta^2 = 0.915$), but not of PICTURE ($F < 1$ and $p > 0.05$), and a significant PICTURE*EMOTION interaction ($F_{2,48} = 3.449$; $p = 0.04$; $p\eta^2 = 0.126$). Post-hoc analysis on the main effect of EMOTION showed higher values for Fear stimuli compared to Happiness ($p = 0.02$) and Neutral ($p < 0.01$), and higher values for Happiness stimuli compared to Neutral stimuli ($p < 0.01$) (see Table 2). As for the interaction effect, greater arousal was found for Fear IAPS stimuli than for

Happiness IAPS and Neutral IAPS stimuli ($p < 0.01$) and for Happiness IAPS relative to Neutral IAPS stimuli ($p < 0.01$). Fear IAPS stimuli also showed greater arousal values than Fear Posture stimuli ($p = 0.01$) whereas no difference between picture types were found for the other two emotion categories (all $p > 0.05$). Fear Posture and Happiness Posture had greater arousal values than Neutral Posture ($p < 0.01$) but did not differ from one another ($p > 0.05$).

	Postures			IAPS		
	Fear	**Happiness**	**Neutral**	**Fear**	**Happiness**	**Neutral**
Accuracy (Questionnaires) (Mean ± S.D.)	90.13± 14.92	89.33± 17.43	95.33± 10.51	81.60± 15.19	93.33± 7.45	97.07± 5.80
Valence (Mean ± S.D.)	2.41± 0.94	7.38± 0.86	5.00± 0.14	2.09± 0.67	7.32± 0.76	5.11± 0.29
Arousal (Mean ± S.D.)	6.08± 1.94	6.26± 1.68	1.61± 1.15	7.20± 0.97	5.88± 1.33	1.57± 1.17

Table 2: Descriptive statistics. In the table are reported all valence and arousal values as well as the accuracy recorded during the questionnaires submitted to the participants. All values are reported as Mean ± S.D.

4.5.3 Response Times and their Coefficient of Variation

The rmANOVA on RTs showed the main effect of PICTURE ($F_{1, 24} = 27.333$; $p < 0.01$; $p\eta^2 = 0.532$) with lower RTs for IAPS compared to Postures; the main effect of CATEGORY ($F_{2,48} = 4.881$; $p = 0.02$; $p\eta^2 = 0.169$), with lower RTs for Negative stimuli compared to Positive and Neutral stimuli (all $p < 0.01$), but no differences between Positive and Neutral stimuli ($p > 0.05$). Remarkably,

the PICTURE*CATEGORY interaction was also significant ($F_{2,48}$ = 12.076; p < 0.01; $p\eta^2$ = 0.335). Post-hoc analysis showed lower RTs for Negative Posture relative to Positive Posture and Neutral Posture (all p < 0.01) which in turn did not differ from one another (p > 0.05). Moreover, no significant differences were found between IAPS emotion categories (p > 0.05; see Figure 2A). In the comparison between picture types (Posture vs. IAPS), Positive and Neutral Postures stimuli had slower RTs than Positive and Neutral IAPS stimuli (all p < 0.01), but no differences were found between Negative Posture and Negative IAPS stimuli (p >0.05; for details see Table 1 and Figure 2A).

Coefficient of variation (CV) of RTs ranged between 20% and 30% (Figure 2B). The rmANOVA on CV showed a significant main effect of PICTURE ($F_{1,24}$ = 9.292; p < 0.01; $p\eta^2$ = 0.279) with higher CV values for Posture than for IAPS stimuli. Moreover, a significant PICTURE*CATEGORY interaction ($F_{2,48}$ = 4.436; p = 0.03; $p\eta^2$ = 0.156) showed higher CVs for Posture compared to IAPS in the Negative (p = 0.03) and Positive condition (p < 0.01), but not Neutral condition (p > 0.05). Moreover, for the Posture category, Positive stimuli had significantly larger CV compared to Neutral (p = 0.01) and marginally larger CV compared to Negative stimuli (p = 0.07) which in turn did not differ from one another (p > 0.05).

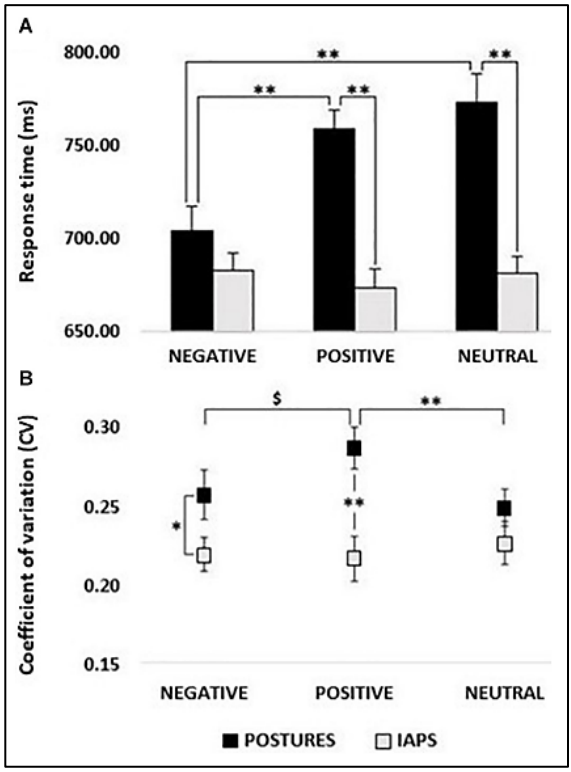

Figure 2: (A) Response times (RTs). Histogram showing a comparison between RTs recorded in postures and IAPS trials. Significant differences were found between negative and positive and negative and neutral for postures, as well as between positive and neutral in the comparison between postures and IAPS, but not for negative. No differences were retrievable between emotions in the IAPS trial. RT values in milliseconds (ms) are reported in the y-axis, and emotions are reported on the x-axis with black bars for postures and gray bars for IAPS. (B) Coefficient of variation; postures vs IAPS. Positive's CV in the posture trial was significantly higher than the one computed for neutral and showed a trend in the comparison with negative, while no differences were found for IAPS. Postures' negative and positive CVs were higher in the comparison with the variation retrieved in the IAPS trials. CV ranges from 0 to 1; on the y-axis, it is possible to observe a partial scale that focuses on the range of the CV found in the experiment. Emotions are reported on the x-axis with a straight line for postures and a dotted line for IAPS. Legend: $* = p < 0.05$, $** = p < 0.01$, $\$ = p > 0.05$.

4.5.4 Correlations

Correlations between valence and arousal ratings are shown in Figure 3. After applying the Bonferroni correction, all correlations found for Happiness pictures survived, showing larger arousal for high-valence stimuli both for Posture ($r = 0.644$, $p < 0.01$) and IAPS categories ($r = 0.483$, $p = 0.02$). Regarding Fear stimuli we found a significant negative correlation for Posture ($r = -0.754$, $p < 0.01$) and, after correction for multiple comparisons, only a marginal trend was retrievable for IAPS stimuli ($r = -0.292$, $p = 0.04$). No correlations were retrievable in the analysis of non-emotional stimuli.

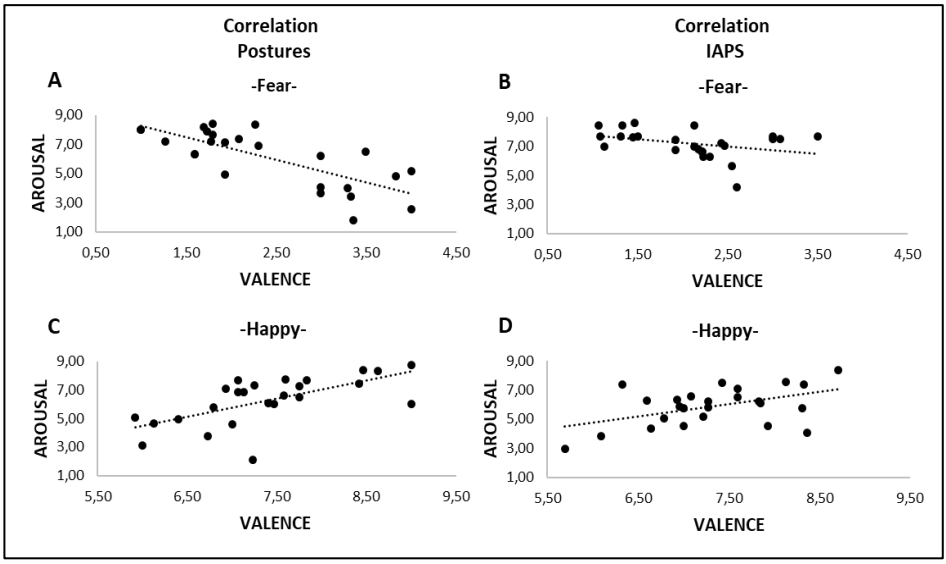

Figure 3: Correlation plots. The picture shows four plots where correlations between valence and arousal are observable. Plots (A) and (C) show the results for the posture trials; (B) and (D) the ones for the IAPS trials. A positive correlation is appreciable for positive emotional pictures (happiness; plots C and D), meaning that higher valence corresponds to higher activation; the opposite is observable for negative pictures (fear; plots A and B), even though for IAPS, stimuli significance was not reached after Bonferroni correction. Both valence and arousal are reported as values on the 0–9 Likert scale. Legend: $ = p > 0.05.

4.5.5 Reliability analysis and PCA

Item analysis on visual stimuli showed an overall good item reliability on valence and arousal ratings for all condition except IAPS neutral stimuli (Table 3). Good reliability is considered when Cronbach's Alpha is greater than 0.8, excellent reliability is when $\alpha > 0.9$, $\alpha > 0.7$ is considered to be acceptable, $\alpha > 0.6$ questionable, $\alpha > 0.5$ poor and $\alpha < 0.5$ unacceptable (Gliem & Gliem, 2003). In order to verify the feasibility of the item analysis and the adequacy of the sample size, eigenvalues of each component were calculated via a principal component analysis (PCA) for both valence and arousal ratings in all conditions (Fear, Happiness and Neutral). For small samples, a first eigenvalue (λ_1) of at least 6 is considered optimal in order to calculate a valid Cronbach's Alpha coefficient, eigenvalues between 3 and 6 have to be considered acceptable but it means that the sample size should be increased in order to give a completely unbiased coefficient (Halil Yurdugül, 2008). Eigenvalues for valence and arousal ratings of body postures showed an adequate first eigenvalues (all $\lambda_1 \geq 6$) for all conditions, meaning that the sample size was correctly estimated in order to verify the reliability of the visual stimuli for emotion and non-emotion detection. IAPS stimulus showed weaker results with $4 \leq \lambda_1 \leq 6$, with the only exception for arousal rating of Neutral stimuli where λ_1 was higher than 6.

	Postures			IAPS		
Cronbach's Alpha	**Fear**	**Happiness**	**Neutral**	**Fear**	**Happiness**	**Neutral**
Valence	α= 0.93	α= 0.88	α= 0.75	α= 0.84	α= 0.77	α= 0.45
Arousal	α= 0.93	α= 0.93	α= 0.92	α= 0.78	α= 0.86	α= 0.97

Table 3: Cronbach's Alpha for Valence and Arousal. The table shows the Cronbach's Alpha computed in order to estimate the reliability of the two sets of images used in the experiment. An overall good reliability is observable for posture's valence as well as for valence for Fear and Happiness in the IAPS trial. The only exception is observable for IAPS Neutral pictures, where reliability for valence is low. Overall reliability for arousal ranges from excellent to good in all emotions and conditions.

4.6. Discussion

The first aim of this study was to assess the capacity to process emotional body postures in a three-choices categorization task. Our findings show significantly lower response times (RTs) for pictures depicting fearful (i.e., negative) body postures when compared to happy (i.e., positive) or neutral postures, suggesting a faster processing of fearful body language.

These results appear in contrast with prior studies investigating processing of emotional facial expressions, where shorter RTs for positive expressions were shown and also respect to previous behavioral studies on emotional body language (Calvo & Beltrán, 2013; Nummenmaa & Calvo, 2015; Van den Stock et al., 2007). Regarding facial processing, this difference in RTs might be explained by peculiar features that are retrievable only in happy faces. As proposed by Ekman and Friesen in 1982, happy faces are characterized by an

increased bilateral activation of the zygomatic major muscles, resulting in what is commonly known as ‘smile’, that makes the facial expression easily recognizable and hardly to be misunderstood (Calvo & Beltrán, 2013; Calvo & Nummenmaa, 2008; Ekman, 1999; Ekman & Friesen, 1982; Frank et al., 1993). These features have a major role in driving the so called ‘positivity offset’ that leads to faster and more accurate processing of positive facial expressions (Calvo & Beltrán, 2013).

In relation to emotional processing of body postures, evidence regarding body language is not so straight-forward and show contrasting results: if on one side neurophysiological studies investigating processing of emotional body postures have shown faster modulation of motor excitability when observing negative emotions, behavioral studies on RTs showed that motor responses are slower for negative whole body expressions and faster for the positive ones (Borgomaneri et al., 2012; Borgomaneri, Vitale, Gazzola, et al., 2015; De Gelder et al., 2004; Huis in ’t Veld et al., 2014; Van den Stock et al., 2007).

Thus, it appears that there is an incongruency between neurophysiological responses and motor outcomes, with the firsts apparently driven by a ‘negativity bias’ an the seconds by an advantage of positive postures, similarly to emotional facial expressions (Cacioppo & Berntson, 1999; De Gelder et al., 2004). It may be argued that this mismatch might be a consequence of the fact that body postures seem not to have peculiar and unambiguous physical features such as the smile that might propend for a positive evaluation so that it is harder to extract precise information on the emotional valence of body language, augmenting the probability to misinterpret it. Furthermore, the variety of basic negative emotions might be considered as another potential confounder in the detection of a specific

emotion, as documented in a study by Van den Stock and colleagues where authors found reduced accuracy in recognizing negative body postures with respect to positive ones (Borgomaneri et al., 2020).

However, studies have also shown that the amount of information carried by postures are as fundamental and complete as the ones deducible from facial expressions (Aviezer et al., 2012; Meeren et al., 2005; Ross & Flack, 2020) and recent models of emotion recognition suggest that the perception of negative expressions in others is able to trigger internal emotional states, which consequently yield to motor responses (i.e. activation of facial or postural muscles) and favor emotional recognition (for a review see Ross & Atkinson, 2020). These considerations, together with the neurophysiological modulation derived from negative emotional processing highlighted above and some methodological considerations that will follow, lead then to a possible explanation of our results.

First of all, differently from prior behavioral studies investigating motor response to emotional postures (Huis In‘t Veld et al., 2014; Van den Stock et al., 2007), in our study we used a set of body stimuli associated with high recognition accuracy (>90%) and no differences between postures types. This was confirmed both in the forced-choice RT task where participants were asked to categorize each visual stimulus as showing negative, positive, or emotionally neutral content by pressing one of the 3 buttons and in the subsequent questionnaire at the end of the experimental session, where they had to categorize the posture using a wider set of emotional categories (including anger, disgust, fear, sadness, surprise and happiness). This suggests that, in general, our images were adequately selected in

order to illustrate negative or positive emotions as well as neutral stimuli. Furthermore, the analysis on accuracy data for the categorization task included in the questionnaires (see Table 2) clearly showed that the body language stimuli we selected were not only recognized as negative, positive or neutral, but they were also correctly identified as belonging to basic emotional states (i.e., fear, happiness, or a neutral state), which prompt us to speculate that our findings could be better ascribed to specific emotional attributes rather than being driven by more general valence effects – although further studies including more emotional postures should be used to address this hypothesis.

In relation to the link between fearful stimuli and motor readiness, our results are consistent with others present in the literature. Fearful body language processing was shown to be linked to action preparation, simulation and execution leading to an early pre-activation of postural and upper and lower limb muscles involved in the emotion observed or to facial muscles involved in the emotion (Huis in 't Veld et al., 2014; Ross & Atkinson, 2020). Viewing of fearful postures is shown to have an effect on motor system at an early time (~70-120ms), where it is observable a suppression of intracortical facilitation of the primary motor cortex and reduced cortico-spinal excitability, suggesting that the motor cortex may undergo a "freezing-like" phenomenon (Borgomaneri, Gazzola, et al., 2015; Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, Gazzola, et al., 2015; Borgomaneri et al., 2017, 2020). Recent evidence on defensive threat reactions show that freezing is not a passive state but rather a parasympathetic brake on the motor system, relevant to perception and action preparation (Gladwin et al., 2016; Hashemi et al., 2019; Roelofs, 2017). Freezing has been conceptualized as an active action preparatory state with a parasympathetic driven

'brake' involving the amygdala and the brainstem (periaqueductal grey) followed by a rapid adaptive response once the brake is 'released' by frontal– amygdala connections (Griebel et al., 1996; Mobbs et al., 2007; Walker & Carrive, 2003).

The open question might then be whether the motor response we found was a consequence of the emotional content of the stimuli we used or a synergic effect of emotion and motor resonance due to the intrinsic movement information expressed inherently in whole-body pictures. To address this issue, the second aim of our experiment was to assess differences in motor response between emotional body postures and IAPS.

First of all, valence and arousal ratings were comparable across the two sets of images with an exception for Fear stimuli, which showed higher arousal for IAPS relative to Posture stimuli (see Table 2). Also, in both sets, Pearson's coefficients showed a similar trend for emotion-matched stimuli, with a negative correlation between valence and arousal for Fear and a positive correlation for Happiness. In sum, accuracy and ratings data show that the pictures selected for both experiments were sufficiently well matched, ruling out that increased perceptual discrimination or attention allocation related to high arousing stimuli could explain the speed up effects we observed on the RTs (Hajcak et al., 2006; Pourtois et al., 2013). The differences found for fearful stimuli does not contradict this statement because the high arousal rating of both negative postures (6.08 ± 1.94) and IAPS (7.20± 0.97) makes all negative pictures belonging to the category of high-arousing stimuli (Lang Bradley, M.M., & Cuthbert, B.N., 2008). Moreover, the item analysis run on the new set of emotional body postures stimuli showed an overall good reliability for valence and an excellent reliability for the

arousal ratings. Thus, each stimulus was correctly recognized as fearful, happy, or neutral by all participants with an overall arousal rating higher than 5 (high-arousing stimuli) for emotional and low-arousal for non-emotional pictures. This result, together with the lower error rate in emotional content categorization for emotional body language, suggests that all the stimuli depicting body language used in this experiment were equally valid and reliable in inducing a specific emotion in all participants as IAPS stimuli, if not even more adequate in evoking a response to basic emotions. Under these premises, we can consider the risk of bias linked to basic differences in categorization and misinterpretation of the emotional content of the stimuli to be low, which leads to our secondary findings.

Our data showed that RTs were longer for positive and neutral body posture in respect to positive and neutral IAPS whereas no difference was found between negative stimuli. An explanation for this result might be linked to the capacity of negative stimuli to allocate attentional resources more rapidly compared to positive and neutral stimuli (Olofsson et al., 2008; van Heijnsbergen et al., 2007). Negative IAPS and body postures images have been shown to modulate early components of event-related potentials already after 100ms from stimulus onset, showing a rapid allocation of attentional resources with a time course similar to that observed for emotional facial expressions (Olofsson et al., 2008; van Heijnsbergen et al., 2007). Such 'negativity bias' for negative scenes and postures has been reported for brain regions involved in emotion processing (e.g. the amygdala, the orbitofrontal cortex or the insula) and motor areas involved in motor representation and planning (e.g. premotor cortex, supplementary motor area and parietal cortex) and the primary motor cortex (Borgomaneri et al., 2012, 2014; Borgomaneri, Vitale, Gazzola, et al., 2015; De Gelder et al., 2004). The

early perceptual categorization in favor of negative emotional body language associated with the early activation of motor and non-motor neural circuits might be the reason why there are no differences in RTs between postures and IAPS and also validates the hypothesis that RTs are primarily driven by the emotional content of the observed picture rather than by the motor information carried by body postures.

On the other hand, there might be also another explanation that potentially raises the issues of comparability of the two sets of pictures and their respective complexity. IAPS pictures present more visual information compared to postures (e.g., more colors, different subjects, objects, and contextual information), and in principle they may contain more elements to disambiguate the emotional content including facial expressions, resulting in shorter RTs. However, this possible explanation alone cannot account for the entire pattern of RTs data we observed. Indeed, RTs to negative body postures did not differ from negative IAPS, suggesting similar processing speed and resource allocation for the two negative categories. On the other hand, CV data speak in favor of a higher complexity of body postures, with larger variability in RTs for body postures than for IAPS stimuli. A possible argument in opposition to this statement might be if we look at CVs for neutral pictures; while there is no difference in RTs' variability, motor response to body postures is significantly longer compared to IAPS. Low CV paired with longer RT may be an indication for low uncertainty in processing the image, but it also means that the image showed needed more time to be correctly interpreted and categorized. This might be explained by the fact that our neutral body postures showed an actor performing non-emotional actions (i.e., higher complexity) which might have led to longer times in order to correctly categorize

the stimulus, but also to a low uncertainty whenever the stimulus was correctly processed. Conversely, the fact that negative postures showed higher variability compared to IAPS, but no differences in RTs may be a sign that, although there might be greater uncertainty in deciphering negative body language, the motor response is accelerated, resulting in a prioritization of attentional allocation when observing a potential threat. It is also true that our results show higher CV and RTs for happy body posture compared to the ones recorded for emotional scenes, and this might suggest that they resulted too complex or ambiguous to be rapidly processed, but the fact that the accuracy in detection the correct emotion in the categorization task is extremely high serves as a counterproof. A possible explanation for this finding might be that the information conveyed by the arms and hands in emotional body language are crucial in order to correctly process some specific emotions (Dael et al., 2012; Ross & Flack, 2020). A closed fist might be an indication of anger (Calbi et al., 2020; Dael et al., 2012) and consequently it might need more time to correctly interpret the whole body posture observed. Considering the facts that the hands draw attentional resources in interpreting the mood expressed in body language and that our happy stimuli depicted mainly the actor with closed fists but in pleasant postures (e.g., jubilation or exultation), it might be plausible to infer that the higher variability and, consequently, the longer RTs retrieved in the happy postures condition are a result of this mismatch between the whole-body posture and the hands. The presence of fists in several pictures of neutral body movements could also account for by RTs in this condition, which is comparable to happy expressions.

Summing up, considering our results and the ones retrievable in literature concerning emotional processing of visual stimuli, it appears that there is a

gradient of complexity where facial expressions are the easiest to process, followed by emotional scenes and lastly postures, which are the hardest. Further studies will be needed in order to deeply explore this issue.

The absence of significant RT differences for IAPS stimuli is in keeping with normative data showing RTs pattern for the subsets of the IAPS pictures we used in our study ("accidents" and "human attack" vs. "families and babies" and "adventure") (Calvo & Avero, 2009). However, the analysis on accuracy during the categorization task showed an error rate higher for Fear IAPS stimuli compared to Happy and Neutral. Although the percentage of correct recognition of the emotional content of the selected fearful stimuli is acceptable, and considering the selection of the IAPS stimuli from a specific fear-related pool (Barke et al., 2012), it appears that the possibility to select a pure set of fearful stimuli in the IAPS database might represent a limitation of this study. It should be noted that the positive IAPS category contained several stimuli depicting smiling individuals. Although happy faces tend to be efficiently recognized (Calvo & Beltrán, 2013; Calvo & Nummenmaa, 2008; Ekman, 1999; Ekman & Friesen, 1982; Frank et al., 1993), their presence in the set of complex scenes we selected was not sufficient to drive an advantage of positive IAPS stimuli relative to negative and neutral IAPS stimuli. Although lack of difference between positive and neutral stimuli was observed also with body stimuli, further studies are needed to clarify whether the inclusion of further negative expressions (e.g., anger or disgust) could counteract the fear specific effects we observed and favor the emergence of a positivity offset similar to that commonly reported in the literature on facial emotion recognition.

In conclusion, the results of this study show that fearful body postures are rapidly recognized and processed, probably thanks to automatic activation of a series of central nervous system structures orchestrating the defensive threat reactions. Neurophysiological and behavioral correlates of fearful body postures processing may be valid tools for the study of psychiatric and neurodegenerative diseases. As an example, these tools might be helpful for a better comprehension of the freezing of gait phenomenon in patients with Parkinson's disease, whose pathophysiology has been recently linked also to a dysfunction in the communication between the limbic system and the basal ganglia (Avanzino et al., 2018; Ehgoetz Martens et al., 2018; Lagravinese et al., 2018).

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5. Experiment 2: Sensorimotor inhibition during emotional processing

Alessandro Botta, Giovanna Lagravinese, Marco Bove, Elisa Pelosin, Gaia Bonassi, Alessio Avenanti and Laura Avanzino.

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5.1. Techniques: Transcranial Magnetic Stimulation

TMS is a non-invasive brain stimulation (NIBS) technique firstly introduced by Barker in 1985 (Barker et al., 1985). In this technique, a large electrical capacitance is charged to a high voltage and then discharged through a coil of insulated copper wire held on the scalp of the subject. A strong transient current flow through the coil and produces a large magnetic field which reaches an apex of intensity of 1.5-2 T in about 50 ms and decays to zero in approximately 5 ms. The result of such an intense magnetic gradient induces an electric current in any neighboring conductive structure which, as a result, induces the depolarization of the stimulated neurons (Rothwell et al., 1999). Before going to the physiological effects of TMS, it is important to understand the physics behind the functioning of such a technique. First of all, a magnetic field is a vector field that describes the magnetic influence on moving electric charges, electric current and magnetic materials (Leech, 1966). A magnetic field is generated by moving charges, hence revealing a close relationship between electrical and magnetic fields. Secondly, TMS is based on the physical phenomenon called

‘electromagnetic induction’, defined by the Maxwell-Faraday law, which states that a time-varying magnetic field always accompanies a spatially varying (also possibly time-varying), non-conservative electric field, and vice versa.

Importantly, the Maxwell-Faraday equation also includes an indication of the direction of the induced electrical current, which is the opposite of the direction of the electrical current generating the magnetic field.

Starting from these assumptions, if we imagine a coil in which runs an electrical current, we now know that a magnetic field will be generated by these moving charges and, in turn, the magnetic field will induce an electrical current in every magnetic material at a certain distance. If we think about a coil positioned on the scalp of a subject, we can expect that the magnetic field generated by the moving charges on the coil itself will induce an opposite electrical current that will influence the cortical neurons undergoing the current (see figure 1). The next question is then: how much of the brain tissues can TMS stimulate? The intensity of the TMS pulse and its brief duration limits the depth and the quantity of tissue stimulated at around 2-to-3 cm below the coil (i.e., from the 3rd to the 5th layer of the brain cortex). In any case, the amount of brain tissue that is stimulated depends on the shape of the coil and on the intensity of the

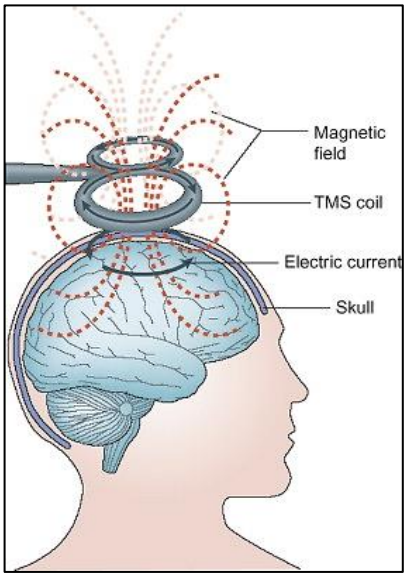

Figure 1: TMS. In this picture it is possible to observe the current flowing in the TMS coil (thin blue line) that generates a magnetic field (red dotted line). As a consequence, the magnetic field will induce an electrical current which will have an opposite direction of the one in the coil. (Spronk et al., 2011)

stimulation (Rothwell et al., 1999). In an interesting study by Deng et al., they studied the tradeoff between depth and focality of TMS; what they observed in their model is that the depth reached by the stimulation depends on the distribution of the induced electrical field (E-field) and, consequently, on the shape of the coil (Deng et al., 2013). As observable in figure 2, the E-field generated by a 'butterfly' coil has its maximum on the junction of the two copper coils, resulting in a more focal stimulation able to reach for deeper brain structures (see fig. 2A). On the other hand, in figure 2B it is possible to observe how, at the same stimulation intensity, the E-field shows a slightly reduced capacity of penetration and a more diffuse E-field (Deng et al., 2013). Summing up, the higher the intensity and the focality of TMS, the deeper the brain tissue will be stimulated.

One important aspect to take into consideration when talking about the structures on which magnetic stimulation have an effect is the type of neuronal population involved. We already said that the depth of stimulation reaches the 5^{th} layer of the cerebral cortex, hence the internal pyramidal layer; this means that, if for example we perform TMS on the motor cortex, we will activate the corticospinal tract and, if the intensity of stimulation is high enough, we will observe a muscle twitch on the muscles encoded for movement in the stimulated brain area. Anyway, the phenomenon by which the TMS evokes a response in the pyramidal tract neurons is peculiar. Depolarization of neurons through NIBS might occur in two different ways.

The first one implies the direct activation of the proximal nodes of the axons of large corticospinal tract cells; this is referred as D-waves and it was firstly observed in electrical stimulation. The second one, on the other hand,

implies the synaptic activation of the output fibers; this is known as indirect activation and results in I-waves in the pyramidal tract (Rothwell et al., 1999). The indirect way is typically associated with low intensity TMS and shows an interesting feature because the I-waves occur at preferential intervals that are synchronized in the population. This means that, when stimulating a certain brain area with TMS, we will observe a series I-waves activating synchronously in a well-defined neuronal pool and resulting in a descendent volley able to induce a motor output in the controlled muscles.

This leads to the last aspect of TMS that will be discussed in this work: what can TMS tells us about the corticospinal tract and how can we measure it? If we take the previous example in which we were performing TMS on the primary motor cortex, we now know that there will be a synchronized synaptic activation of the underlying pyramidal neurons that will result in a descendent volley directed to the muscles innervated by the neurons themselves. This descendent volley will produce a muscle twitch at a certain point in time after the stimulation and the muscular contraction, which we can record via electromyographic (EMG) electrodes, will tell us two main things: the first one is the time of conduction (or central motor conduction time), or the time it takes for the signal to propagate from the pyramidal neurons in the cortex to the neuromuscular junction; the second one is the intensity of the muscular contraction evoked by TMS.

Regarding the central motor conduction time (CMCT), the interval from the application of the magnetic stimulus to the onset of EMG activity in the target muscle is about 20 ms for the muscles of the arm and 30 ms for the muscles of

the leg. This total latency is made up of two main components: the first one is the time to activate spinal alpha motoneurons and it's called the central delay, and the second one is the time from the activation of spinal motoneurons to the muscle response, that is called peripheral delay. The importance of the CMCT relies in the fact that it can give an estimate of the general conditions of the corticospinal tract. In fact, the aforementioned intervals (i.e., 20-30 ms for the upper and lower limb respectively) are intended for healthy subjects; the measurement of the CMCT in pathological conditions might show longer latencies depending on the severity and on the level of the damage, hence assuming a clinical relevance (Udupa & Chen, 2013). In order to estimate the CMCT, it is crucial to have a reliable measure of the peripheral muscular activity, and this is possible by recording the intensity of the contraction by EMG. After the stimulation of a certain area of the primary motor cortex we expect to see a muscle twitch. The EMG recording of this muscular activity is called motor evoked potential (MEP) and it gives an indication of the strength of the contraction itself (see figure 3B).

Regarding the central motor conduction time (CMCT), the interval from the application of the magnetic stimulus to the onset of EMG activity in the target muscle is about 20 ms for the muscles of the arm and 30 ms for the muscles of the leg. This total latency is made up of two main components: the first one is the time to activate spinal alpha motoneurons and it's called the central delay, and the second one is the time from the activation of spinal motoneurons to the muscle response, that is called peripheral delay. The importance of the CMCT relies in the fact that it can give an estimate of the general conditions of the corticospinal tract.

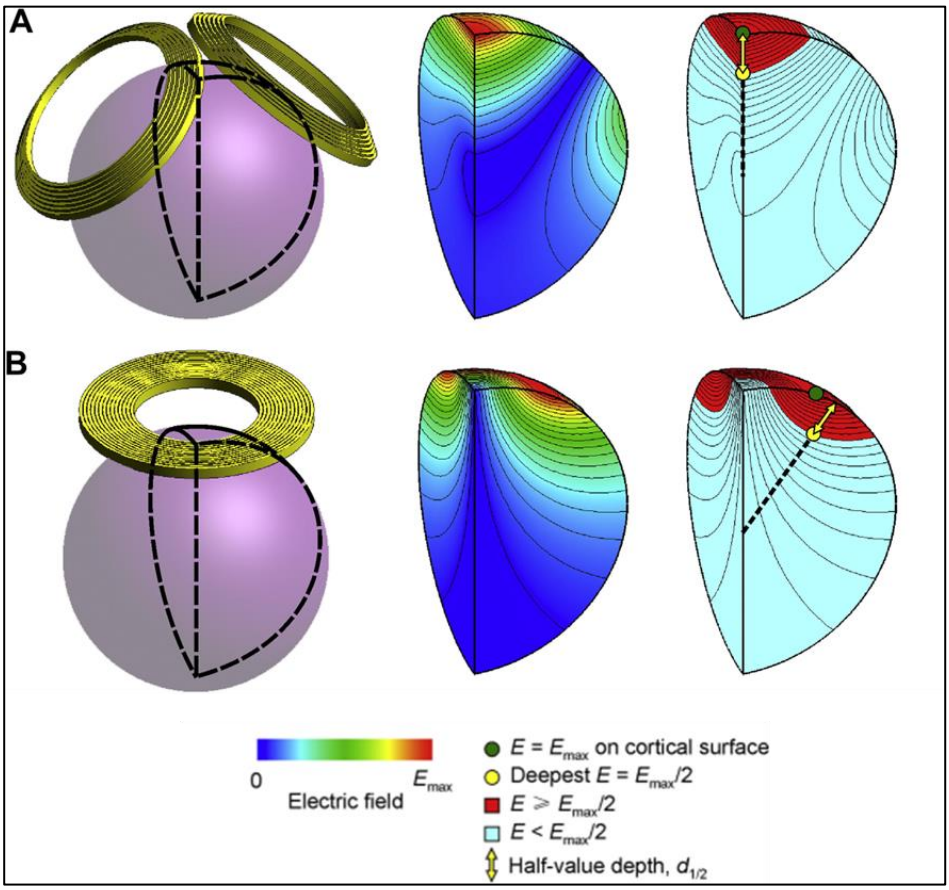

Figure 2: Examples of electric field characterization. The left column shows the respective coil and the spherical model representation of the brain. The middle column shows the electric field strength contour and color maps on the quarter-sphere segment of the brain outlined in black on the left. The right column shows the location of the maximum induced electric field on the brain surface, Emax (green circle), and the location of the deepest point where the electric field strength is Emax/2 (yellow circle). The yellow arrow represents the radial distance from the cortical surface to the deepest point where the electric field strength is half of its maximum value on the cortical surface. The red portions of the quarter-sphere indicate the regions of the brain exposed to electric field (Deng et al., 2013).

In fact, the aforementioned intervals (i.e., 20-30 ms for the upper and lower limb respectively) are intended for healthy subjects; the measurement of the CMCT in pathological conditions might show longer latencies depending on the severity and on the level of the damage, hence assuming a clinical relevance (Udupa & Chen, 2013). In order to estimate the CMCT, it is crucial to have a reliable measure of the peripheral muscular activity, and this is possible by recording the intensity of the contraction by EMG. After the stimulation of a certain area of the primary motor cortex we expect to see a muscle twitch. The EMG recording of this muscular activity is called motor evoked potential (MEP) and it gives an indication of the strength of the contraction itself (see figure 3B).

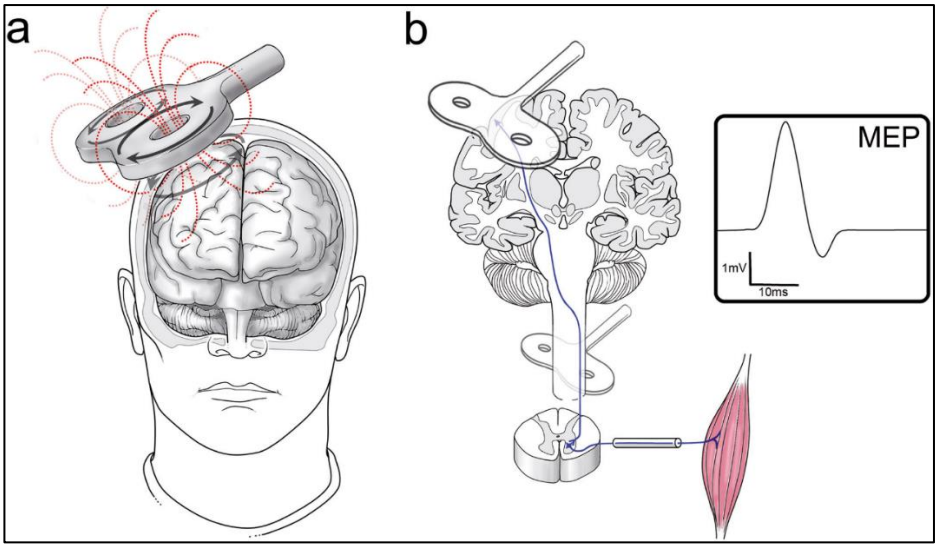

Figure 3: Transcranial magnetic stimulation (TMS). (a) Visualization of transcranial magnetic stimulation (b) of the motor cortex and the spinal cord. The effects of TMS are quantified by recording of motor evoked potentials (MEPs) (Vlachos et al., 2017).

In order to evoke a MEP, anyway, TMS at a random intensity is not enough. Firstly, it is important to assess the motor threshold of the subjects undergoing the stimulation. There are mainly two types if threshold: the resting motor threshold (rMT), which is measured in a relaxed muscle, and the active motor threshold (aMT), measures in an active muscle. In order to measure the threshold for evoking a response in target muscles, the coil must firstly be placed over the most effective point (hotspot) on the scalp for eliciting any response at all. When the hotspot is found, the stimulus intensity must be progressively reduced in 2% or 5% steps until a level is reached below which reliable EMG responses disappear. The rate of stimulation is relevant and there should be more than 4 s between consecutive stimuli. In the case of the rMT, a reliable response can be defined as an MEP of 50 ± 100 mV occurring in at least half of 10 to 20 consecutive trials. For aMT, the minimal response size may be around 200 ± 300 mV because of the difficulty in distinguishing it from the background activity (Rothwell et al., 1999).

A correct localization of the hotspot and of the motor threshold is crucial in order to properly assess corticospinal excitability and is a mandatory first step in all TMS protocols, included the one assessing sensorimotor inhibition.

5.1.1 Short-latency afferent inhibition

In the first chapter of this work, we saw how somatosensory peripheral stimulation is able to inhibit motor response (see section 2.1). In this section we are going to focus on the experimental protocols implemented in order to assess sensorimotor inhibition.

In 1990, Delwaide and Olivier reported that electrical stimulation (ES) of the median nerve at the wrist could profoundly suppress electromyographic responses evoked in relaxed hand muscles evoked by TMS stimulation at 18-21 ms after peripheral stimulation, and they suggested that the effect occurred at the cortical rather than the spinal level (Delwaide & Olivier, 1990). Moreover, some years later, Bertolasi et al. found that stimulation of muscle afferents in the median nerve could suppress the excitability of cortical projections to forearm extensor muscles, while radial stimulation suppressed the excitability of cortical projections to forearm flexor muscles (Bertolasi et al., 1998). On the impulse of this work and of the diffusion of TMS, Tokimura and colleagues designed an experiment in which they aimed to confirm the findings of Delwaide and Olivier and to definitely demonstrate that the inhibitory effect of the peripheral stimulation was a cortical phenomenon appearing in a defined time window (Tokimura et al., 2000).

In a series of experiments, Tokimura et. al tested how the ES of the median (MN) and digital (DN) nerves can modulate the MEPs in the first dorsal interosseous muscle (FDI) and in the abductor pollicis brevis (APB) at different timepoints. The peripheral electrical stimulation was used as conditioning stimulus, while TMS over the primary motor cortex areas encoding for FDI and APB was used as test stimulus. The reason why they tested the influence on corticospinal excitability of two nerves is related to the differences in the composition of the nerves themselves. The DN is a cutaneous nerve, meaning that they receive afferences only by cutaneous mechanoreceptors, while the MN is a mixed nerve which includes afferents coming from joints, muscles, and cutaneous mechanoreceptors. It has been observed that DN stimulation of the second digit

evokes SAI when the TMS stimulus was provided from 20 to 50ms after ES in APB and FDI, while, in contrast, following stimulation of the mixed MN at the wrist level, SAI is observed over a smaller ISI range extending from 18 to 28ms in FDI and APB. Although both nerve types contain cutaneous afferents, only mixed nerves contain the largest and fastest conducting muscle/joint afferents that may shorten the temporal window in which SAI occurs. A possible mechanism underlying this phenomenon may be related to the fact that S1 neurons receiving fast-conducting proprioceptive input experience post-excitation inhibition, therefore the arrival of subsequent slower conducting afferents terminate on hyperpolarized neurons (Turco et al., 2018).

Tokimura and colleagues found that the higher inhibition of motor output for FDI after ES of the median nerve was when the peripheral stimulation was performed from 20 ms before TMS onward, confirming the timing previously found by Delwaide and Olivier (see fig. 4). Furthermore, by stimulating the cortical areas responsible for the FDI muscle, they also confirmed the fact that a major part of the median nerve inhibition is primarily of cortical origin.

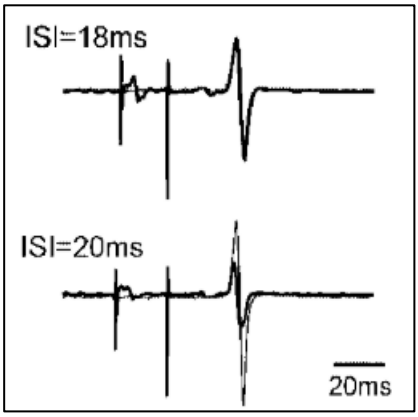

Figure 4: MEPs recorded in the FDI after electrical stimulation of the MN at an ISI of 18 and 20 ms (Tokimura et al., 2000).

As for the DN, they found similar results as the ones found for the MN, but with 2-3 ms of delay in the EMG response and with a longer inhibitory effect (Tokimura et al., 2000).

In conclusion, the SAI protocol validated by Tokimura et al., and used in the following experiment, involves a double stimulation, the first one provided by ES of the MN at the wrist level (or of the DN at the finger level) followed after at least 20 ms by a TMS pulse on the cerebral area encoding for the studied muscle (preferably the FDI or the APB).

5.2 Rationale

Appropriate motor responses to potential threats require rapid evaluation of the menace's features and the surrounding environment (Gladwin et al., 2016). Being able to direct attention toward relevant sensory stimuli, as well as to correctly read emotional signals in others, is crucial in order to plan and consequently choose the most appropriate reaction (Roelofs, 2017; Smith et al., 2003; van Heijnsbergen et al., 2007; Vuilleumier, 2005). Threat-related visual stimuli are rapidly processed in the brain as shown by early modulations of event-related potentials (ERPs) (De Gelder, 2009; Meeren et al., 2005; van Heijnsbergen et al., 2007); these stimuli are thought to increase sensory vigilance and grab attention (Olofsson & Polich, 2007; Schupp et al., 2003; Smith et al., 2003). In turn, perceived threats rapidly prime the body for action, as shown by both behavioral and electrophysiological evidence (Frijda, 2010; Tamietto & De Gelder, 2010). Rapid motor responses to emotional stimuli have been often reported using motor-evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) of the primary motor cortex (M1), which offers the possibility to probe the motor system with high temporal resolution (Borgomaneri, Vitale, &

Avenanti, 2015; Borgomaneri, Vitale, et al., 2020; Borgomaneri, Vitale, Gazzola, et al., 2015).

These studies have shown that emotional body language (EBL) – and in particular, threat-related expressions, such as fearful body postures – modulate corticospinal excitability (CSE) and intracortical M1 processes such as intracortical facilitation (ICF) (Borgomaneri et al., 2017; Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, et al., 2020; Borgomaneri, Vitale, Gazzola, et al., 2015). Also, emotional stimuli like emotional facial expressions or more complex stimuli like natural emotional scenes from the International Affective Pictures System (IAPS) have been shown to modulate CSE (Borgomaneri et al., 2012, 2014, 2021; Coelho et al., 2010; Ekman & Rosenberg, 2005; Lang Bradley, M.M., & Cuthbert, B.N., 2008; Schutter et al., 2008). These studies have often reported two types of motor modulations, i.e., early inhibitory effects (i.e. when CSE or ICF was probed 70-150 ms after stimulus onset) that are thought to reflect early freezing-like orienting to the possible source of threat and later (>150 ms) motor facilitations, reflecting increased motor readiness to emotional stimuli (Borgomaneri et al., 2017; Borgomaneri, Gazzola, et al., 2015; Borgomaneri, Vitale, Gazzola, et al., 2015; Botta et al., 2021; Coelho et al., 2010; Schutter et al., 2008).

Motor preparation requires fine tuning of sensorimotor interactions between somatosensory and motor regions (Gale et al., 2021; Rens et al., 2020). However, despite the relevance of sensorimotor integration to efficient action processing (Catmur et al., 2007; Maffongelli et al., 2020; Rens et al., 2020), to

date, little is known about how the underlying sensory-to-motor interactions are affected by emotional information in humans.

Sensorimotor system has been shown to be sensitive to emotions to the extent that emotional recognition processes are able to induce phenomena of motor mimicry and somatosensory simulation, which activate the motor and the sensory system (Huis In‘t Veld et al., 2014; Ross & Atkinson, 2020). Furthermore, it has also been shown that these effects are more evident when the observer is confronted with negative emotions (e.g., fear or anger) which are able to trigger similar internal emotional states and in turn enhance sensorimotor system’s readiness (Ross & Atkinson, 2020).

A valuable method to non-invasively probe sensorimotor integration at the cortical level is the so called short-latency afferent inhibition (SAI) TMS protocol (Tokimura et al., 2000) which involves pairing electrical stimulation of peripheral somatosensory afferents with focal TMS targeting of the contralateral M1.

Studies using the SAI protocol have shown that peripheral nerve stimulation at the contralateral wrist reduces the amplitude of MEPs when the TMS pulse is given to M1 2–8 ms after the arrival of the afferent volley in cortex. The circuitry involved in SAI is complex. The sensory input exerts its inhibitory effects on the corticospinal neurons through γ-Aminobutyric acid (GABA)-ergic intracortical circuits (V. Di Lazzaro et al., 2005; Vincenzo Di Lazzaro & Ziemann, 2013), but its magnitude is also modulated by dopaminergic (Sailer et al., 2003) and cholinergic neuromodulatory circuits (V. Di Lazzaro et al., 2000).

As previously stated, although the exact mechanisms of SAI are still not clear, the contributions of several neurotransmitters in the phenomenon have been

studied. It has been shown by Di Lazzaro et al. that SAI was reduced (i.e., less sensorimotor inhibition) when subjects received benzodiazepines binding the α1-subunit of the $GABA_A$ receptors (e.g., Zolpidem) (V. Di Lazzaro et al., 2007), while it was found to be increased after the administration of acetylcholinesterase inhibitors (Vincenzo Di Lazzaro et al., 2005). Thus, as suggested in a review by Turco and colleagues, SAI generation is probably related to specific inhibitory cells activated by cholinergic inputs (Turco et al., 2018). It has been also shown that SAI was reduced in PD patients under L-Dopa medication in the ON phase, showing a potential detrimental effect on sensorimotor inhibition of high concentration of Dopamine (Sailer et al., 2003). If we consider the fact that cholinergic efferent projections of the basal forebrain nuclei to the cortex play a critical role in functions such as arousal, attention, cognitive and emotional processes (Davis & Whalen, 2001) and that SAI has been shown to be dynamically modulated by cognitive processes, for instance working memory and attention (Bonnì et al., 2017; Mirdamadi et al., 2017; Suzuki & Meehan, 2018) and planning of both executed and imagined movements (Asmussen et al., 2013; Bonassi et al., 2019), it appears plausible that it is a suitable tool to explore dynamic modulation of cholinergic networks during higher-order functions such as emotional processing.

5.3 Aim of study

The main aim of this study was to assess SAI while healthy participants were observing fear-related and happiness-related EBL and IAPS pictures and neutral controls. Our hypothesis was that processing emotional signals – which are known to affect perceptual and motor as well as cognitive processes – would also influence sensorimotor integration. While prior work exploring early motor response to emotional stimuli have typically used EBL stimuli – which are known to induce emotion-related but also motor resonance effects at the CSE level (Borgomaneri et al., 2012; Borgomaneri, Vitale, Gazzola, et al., 2015; L Fadiga et al., 1995; Luciano Fadiga et al., 2005) – in the present research we tested both EBL and IAPS pictures so to assess the generalizability of the effects and disentangle whether any modulation of the SAI protocol could be related to emotional modulation, motor resonance or other general effects. More specifically, we aimed to compare complex emotional scenes, controlled in order to be embedded with minimal motor information, with emotional body postures, who intrinsically carry both motor and emotional information, in order to understand if the prevalence of one of these two types of information (i.e., emotional or motor) is responsible for the modulation of the sensorimotor network.

Building on prior work showing early and later changes in MEPs during emotional processing (e.g., (Borgomaneri, Vitale, Gazzola, et al., 2015),(Borgomaneri, Bolloni, et al., 2020; Borgomaneri, Gazzola, et al., 2015; Borgomaneri, Vitale, & Avenanti, 2015)), here, we tested SAI at two different

time-points, i.e., at 120ms and 300ms from picture onset (as performed by Borgomaneri et al. in the studies (Borgomaneri, Vitale, Gazzola, et al., 2015) and (Borgomaneri, Gazzola, et al., 2015) respectively). While the 120 ms time point allows us to assess early subcortical-cortical automatic response to emotional stimuli (Borgomaneri, Vitale, Gazzola, et al., 2015), the 300 ms timepoint allows assessing later, cognitive processing of motor and emotional information (Borgomaneri, Gazzola, et al., 2015; Borgomaneri, Vitale, Gazzola, et al., 2015). Building on the evidence that threat-related signals triggers early response in cortico-subcortical networks involved in emotion processing and action planning (Oya et al., 2002; Sagaspe et al., 2011; Vuilleumier et al., 2003) and that early response to emotional signals – in particular to fear-related signals – may involve inhibitory motor processes (Borgomaneri, Gazzola, et al., 2015; Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, Gazzola, et al., 2015), we expected that fear-related pictures would enhance SAI effects in the early time point.

A control experiment was also performed to test whether a simple, non-emotional, visual stimulus (i.e., while observing a black screen at rest) was able to modulate SAI at 120 and 300ms differently from the SAI recorded at baseline. In this case, we aimed to exclude that the modulation of sensorimotor inhibition could have been a consequence of the mere observation of a visual stimulus itself, possibly driven by light changes and not by the increased attentional load derived from the observation of more complex emotional stimuli.

Finally, we also considered the fact that motor reactivity during perception of social and emotional information can be largely affected by inter-individual

differences in specific personality traits (Avenanti et al., 2009; Borgomaneri et al., 2021; Minio-Paluello et al., 2009). In particular prior work has shown an association between behavioral inhibition (BIS) and the magnitude of motor response to EBL, with larger M1 suppression due to fear-related EBL in participants showing higher scores at the BIS scale (Borgomaneri et al., 2017). Therefore, we also submitted participants to BIS and behavioral approach system (BAS) (Carver & White, 1994; Leone et al., 2002). The BIS is described as an attentional system that disrupts ongoing behavioral response in order to allow the subject to focus on relevant cues (e.g. punishment, non-reward or novelty), while the BAS is linked to positive feelings such as joy or optimism or negative such as rage and aggression; and BIS/BAS scales have been used in several neuroscientific studies (Amodio et al., 2008; Kennis et al., 2013). In this way, we tested whether interindividual differences in BIS/BAS predict magnitude of sensorimotor integration as indexed by the SAI effects.

5.4 Experimental procedure

5.4.1 Participants

Thirty healthy participants (15 males, mean age ± S.D.: 23.7 y ± 3.3) were enrolled in the main study (16 for the EBL experiment and 14 for the IAPS experiment) and 10 additional participants (6 males, 28.3 y ± 2.1) took part in a control experiment in which sensorimotor modulation was investigated during observation of a non-emotional visual stimulus (see “Visual stimuli”).

In the main study, 16 participants (6 males, mean age ± S.D.: 24.6 y ± 3.9) were tested with EBL stimuli and 14 participants (9 males, mean age ± S.D.: 22.5 y ± 2.3) were tested with IAPS stimuli. All participants were in good health, without any nervous, muscular, orthopedic, or cognitive disorders. Right arm dominance was determined by means of the Edinburgh Handedness inventory (Oldfield, 1971). Prior to the experimental session, all participants gave written informed consent and filled out a TMS safety, 13-items screening questionnaire (Rossi et al., 2011). The experimental protocol was approved by the ethics committee of the University of Genoa and was carried out in agreement with legal requirements and international norms stated in the adjourned declaration of Helsinki (Association, 2001).

Considering the impossibility to retrieve previous experimental SAI data recorded in a similar study design, in the main experiment we opted for a post-hoc power analysis in order to assess if the sample size we used in our study was appropriate. Results of the post-hoc power analysis showed that the sample size used for the EBL experiment (N=16) was appropriate with a power of 95% ($1-\beta = 0.95$; $\alpha = 0.05$; effect size = 0.97), while for the IAPS experiment (N=14) we retrieved a power of 94% ($1-\beta = 0.94$; $\alpha = 0.05$; effect size = 1.00). Power analysis was computed on G*Power 3.1.

5.4.2 Visual stimuli

Visual stimuli were presented on a 22-inches computer screen (resolution: 1680x1050, refresh rate: 60.0Hz; 16.67ms ± 12.37ms) located at 1.5 m away from

the participants. Refresh rate was assessed via a photosensor and corresponded to normative values (Garaizar et al., 2014).

A total of 90 emotional visual stimuli were used for the main study, 45 for the EBL experiment and 45 for the IAPS experiment (see Fig. 5). For the EBL experiment, emotional posture pictures were selected from a validated EBL database (Borgomaneri et al., 2012; Borgomaneri, Vitale, Gazzola, et al., 2015). EBL pictures depict four actors in different postures with emotional and neutral valence, thirty depicting negative (fear) and positive (happiness) movements and fifteen with no emotional significance (neutral). The actors were not handling objects and their face was blanked out.

Regarding the IAPS experiment, 45 stimuli were taken from the IAPS database, fifteen with negative emotional valence (fear), fifteen with positive valence (happiness) and fifteen neutral pictures (neutral). Fear evoking IAPS pictures were extrapolated from the study of Barke and colleagues in order to select fear-related stimuli, not contaminated by other negative emotions (e.g. disgust or rage) (Barke et al., 2012).

Furthermore, considering that the goal of the IAPS experiment was to test whether emotional scenes, with little or no motor information, was able to induce a modulation in the sensorimotor circuit, we excluded all the IAPS pictures that depicted whole human bodies involved in some kind of actions.

For the control experiment, the visual stimulus was a black cross on a white screen, with controlled resolution and refresh rate as for the main experiment.

Each experimental stimulus (EBL and IAPS pictures in the main experiment, black cross in the control experiment) lasted 1000 ms, with an inter-stimulus interval of 4000 ms.

5.4.3 Transcranial magnetic stimulation (TMS)

Single-pulse TMS was delivered using a Magstim 200 stimulator (Magstim, UK) with a monophasic current waveform connected to a figure-of-eight-shaped coil (external diameter of each loop, 9 cm) held tangentially to the scalp. The center of the junction of the coil was placed over the hand area of the contralateral M1 (i.e., left M1) at the optimal position (hotspot) to elicit MEPs in the right FDI, with the handle pointing backwards and a 45° away from the midline. With this coil orientation, the induced current flowed in an anterior–medial direction approximately perpendicular to the central sulcus. The optimal coil location was searched by slightly moving the coil over the left M1 area until MEPs of maximal amplitude and lowest threshold in the right FDI were elicited. The exact coil position was marked by an inking pen to ensure an accurate positioning of the coil throughout the experiment.

5.4.4 Short-latency afferent inhibition (SAI) protocol

SAI was tested following the validated protocol by Tokimura and colleagues (Tokimura et al., 2000).

The SAI effect was tested with a supra-threshold test TMS stimulus over the M1 representation of right FDI adjusted to produce MEPs of ~1 mV (peak-to-peak amplitude), preceded (0 ms inter-stimulus interval) by an electrical conditioning stimulus over the contralateral median nerve at the right wrist. This conditioning interval was shown to be optimal to evoke MEP inhibition in several studies (Fischer & Orth, 2011; Tokimura et al., 2000). Electrical stimulation (ES) was delivered through a bipolar electrode (cathode proximal) using a square-wave pulse (duration, 200 μs) set at intensity just above the threshold for evoking a small twitch in the *opponens pollicis* muscle (Digitimer D180 high voltage electric stimulator). In each experiment (EBL, IAPS) of the main study, a total of 90 trials were performed, of which 45 were conditioned trials (COND: ES+TMS) and 45 unconditioned trials (TEST: TMS).

In the control experiment, a total of 30 trials were performed (15 TEST and 15 COND). For all conditions, in the main experiment and in the control experiment, SAI was tested at two time-intervals: 120 ms and 300 ms after visual stimulus onset (see fig. 5). Additionally, two baselines recording of 30 trials each were recorded before (PRE) and after (POST) the experimental session.

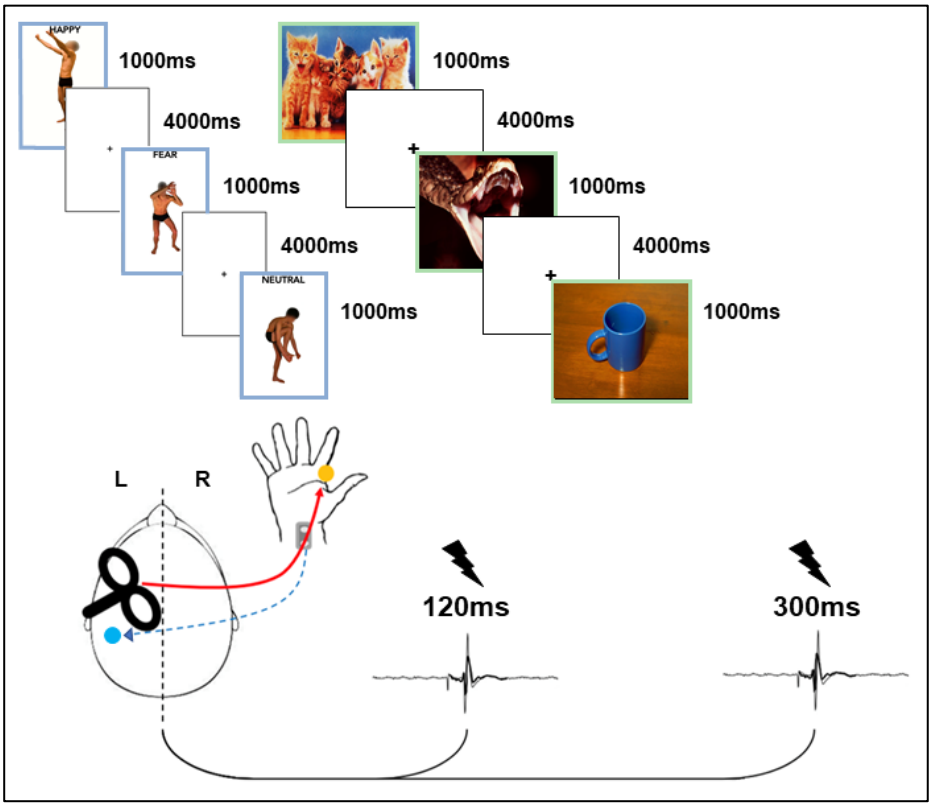

Figure 5: Experimental design. SAI was tested via an electrical stimulus (ES) delivered over of the median nerve (dashed blue arrow), followed 20 ms later by a transcranial magnetic stimulus (TMS) stimulus (red arrow) over the left first dorsal interosseus (FDI) cortical area at two timepoints, 120 and 300ms after visual stimulus onset (black lightning). The blue dot indicates the somatosensory afferences to the sensorimotor cortex, while the yellow dot represents the FDI muscle. Each visual emotional stimulus had duration of 1000ms, interspersed by a blank, white fixation screen of 4000ms, for a total of 45 stimuli for each trial [emotional body language (EBL) and International Affective Picture System (IAPS)]. The same experimental design was followed also for the control experiment, with the emotional visual stimuli replaced by a black cross on a white screen.

5.4.5 Electromyographic (EMG) recording

EMG was recorded with silver disc surface electrodes placed with a belly-tendon montage over the right first dorsal interosseus (FDI) muscle. Electromyography signals were amplified and filtered (20 Hz to 1 kHz) with a D360 amplifier (Digitimer). The signals were sampled at 5000 Hz, digitized with a laboratory interface (Power 1401, Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer for display and later offline data analysis.

5.4.6 Experimental procedures

In all experiments, participants were comfortably sitting on a chair at approximately 1.5m from the computer screen where the visual emotional or neutral stimuli (main experiment) or the non-emotional stimulus (control experiment) were presented. The experiment was programmed using Matlab (version 9.9.0.1467703, MathWorks, Natick, MA, USA) software to control picture presentation and to trigger ES and TMS.

MEPs were collected in 4 blocks (two baseline blocks and two experimental blocks). The baseline blocks were the first (PRE) and the last (POST) (30 trials each): participants were asked to keep their eyes open with the instruction to watch a black screen while receiving TMS over M1 (interpulse interval 4000 ms). The interpulse interval was determined following the Guidelines of the International Federation of Clinical Neurophysiology (Rothwell et al., 1999). Fifteen COND trials and 15 TEST trials were collected in each block.

In the experimental blocks (90 trials each) participants were asked to stay still and focus on the pictures sliding on the screen. In these two blocks, TMS pulse was delivered at 120 or 300 ms after picture onset and the order of the blocks was randomized. The trials in the experimental blocks were divided in 45 COND trials and 45 TEST trials, which in turn provided for 30 emotional stimuli (15 for fear and 15 for happiness) and 15 neutral stimuli each.

Participants were asked to stop the experiments whenever they felt discomfort or pain. They were also invited to maintain attention to the stimuli at regular intervals throughout the experiment and a verbal comment was asked at the end of each trial were all participants had to tell if they perceived any emotion on the observed pictures. A pause of 3-4 minutes between blocks was allowed to prevent fatigue or lack of attention. The experiment lasted for approximately 120 minutes.

A similar protocol was used for the control experiment, where 30 trials (15 COND and 15 TEST) were performed for the two baseline blocks (PRE and POST) and 30 trials were performed at 120 and 300ms after stimulus onset.

Finally, the Italian version of the BIS and BAS questionnaire was acquired submitted via email to all main experiment's participants in order to acquire individuals' specific personality dimensions (Leone et al., 2002). The whole questionnaire consists of 20 items of which the firsts seven give the BIS score (maximum 35 points) and the remaining give the BAS score (maximum 65 points). The BAS scale is divided in three main subclasses as follow: BAS Reward from item 8 to item 12, BAS Drive from item 13 to item 16 and BAS Fun from item 17 to item 20. The subject had to give a rating from 1 to 5, where 1 stood for

"It doesn't describe me at all" and 5 stood for "It describes me completely". A high score in the BIS scale indicates an increased propensity in withdrawal or inhibitory behavior when facing a potential threat, while a high score in the BAS scale is an indication of action engagement (Amodio et al., 2008; Gray, 1990).

5.4.7 Data analysis

Neurophysiological data were analyzed offline. The mean peak-to-peak MEP amplitude (in mV) was computed for each condition, with or without peripheral stimulation (MEPs COND and MEPs TEST). To evaluate the SAI effect, conditioned MEPs were expressed relative to unconditioned MEPs (SAI = COND/TEST). Moreover, SAI value at each testing time (120 and 300 ms) was normalized to SAI recorded at baseline (SAI Ratio = $SAI_{Experiment}$/ $SAI_{Baseline}$). This normalization shows data as an increased or decreased SAI compared to the SAI recorded at baseline. A value greater than 1 indicates a SAI reduction in relation to baseline (i.e., reduced sensorimotor inhibition), whereas a value lower than 1 indicates an increase of SAI in relation to baseline (i.e., increased sensorimotor inhibition) (Asmussen et al., 2014; Bonassi et al., 2019). The same analysis was run also for the data acquired in the control experiment.

5.4.8 Statistical analysis

Before performing further statistical analysis, we checked that all variables were normally distributed (Shapiro–Wilk test) and that sphericity was respected (Mauchly test). Levene's test of homogeneity of variances was also

performed in order to assess the comparability of the two datasets in the main experiment (EBL and IAPS).

The effects of electrical conditioning on MEP amplitudes in the two baseline blocks were tested using a mixed factors ANOVA with BLOCK (PRE and POST) and TYPE OF STIMULATION (TEST and COND) as within-subjects factors and GROUP (EBLs and IAPS) as between-subjects factor.

For the main study, SAI Ratio data were analyzed via a 3 x 2 x 2 mixed factors ANOVA, with EMOTION (fear, happiness and neutral) and TIME (120 ms and 300 ms) as within-subject factors and GROUP (EBL and IAPS) as between-subject effect. Post-hoc analysis was performed by means of Bonferroni correction method for multiple comparison.

We also performed a 3 x 2 x 2 mixed factors ANOVA, with the same within-subjects factors (EMOTION and TIME) and GENDER (Male and Female) as between-subjects effect, for both EBL and IAPS stimuli.

In order to assess whether there was a difference between the unconditioned MEPs amplitudes (MEPs TEST) and SAI effects (COND/TEST) recorded while processing EBL and IAPS pictures and the ones recorded at baseline, we performed a 3 x 2 mixed factors ANOVAs with CONDITION (Baseline, 120ms and 300ms) as within-subjects factor and GROUP (EBLs and IAPS) as between-subjects factor separately for each emotion (fear, happiness and neutral). We opted to name differently the TIME and CONDITION factors in order to highlight the presence of baseline data in CONDITION. Bonferroni corrected post-hoc tests were used to test significant effects.

In the control experiment, MEPs TEST and SAI data were analyzed via a repeated measure ANOVA with CONDITION (Baseline, 120ms and 300ms) as within-subjects factor.

Finally, to investigate a possible relationship between SAI modulation and personality trait in relation to emotional processing, we performed a Pearson's correlation between BIS/BAS questionnaire scores and SAI Ratio for normal distributed data and otherwise a Spearman's Rho correlation test was run.

Statistical analysis was performed with SPSS 25. P-values of 0.05 were considered as threshold for statistical significance.

5.5 Results

At baseline, when TMS was preceded by ES, MEPs amplitude consistently decreased relative to TMS alone (Table 1), thus replicating the typical SAI effects. Accordingly, the statistical analysis showed a strong effect of TYPE OF STIMULATION ($F [1, 28 = 324.567$; $p < 0.01$; $p\eta 2 = 0.921$), with lower amplitude in the COND condition (mean ± S.E.M. = 0.468 ± 0.027) compared to the TEST condition (1,27 ± 0,048), while no influence of the factors BLOCK or GROUP were found (all $F \leq 2,23$; all $p > 0.05$), thus suggesting no change in motor excitability over time in the two experimental groups submitted to the observation of EBL and IAPS stimuli. Furthermore, considering that no influence of BLOCK was observed, indicating no changes in motor excitability over time, the baseline blocks were averaged for computing the SAI Ratio (see next section).

A	EBL						
		Fear		Happy		Neutral	
	Baseline	120ms	300ms	120ms	300ms	120ms	300ms
MEP Test Stimulus (mV)	1.17±0.23	1.07±0.32	1.24±0.41	1.03±0.32	1.17±0.38	1.03±0.22	1.20±0.38
MEP Conditioning Stimulus (mV)	0.44±0.03	0.33±0.05	0.50±0.05	0.40±0.06	0.50±0.05	0.42±0.06	0.52±0.05
SAI	0.37±0.03	0.31±0.03	0.41±0.04	0.39±0.04	0.43±0.03	0.40±0.04	0.44±0.04
B	**IAPS**						
		Fear		Happy		Neutral	
	Baseline	120ms	300ms	120ms	300ms	120ms	300ms
MEP Test Stimulus (mV)	1.35±0.08	1.34±0.14	1.31±0.11	1.30±0.12	1.36±0.09	1.31±0.12	1.34±0.11
MEP Conditioning Stimulus (mV)	0.50±0.05	0.37±0.03	0.56±0.07	0.50±0.06	0.64±0.09	0.51±0.06	0.5±0.07
SAI	0.38±0.03	0.29±0.02	0.44±0.04	0.41±0.04	0.46±0.04	0.41±0.04	0.46±0.04

Table 1: MEPs and SAI data in the main experiment. In the table, MEPs values for the Test and Conditioning stimuli and SAI data are reported for EBL (1A) and IAPS (1B). All values are reported as Mean ± Standard Error of the Mean (S.E.M.).

5.5.1 Main experiment

Modulation of sensorimotor integration during perception of emotional pictures: comparison between emotions

Statistical analysis on SAI ratio values showed that sensorimotor modulation occurred at 120 ms specifically for fearful stimuli and at 300 ms for all the emotional stimuli (see Figure 6). RM ANOVA on SAI Ratio data showed significant results for both main effects EMOTION ($F [2, 56] = 9.723$; $p < 0.01$; $p\eta 2 = 0.258$) and TIME ($F [1, 28] = 17.709$; $p < 0.01$; $p\eta 2 = 0.387$), which were

qualified by the interaction effect EMOTION*TIME ($F [2, 56] = 4.079$; $p = 0.035$; $p\eta 2 = 0.127$). All the effects involving the factor GROUP were not significant in the ANOVA (all $F \leq 0.508$; all $p > 0.05$), suggesting comparable sensorimotor modulations with the two sets of pictures. Post-hoc analysis of the EMOTION*TIME interaction showed that only during processing of fearful stimuli at 120 ms from the stimulus onset SAI increased (i.e., larger inhibition) respect to the same timing for happiness (120 ms, $p < 0.01$) and neutral (120 ms, $p < 0.01$). Furthermore, only for fear and happiness, SAI recorded at 120 ms after stimulus onset was significantly higher compared with SAI recorded at 300ms ($p < 0.01$ for fear; $p = 0.012$ for happiness).

No effects for GENDER were retrieved in the analysis for EBL or IAPS stimuli (all $F \leq 0.508$; all $p > 0.05$).

Modulation of corticospinal excitability and sensorimotor integration during perception of emotional pictures

Considering that the analysis on SAI Ratio data showed an influence of EMOTION, which was different for the early and later time points considered (i.e., EMOTION*TIME interaction) we further investigated SAI modulation during observation of emotional stimuli performing additional analyses. The ANOVAs on MEPs TEST showed no significant main effects or interaction of the factors CONDITION or GROUP across the three emotions (fear, happiness and neutral; all $F \leq 1$; all $p > 0.05$).

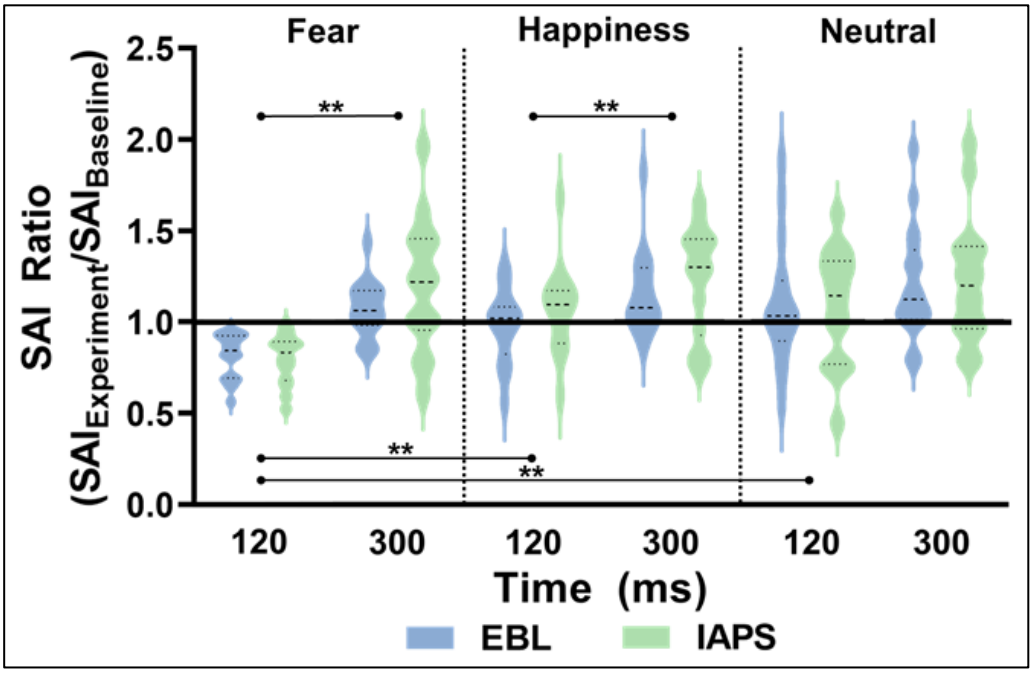

*Figure 6: SAI Ratio data. The picture shows the violin plots all SAI Ratio data for EBL and IAPS. A value greater than 1 (black thick line) indicates a decreased inhibition (SAI reduction) in relation to baseline, whereas a value lower than 1 indicates an augmented inhibition (SAI increase) in relation to SAI recorded at baseline. As observable, the only increase of SAI was at 120ms after stimulus onset for fearful emotional stimuli. All SAI Ratio data computed at 300ms showed a decreased SAI across emotional and neutral stimuli in both EBL and IAPS. SAI Ratio data are reported on the y-axis, while the two timepoints 120 and 300ms are reported on the x-axis, separately for every condition. **= p< 0.01.*

In contrast, the ANOVA on SAI data for fear showed significant effect of CONDITION ($F [2, 56] = 28.814$; $p < 0.01$; $p\eta 2 = 0.507$), but no main effect or interaction involving the factor GROUP ($p > 0.05$). Post-hoc analysis (see Figure 7A) showed that the SAI effect increased (i.e., lower SAI values) in the early time point (120 ms) relative to the later time point (300 ms) and baseline (all $p < 0.01$). Furthermore, at 300 ms after fearful stimuli onset, SAI was significantly lower relative to baseline ($p < 0.01$).

The ANOVA on SAI effects for the other two conditions (Happiness, Figure 7B; Neutral stimuli, Figure 7C) showed a significant effect of CONDITION for both happiness ($F [2, 56] =9.669$; $p < 0.01$; $p\eta 2 = 0.257$) and neutral stimuli ($F [2, 56] = 5.824$; $p < 0.01$; $p\eta 2 = 0.172$), but not main effect or interaction involving the factor GROUP (all $F \leq 1$; all $p > 0.05$). Post-hoc analysis showed that for both happiness-related and neutral stimuli, SAI significantly decreased at 300 ms relative baseline levels (all $p < 0.01$) and showed similar trend relative to the 120 ms condition (happiness-related stimuli: $p = 0.016$; neutral stimuli $p = 0.082$).

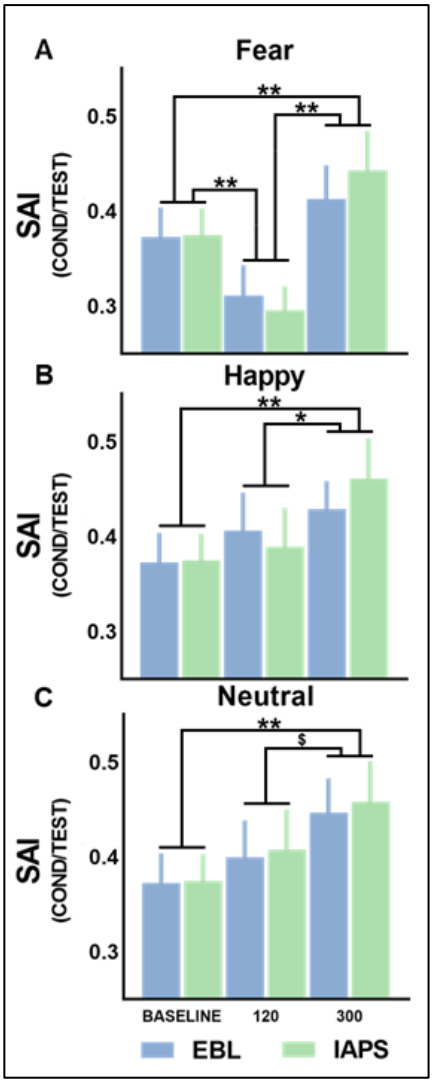

*Figure 7: SAI data. All SAI data are graphically reported. In figure 3A, the increase of SAI at 120ms after stimulus onset for fearful stimuli is observable in both conditions (EBL and IAPS) in the comparison with SAI recorded at baseline (no visual stimuli). At 300ms after stimulus onset, SAI was decreased compared to baseline for all emotional (3A and 3B) and neutral stimuli (3C) in both conditions (EBL and IAPS). SAI data (MEP conditioned/MEP test) are reported on the y-axis, while the three timepoints at which SAI was tested (Baseline, 120ms and 300ms) are reported on the x-axis. *= p< 0.05, **= p< 0.01, $= p >0.05 (trend).*

5.5.2 Correlation analysis

Twenty-one out of 30 participants accepted to compile the BIS/BAS questionnaires and were included in the correlation analysis. Three of them were excluded as outliers (distance from the trend-line higher than 2.5 standard deviations), for a total of 18 participants from both the EBL and the IAPS experiments.

Since, statistical analysis on SAI Ratio data did not show any significant difference between groups (EBL and IAPS), before running the correlation analysis, we performed a linear regression analysis to verify that the GROUP was not a significant predictor for BIS, BAS and SAI RATIO data. Statistical analysis showed that GROUP was never a significant predictor, thus we run the correlation analysis combining the data of EBL and IAPS conditions.

Pearson's correlation was run separately for the BIS and BAS questionnaire scores that presented normal distribution.

Results showed that the only significant correlation we found was a positive correlation between BIS and SAI Ratio data for fearful stimuli at 120ms post-stimulus ($p = 0.003$, $r = 0.627$) (Figure 8). Correction for multiple comparisons was run in accordance with the following formula from the work by Curtin and Schulz (Curtin & Schulz, 1998):

$$\alpha' = 1 - (1 - \alpha)^{1/k}$$

where α' will be the new alpha-value corrected for multiple comparisons, α is the value for significance previously set in our study and k is the number of comparisons on the same dependent variable (which in our case is equal to 10).

Therefore, the new alpha-value is set at 0.005. Acknowledged that the p-value computed for the correlation between the BIS value and the SAI Ratio data for fearful stimuli at 120ms post-stimulus is still significant after multiple comparisons correction ($p = 0.003$), we can say that the positive correlations we found indicates that participants with augmented SAI at 120 ms from the picture onset had a lower BIS score. All other correlations for the BIS score, as well for the BAS score, were not significant (all $p > 0.05$).

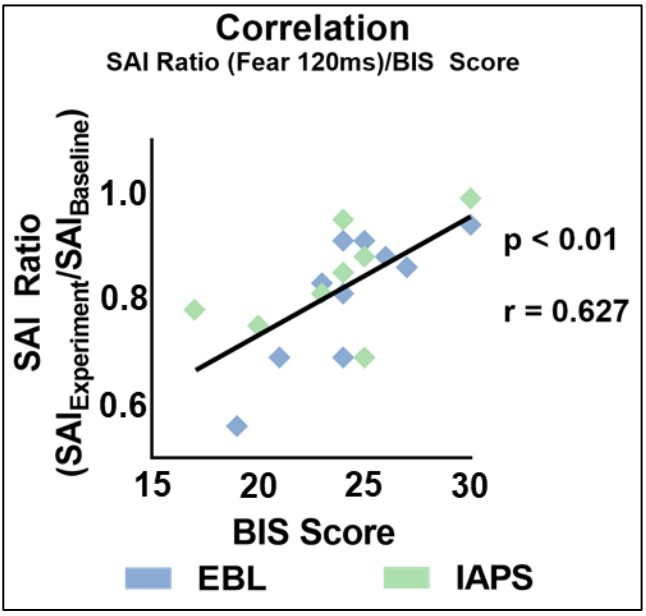

Figure 8: Correlation analysis on SAI Ratio and BIS. Pearson's correlation analysis for SAI Ratio computed for fearful stimuli at 120ms after stimulus onset and BIS scores of participants from both EBL and IAPS. A positive correlation is observable between the two sets of data, meaning that at a higher BIS score corresponded a decreased SAI and vice versa.

5.5.3 Control experiment

Statistical analyses on MEPs TEST and SAI data for the control experiment showed no significant differences for CONDITION ($p > 0.05$), meaning that all MEPs and their relative SAI during the observation of a non-emotional visual stimulus were comparable at baseline and at 120ms or 3 00ms after the stimulus onset (see Table 2).

	Control Experiment		
	Baseline	**120ms**	**300ms**
MEP Test Stimulus (mV)	0.98 ± 0.03	1.00 ± 0.08	0.94 ± 0.08
SAI	0.44 ± 0.04	0.46 ± 0.04	0.47 ± 0.05
SAI Ratio	N.A.	1.07 ± 0.05	1.07 ± 0.03

Table 2: MEPs and SAI data in the control experiment. In the table, MEPs values for the Test stimulus, SAI data and SAI Ratio data are reported for baseline and for the two timepoints considered in the main study (i.e., 120 and 300ms after stimulus onset). For the SAI Ratio data, no baseline is retrievable because it was already used for its computation. All values are reported as Mean ± Standard Error of the Mean (S.E.M.).

5.6 Discussion

The main aim of this study was to investigate sensorimotor integration in healthy adults while observing EBL and IAPS pictures. Furthermore, we explored whether interindividual differences in BIS/BAS personality traits predict the magnitude of sensorimotor integration mechanisms as tapped by the SAI effect.

The results showed a consistent SAI effect on single pulse TMS when ES were administered over the median nerve. Remarkably, we observed the

following sets of novel findings: (i) only while processing fearful stimuli, sensorimotor inhibition (i.e., the SAI effect) increased in magnitude, at earlier latencies (120 ms after the stimulus onset) irrespective of the type of picture (EBL or IAPS); (ii) at 300 ms after the stimulus onset, sensorimotor inhibition decreased while processing all kind of emotional stimuli (fear, happiness, neutral) again irrespective of the type of picture (EBL or IAPS); (iii) lastly, there was a correlation between sensorimotor inhibition at earlier latencies (120 ms after the stimulus onset) while processing fearful stimuli and BIS score.

There are different possible explanations accounting for the enhanced SAI effects – that is, increased sensorimotor inhibition – observed at 120 ms after the stimulus onset only for fearful stimuli, and these accounts deal with modulation of sensory afference and activity in the attentional circuits. Indeed, the circuitry underlying SAI is complex. A recent neuronal model of SAI proposes that sensory afference drives corticospinal activity through a perisomatic inhibitory projection as well as the modulation of distal dendritic excitatory input from the I-wave generators (Turco et al., 2018). The perisomatic inhibitory input is proposed to originate from ɣ-amino butyric acid (GABA) basket cells located in layer IV of the motor cortex. These basket cells are hypothesized to receive excitatory input from somatosensory pyramidal neurons as well as from the thalamus and are sensitive to cholinergic inputs coming from the basal forebrain (BF) (Szabó et al., 2010; Tremblay et al., 1990). The BF cholinergic system has extensive diffuse projections to neocortex as well as projections to and from basolateral amygdala and olfactory bulb and is strongly implicated in attention (Davis & Whalen, 2001; Tamietto & De Gelder, 2010).

Studies suggest that increased sensorimotor inhibition may derive from an augmentation of the sensory afferent volleys to the cortex. Indeed, it has been consistently shown that the greater the afferent volley evoked by peripheral stimulation, the stronger is the magnitude of SAI (for a review see (Turco et al., 2018)). However, modulation of sensorimotor inhibition may also stem from the engagement of attentional circuits. Indeed, paying attention to the body part tested in the SAI protocol (hand) during an attentional task (internal focus) leads to a greater SAI with respect to what observed when the focus is external (L. Y. Suzuki & Meehan, 2020). Such an effect was explained by observing that internal focus might result in an augmentation of somatosensory volleys from the specific body part, which is in keeping with the notion that observing and paying attention to a specific body part enhance the somatosensory representation of that body part (Taylor-Clarke et al., 2002). In a similar vein, observing a touch onto another body – which is known to engage the activation of the somatosensory cortex through a somatosensory resonance mechanism (Bufalari et al., 2007; Keysers et al., 2010), also increase the SAI effect (Wood et al., 2010). On the other hand, SAI effects may also be modulated by more general attentional mechanisms as well. In a recent study, increase of SAI was observed during a computerized non-verbal recognition memory task that requires recognizing previously encoded faces, in the retrieval phase (Bonnì et al., 2017). The authors proposed that the retrieval phase of the visual memory task could evoke a considerable activation of widespread cholinergic projections from the BF to the cortex (Bonnì et al., 2017).

In a more speculative way, we can also ask ourselves what is the structure that may theoretically drive these mechanisms (modulation of sensory afference

and/or recruitment of attentional circuits) during processing of emotional fearful aversive stimuli? Although in our study we cannot directly address this issue, we know that a pivotal structure in decoding emotional information, specifically for threat-related stimuli, with widespread cortical and subcortical interactions is the amygdala (AMG). AMG has been shown to receive complex sensory information from the periphery and process them in its lateral and basolateral nuclei, which in turn project to its central nucleus (Lang et al., 2000). Then, the central nucleus of the AMG sends projections to a widespread network involving a multitude of structures which are involved in both motor and non-motor responses at short and long latencies (Grèzes et al., 2014; Petrovich et al., 2009; van Heijnsbergen et al., 2007; Vuilleumier, 2005). More specifically, in response to aversive stimuli, the central nucleus of the AMG has been shown to project to several subcortical structures involved in attentional processes such as the BF and the locus coeruleus. Through its projections toward cholinergic nuclei located in the BF, AMG is able to influence an extended cortical and subcortical network which in turn increases the attentional control over a stimulus (Davis & Whalen, 2001; Vuilleumier, 2005). Furthermore, this widespread cholinergic activity might be able not only to increase attention, but also to lower the threshold of the sensory system in order to focus attentional resources over threat-related stimuli and consequently potentiate sensory afferences processing (Davis & Whalen, 2001). Projections from the central nucleus of the AMG have also an influence over the noradrenergic cells of the locus coeruleus, structure responsible for stress and conditioned fear response as well as for autonomic activity (Aston-Jones et al., 2007; Davis & Whalen, 2001; Gu et al., 2020).

Taken all together, the engagement of cholinergic circuits secondary to emotional stimulation coupled together with the activation of the locus coeruleus and the concomitant activation of the sensory thalamus may lead to an increased state of vigilance which directs attention toward strongly arousing stimuli with aversive properties (Davis & Whalen, 2001; Gu et al., 2020; Tamietto & De Gelder, 2010; Vuilleumier, 2005).

Related to the temporal dimension of the activation of the AMG, it has been shown that the AMG promptly reacts to fearful signals, for example showing a short-latency activation at already ~70 ms from presentation of a fearful face – which might be linked to an evolutionary mechanism for processing threat-related stimuli, in order to rapidly react to a potential menace (Méndez-Bértolo et al., 2016). Such a mechanism was referred as 'preparedness', which is defined as an instinctive response to known aversive stimuli that does not show an elaborated cognitive processing of the afferent information (Seligman, 2016). So far, this phenomenon has been described in processing emotional facial expressions (Méndez-Bértolo et al., 2016), but since subcortical areas activated during facial expression processing are partially overlapping with those engaged during processing of EBL and IAPS (one over all is AMG), it is plausible to expect similar pattern of activation for both categories of emotional stimuli (De Gelder, 2009; Johnston et al., 2013; Meeren et al., 2008).

Based on the aforesaid premises and on our results, it appears reasonable to speculate that the rapid amygdala activation, as described for fearful facial expressions, might be a key component for the sensorimotor modulation we found for fearful pictures, acting via the same principle of 'preparedness' observed for

threat-related faces, even though specific imaging studies are needed in order to confirm these supposed mechanisms.

Moreover, the fact that we observed such a fast increase of SAI for all fearful pictures, being them EBL or IAPS, might be related to the fact that an aversive emotional content is able to rapidly activate a vast and intertwined network capable of focusing attentional resources over a potential threat via the induction of sensory vigilance and hence inducing a transient and short-latency freezing-like phenomenon of the motor cortex (Borgomaneri, Vitale, Gazzola, et al., 2015; Méndez-Bértolo et al., 2016).

The second result of our study was that at longer latency (i.e., 300 ms after the stimulus onset) sensorimotor inhibition decreased while processing all kind of emotional stimuli (fear, happiness, neutral) conveyed by all the pictures (EBL and IAPS). First, it is worthy to note that in our control experiment, planned to test SAI at 120 and 300 ms after the onset of a picture representing a black cross on a white screen, we did not find a modulation of SAI. This result mostly excludes that solely being presented with a visual stimulus shown on a screen may modulate sensorimotor inhibition (for a detailed analysis on the topic see 'Limitations').

As an example of sensorimotor modulation derived from higher order cognitive processes, it has been observed that decreased sensorimotor inhibition has been observed during a verbal working memory task, with increasing memory set size (Lorraine Y. Suzuki & Meehan, 2018). Indeed, SAI was significantly reduced during memory maintenance of six-digit compared to two-digit set (Lorraine Y. Suzuki & Meehan, 2018). This result was interpreted as the necessity

to increase the suppression of the task-irrelevant somatosensory afferent projections to motor cortex during the maintenance period of the verbal working memory task. Following this finding, here we showed no modulation during visual processing of a blank screen, but when the visual information increased in complexity, as for IAPS and EBL pictures, SAI decreased at 300ms. We might propose that even during passive visual processing of images of different complexity, decreased sensorimotor inhibition may represents the suppression of the task-irrelevant somatosensory afferent projections to motor cortex, similarly to what happens during a higher order cognitive processing such as a visual working memory task of increasing complexity. Indeed, 300 ms latency is consistent with cognitive processing of the visual stimuli. Event-related potentials studies showed during visual processing of a variety of stimuli a consistent component related to cognitive control, that is the P300, a positive wave that occurs at around 300–650 ms after stimulus presentation and which is maximal over the central-parietal region (Barceló & Cooper, 2018; Gontier et al., 2007). Major determinants of P300 amplitude during visual processing are reward value, affective significance, and the influence of these factors on attention resource allocation (Duncan-Johnson & Donchin, 1977; Olofsson & Polich, 2007; Polich & Kok, 1995).

The third result of our study was that sensorimotor modulation during processing of fearful stimuli at 120 ms correlated with the BIS score: the more the increase in sensorimotor inhibition at 120 ms while processing fearful stimuli, the lower the BIS score. A low score in the BIS questionnaire indicates an individual minor propensity in withdrawal behavior when facing a potential threat. If modulation of sensorimotor inhibition is driven by the activation of the AMG

orchestrating a cascade of events including increased attention with higher sensory vigilance, we can hypothesize that these processes are linked to behavior, including propensity in withdrawal behavior. Greater attention and sensory vigilance may guide a higher propensity in facing a potential threat, rather than withdraw from it. This speculation is in line with recent behavioral findings of our group: by using the same sets of EBL and IAPS images adopted here and analyzing response time in a recognition task, we found that response time for fear pictures were lower than for happy and neutral, particularly for EBL pictures (Botta et al., 2021). Possible explanations for this finding may lie on shorter time for processing fear respect to happy pictures, but also on fear stimuli facilitating faster motor commands with respect to happy and neutral.

In any case it is important to note that, although the results of the correlation analysis resulted significant for multiple comparisons correction, deeper investigations are needed in order to confirm our findings with a bigger sample size and separately for EBL and IAPS.

In conclusion, here we showed that sensorimotor inhibition, depending on the magnitude of afferent sensory volley and on the activity of cholinergic circuits, is dynamically modulated during visual processing of emotional stimuli. At short latency from picture onset, reflecting automatic processing of emotional information, only negative, fearful stimuli induced a modulation of sensorimotor inhibition. At 300 ms from picture onset, reflecting cognitive appraisal of visual information, SAI appeared to decrease in relation to the complexity of the image, rather than its emotional content. Sensorimotor inhibition has been used so far as a tool to explore cognitive-motor interaction, but the results of this study suggest

that it may also be used to explore emotional-motor interaction, in physiological and pathological conditions.

5.7 References

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6. Experiment 3: Early modulations of neural oscillations during processing of emotional body language

6.1 Techniques: Electroencephalography

Electroencephalography (EEG) is a non-invasive technique aimed to record the electrical neuronal activity on the scalp. The first EEG recording was performed by the neuropsychiatrist Hans Berger back in 1929; the scientist, through the use of a series of galvanometers placed on the scalp of his son, recorded for the first time a rhythm at 10 Hz which will later be called the 'alpha' rhythm (see fig. 1) (Niedermeyer & da Silva, 2005). Throughout the following decades, EEG techniques were perfectioned and started to attract clinicians and scientists whose aim was to study the electrophysiological correlates of brain activity in different populations and in the most disparate experimental conditions.

In general, EEG is a graphic representation of the differences in electrical potential between two different cerebral locations, recorded on the scalp and plotted over time (Olejniczak, 2006). Anyway, the recording is not trivial and, in order to properly record the potentials of cortical neurons, several aspects have to be taken into account so that to make it possible to 'read' cortical electrical activity. First of all, the electrical signals recorded at the scalp level have to be generated by groups of cerebral cortical neurons which are similarly oriented, show a polarized 'geometry', are localized in the proximity of the scalp (and of

the recording electrode) and have to be able to activate synchronously (Britton et al., 2016).

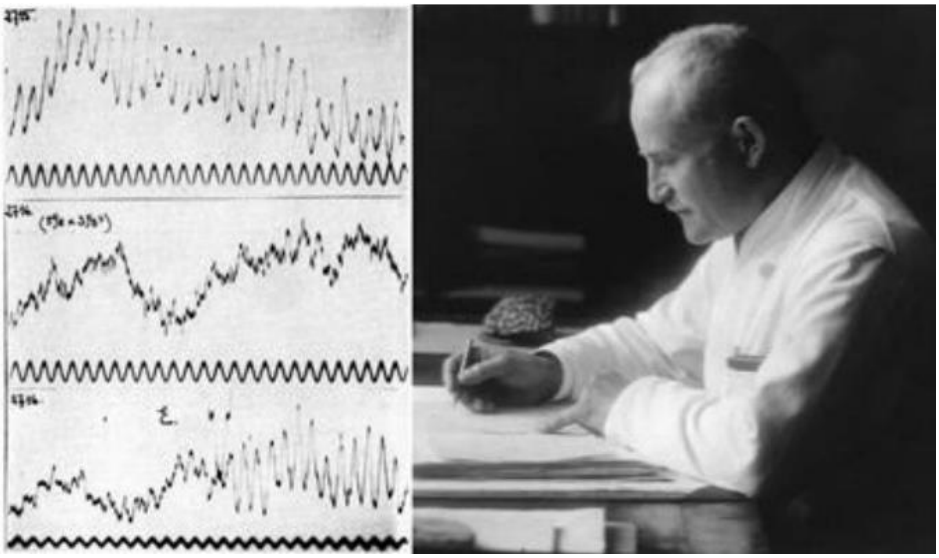

Figure 1: Hans Berger and the first EEG recording. On the left it is possible to observe the recorded oscillatory activity (upper signal), showing a more random and asymmetrical activity, and the regular 10 Hz wave (lower signal) used by Berger as reference. The man on the right is Hans Berger (picture modified from the original by Niedermeyer and da Silva, 2005)

The main class of neurons displaying all the aforementioned features are the pyramidal neurons. The peculiar oblong geometry of their cell body allows them to behave like an electrical dipole, hence showing the ability to be polarized depending on the type of synapse, being it excitatory or inhibitory. Both excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) contribute to the synaptic activity recorded by EEG, but the result will differ in

terms of polarity. If we consider an EPSP, hence a synapse involving the inward flowing of positive ions such as Na^+ toward the intracellular space which causes neuronal depolarization, the oblong shape of the pyramidal neurons will cause a distribution of negative charges in the extracellular space nearby the locus of the synapse and an accumulation of positive charges distally (see fig. 2) (Holmes & Khazipov, 2007). But in order to record the electrical activity of the cortex, neurons have to show not only a polarized 'geometry', but they also have to be oriented in the same direction and to be in the proximity of the scalp, so to allow spatial electrical summation. Luckily for us, the distribution of the pyramidal neurons in the cortex sheets presents a columnar structure, meaning that the neuronal cells bodies are in the deeper laminae and the dendritic arbors extend upward toward the scalp and all neurons point in the same direction (as observable in fig. 2).

Taken all together, these spatial and structural characteristics of the pyramidal neurons should make it possible to record an electrical field at the level of the scalp, but there is one last thing missing. The potential generated by one neuron, or few neurons, is not big enough in order to be recorded by an external electrode at the level of the scalp. It then comes the last feature that we previously discussed that is the capacity to fire synchronously. It takes a combined synchronous electrical activity of at least a pool of 108 neurons in an area of 6 cm^2 in in order to create a visible EEG signal (Olejniczak, 2006).

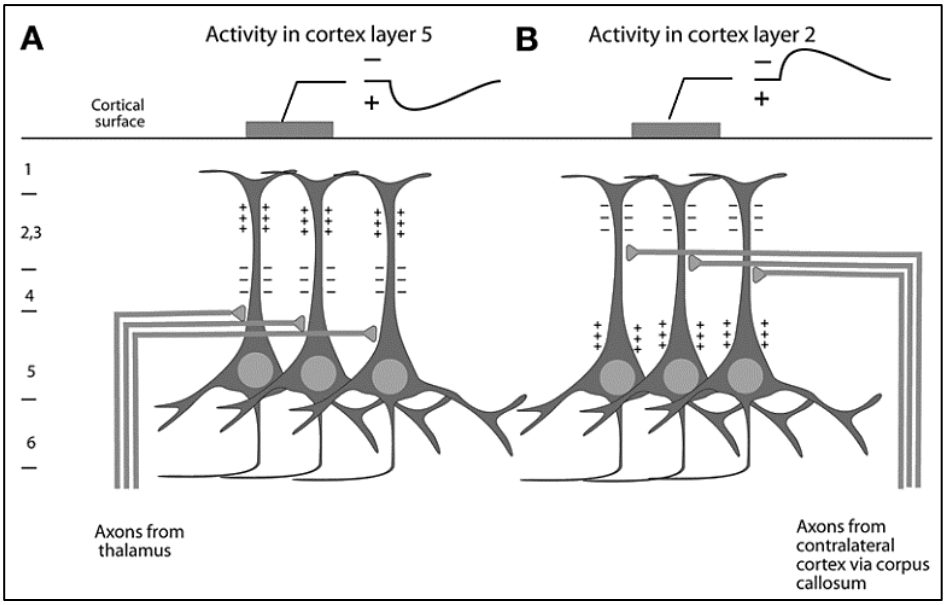

Figure 2: Pyramidal neurons cortical organization and polarity of the surface EEG depending on the location of the synaptic activity within the cortex (Kandel et al., 2000).

Thus, in order to obtain a cogent EEG signal we need a significant amount of synchrony between thousands of neurons, which, as a fortunate coincidence, is what happens in neuronal pools specialized in a specific function or in brain networks (Holmes & Khazipov, 2007). If, for example, we take the first EEG recording made by Hans Berger as we said he recorded an alpha rhythm. The alpha rhythm (which encompasses frequencies between 8 and 13 Hz) is one of the basic frequency bands in which neuronal activity resonates together with the delta (0.5-4 Hz), the theta (4-7 Hz), the beta (13-30 Hz) and the gamma bands (>30 Hz). Without going through the functional significance of those rhythm, it suffices to acknowledge that if a neuronal pool fires at a determined frequency rate it

means that their activity is synchronous and, if we record its electrical activity, we will observe a signal characterized by defined and periodic oscillations (see fig. 3). So, coming back to Berger, he was able to record the activity of a pool of occipital neurons resonating at a frequency of 10 Hz only because these neurons were 'acting' synchronously and under the same condition. Interestingly, in the second and third panel on the left of figure 1 it is also possible to observe the so called 'alpha blocking' which is an example of desynchronized neuronal activity occurring when the subject focuses on a cognitive task or is simply with his eyes open. Contrarily to synchronization, desynchronization is always an expression of neuronal activity but it consists in a reduced spectral power in determined circumstances, involving a reduction of amplitude and an increase of frequency, usually occurring during the execution of cognitive tasks and under the influence of different subcortical structures such as the basal forebrain and the locus coeruleus (Olejniczak, 2006).

From a technical point of view, EEG systems consist of different parts enumerated in the following list (for the aim of this thesis, I will only explain some of the main technical aspects and precautions relevant for EEG recording):

- Electrodes
- Amplifier
- A/D converter
- Recording device (e.g., laptop)

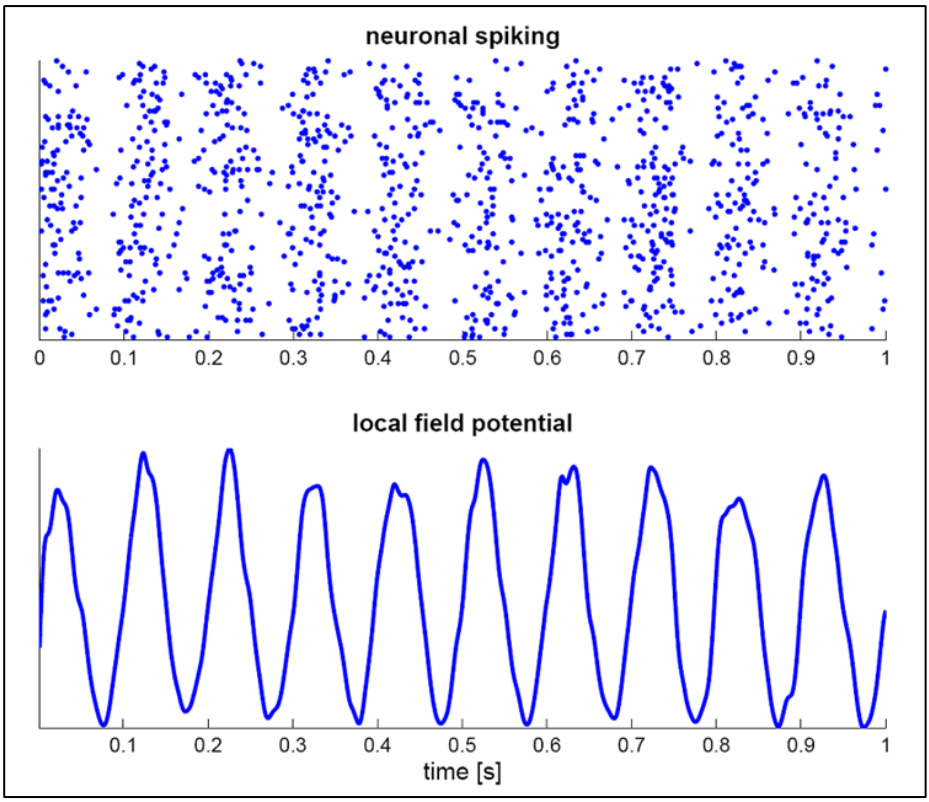

Figure 3: Relation between neuronal spiking and local field potentials. In the picture it is possible to observe the how synchronized neuronal firing at a frequency of 10 Hz is represented during EEG recording in terms of electrical activity and local field potential. From: en.wikipedia.org/wiki/Neural_Oscillation

Electrodes are the units 'reading' the signal from the scalp which then reaches the amplifier that elaborates and, obviously, amplifies the signal so to allow the signal to be accurately digitalized (EEG signals are on the order of the microvolts). The A/D converter changes the signal from the analog to the digital form and, lastly, the signal reaches the recording device where data are stored.

High density EEG (hdEEG) signal is recorded via 128 channels mounted over an elastic cap, with distances between the electrodes defined through the international 10-20 system. Electrode placements are labelled according to adjacent brain areas: F (frontal), C (central), T (temporal), P (posterior), and O (occipital). The letters are accompanied by odd numbers at the left side of the head and with even numbers on the right side (see fig. 4). Two extra electrodes are used, one with the role of reference and the other as a ground electrode needed for getting differential voltage by subtracting the same voltages showing at active and reference points. There are several different types of electrodes, but the most used are usually active electrodes with an Ag-AgCl or silver-silver disks of 1-to-3 mm of diameter as recording 'core'. The material of the disk is extremely important because it has to be sensitive to extremely small variation in the electrical field. In fact, EEG recording is based on small differences in electrical potentials between the reference and the active electrodes placed all over the scalp, corrected through the signal recorded by the ground electrode (via the following formula: [channel – ground] – [online reference – ground] = channel – online reference) (Leuchs, 2019).

An issue that has to be taken into account when recording EEG data through active electrodes is impedance. The concept of impedance is basically the same defined for electrical resistance, but applied on alternating current, as it is the current recorded by EEG; the higher the impedance, the lower will be the quality of the signal, leading to distortions that might be extremely difficult to correct. In order to prevent signal distortions, impedance at each electrode contact with the scalp should all be below 5 KΩ (Teplan, 2002).

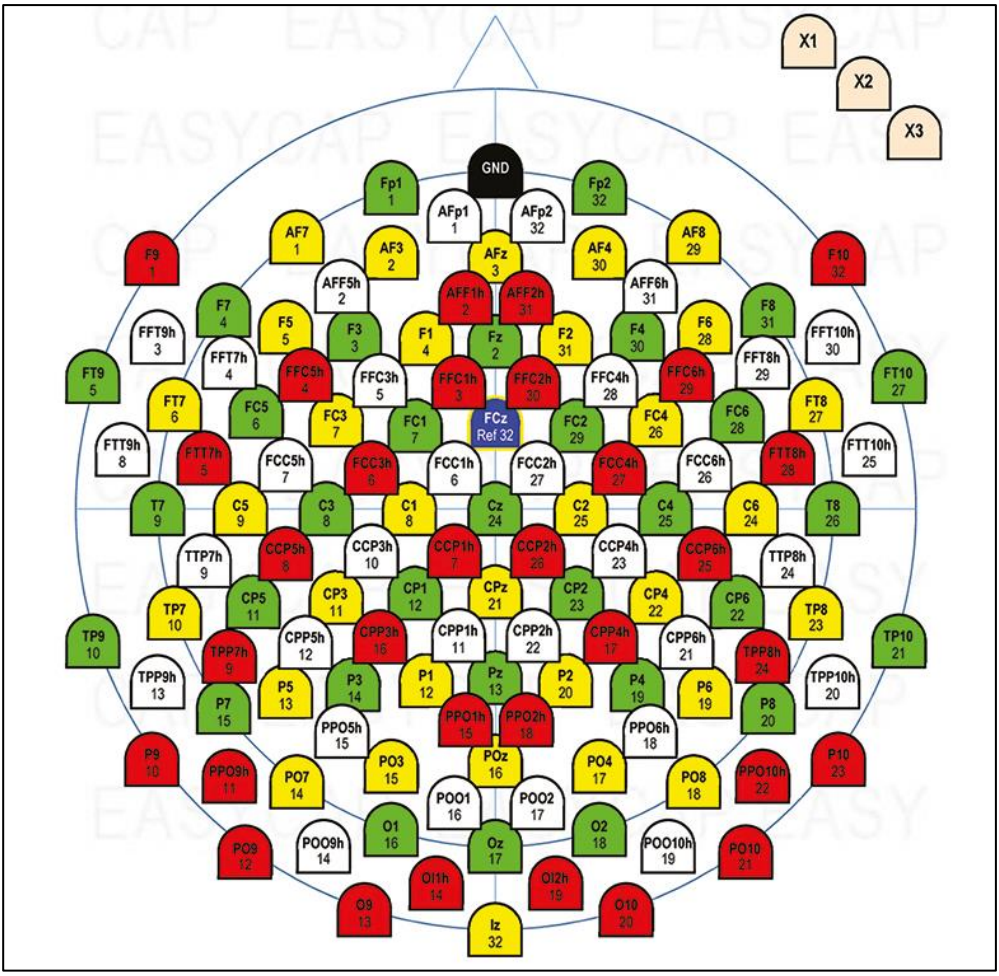

Figure 4: 128 channels montage. In the picture it is possible to observe the standard montage of an hdEEG system on an elastic cap. In black it is highlighted the ground electrode, while in blue (FCz) the reference. The X1, X2 and X3 stand for the electrodes recording EMG activity from eye movements and their relative ground electrode. The image is taken and modified from the Brain Products Press Release 'actiCAP active electrode walkthrough' (2017).

Impedance can be lowered first of all by accurately cleaning the skin surface on the scalp of the participants, so that to remove all grease and dead skin particles. Secondarily, after the apposition of the elastic cap mounted with the

electrodes on the head of the participants, conductive hydrogel-based gel is placed in between the scalp and the recording electrode, so that to further lower the impedance for recording. After checking for impedance, the last thing that might represent a potential nuisance in EEG recording are artifacts. The main artifacts retrievable in EEG signals come from the electrical grid and muscular contractions. The first one is easily removed from the signal by applying a notch filter which deletes all frequencies at 50 Hz, that is the frequency of the alternating current in Europe. As for the second, the positioning of electromyographic recording electrodes is necessary so to monitor eyes movement and consequently correct the recorded signal through an independent component analysis aimed to delete muscular artifacts (Leuchs, 2019; Teplan, 2002) (the exact method for artifact correction will be explained in the 'Experimental procedures' section of this experiment).

6.2 Rationale

The understanding of nonverbal emotional cues has become a cornerstone of cognitive neuroscience since the first studies of Darwin in the 19th century (Darwin, 1872). Although for decades the main interest of researchers was focused on emotional facial expressions, studies on emotional body language (EBL) are now attracting almost as much attention as their facial counterpart (De Gelder, 2009). EBL have been shown to be rapidly recognized (Borhani et al., 2015; van Heijnsbergen et al., 2007) and, especially when it comes to fearful behavior, its perceptual processing displays an effect on sensorimotor system

already at 100-120ms after stimulus presentation (Borgomaneri, Vitale, Gazzola, et al., 2015; Botta et al., 2022; van Heijnsbergen et al., 2007).

Recent studies have shown that threat-related (i.e., fearful) EBL static pictures exert a modulatory, rapid effect on corticospinal excitability as well as on intracortical facilitatory mechanisms (Borgomaneri et al., 2017; Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, Gazzola, et al., 2015) and can induce reduced response times, hence faster motor response, when compared to positive EBL an non-emotional body language (Botta et al., 2021). Furthermore, in a previous work by our group, we found that fearful EBL stimuli, differently from positive and non-emotional stimuli, are able to induce an increase of short-latency somatosensory afferent inhibition (SAI) at 120ms after the stimulus onset, hence demonstrating that emotional processing of fearful information produces an early reduction of primary motor cortex (M1) excitability operated by the primary somatosensory cortex (S1) (Botta et al., 2022). It appears then that the observation of fear-related behavior induces an early, transient, decrease of M1 excitability which can be referred as a 'freezing-like' phenomenon of motor cortex which in turn produces faster motor response at longer latencies (i.e., 700-800ms).

This early responsiveness of sensorimotor network highlights also another aspect of EBL processing. In order to result in such a fast modulation, fearful motor behavior as to be rapidly detected by the observer. Electroencephalography (EEG) studies on event related potentials (ERPs) during EBL observation have shown that the first response to fearful stimuli is retrievable at 110ms after picture onset on posterior cortical areas (Meeren et al., 2005; van Heijnsbergen et al.,

2007). Moreover, in the comparison between non-emotional and fearful body language they observed longer latencies for neutral stimuli but no differences in terms of P100's amplitudes, interpreting such results as a perceptual advantage of fearful EBL that did not result in an increased attentional resources allocation, at least at short latencies (van Heijnsbergen et al., 2007). It is also to consider another aspect: EBL inevitably carries intrinsic motor information, which can be reflected in a more central modulation of neural activity. In a series of studies, Kiefer and collaborators described an early ERP component in central motor and premotor areas which peaked during action related and visual sensory processing; the authors refers to it as 'fronto-central P100' and it is retrieved in a time window ranging from 85 to 115ms after stimulus onset and, differently from the canonical posterior P100, shows a negative deflection (Markus Kiefer et al., 2011; Sim et al., 2015). Despite the presence of several works showing the link between emotion recognition and motor mimicry (for a review see Ross & Atkinson, 2020), little is known about the differences between emotional and non-emotional EBL in terms of latencies and magnitude of EEG recorded cortical responses in the motor cortex. Considering the neurophysiological evidence on sensorimotor inhibition induced by fearful behavior (Botta et al., 2022) and on temporal dynamics of body language perception (van Heijnsbergen et al., 2007), it seems plausible to expect a decreased short-latency activity of M1 at the expenses of fearful EBL, driven by the activity of inhibitory interneurons between S1 and M1, but not a delayed fronto-central P100 response in the comparison between the two conditions (i.e., fear and neutral).

Another index of cortical and network activity that could support this hypothesis might come from recent studies on mu rhythm suppression and low-

beta rhythm. Mu rhythm is an oscillation within the standard alpha frequency (i.e., 8-13 Hz) recorded over the central scalp location, which shows a decreased power during action execution and action observation (Oberman et al., 2005; Pineda, 2005) and during the observation of EBL (Schubring & Schupp, 2019; Siqi-Liu et al., 2018). It has been hypothesized that mu desynchronization reflect the activity of the mirror neuron system, hence reflecting not only phenomena of motor resonance and sensorimotor simulation but also of internal representation of others' emotional state (Moody et al., 2007, 2018; Siqi-Liu et al., 2018). Similarly, an influence on brain oscillation has also been showed as a decreased fronto-central desynchronized activity on the lower beta band (16-20 Hz) produced by negative EBL (Siqi-Liu et al., 2018).

6.3 Aim of study

The results retrievable in literature for brain oscillations modulations during processing of EBL mainly focused on longer latencies (Schubring & Schupp, 2019; Siqi-Liu et al., 2018) compared to the ones in which fearful EBL showed modulatory effects on the sensorimotor system, and, moreover, the studies available did not focus on fearful stimuli. For these reasons, in this study we aimed to explore cortical oscillations in the μ-alpha and β frequency bands in the first 200 ms after stimulus onset in order to understand whether the somatosensory inhibition over M1 retrieved in our study 'Sensorimotor inhibition during emotional processing' (Botta et al., 2022) induced by fearful EBL shows a modulation on cerebral rhythms linked to the sensorimotor system.

Furthermore, by investigating the event-related potential component P100 over fronto-central cortical areas, we also aimed to understand if the mere observation of fearful EBL shows reduced activity on motor areas when compared to neutral EBL, thus giving a more depth insight on sensorimotor inhibition induced by emotional manipulation.

6.4 Experimental procedure

6.4.1 Participants

Seventeen healthy, right-handed, individuals (9 females, mean age ± SD: 22.9 ± 3.3 years) were enrolled in the study. All participants were in good health, without any nervous, muscular, orthopedic, or cognitive disorders. Right-handedness was assessed by the Edinburgh Handedness inventory (Oldfield, 1971). The experimental protocol was approved by the ethics committee of the University of Genoa and was performed in agreement with legal requirements and international norms stated in the adjourned declaration of Helsinki (World Medical Association, 2001).

6.4.2 Experimental design and procedure

All participants were asked to sit on an armless chair and to passively observe the 15.6 inches screen located at a distance of 1 meter in front of them. After a brief explanation of the experiment, the 128-channel hdEEG montage was completed. The experiment was composed of three trials of 100 randomized

fearful/neutral visual stimuli, for a total of 300 EBL stimuli (150 fearful and 150 non-emotional). Each visual stimulus had a duration of 500 ms with an ISI of 1500 ms where a black cross on a white blank screen was shown to the participants. The duration of the whole experiment was approximately 80 minutes.

6.4.3 Visual stimuli

Visual stimuli were presented on a 15.6-inches computer screen (resolution: 1920x1200, refresh rate: 60.0Hz) located at 60 cm away from the participants.

A total of 60, randomized, visual stimuli were used (30 fearful and 30 neutral). Half of the stimuli were the original pictures from both databases and the other half were mirror-reflected copies. EBL emotional pictures were selected from a validated EBL database (Borgomaneri et al., 2012; Botta et al., 2021). EBL pictures depict four actors in different postures with negative and neutral valence, fifteen depicting fearful EBL and fifteen with no emotional significance. The actors were not handling objects and their face was blanked out. In order to reach a congruous number of trials, all pictures were pseudorandomized so that the total number of stimuli per trial was sufficient in order to study electrophysiological correlates of emotional processes and weighted in order to maintain the same number of visual stimuli for the two conditions examined (i.e., fear and neutral). Visual stimuli were presented via E-Prime 3.0 (Psychology Software Tools, Pittsburgh, PA, United States).

6.4.4 Data collection

EEG data were recorded via a 128-channel hdEEG data at 1 KHz sampling rate amplified by an ActiCHamp amplifier (Brain Products GmbH, Germany). Electrode impedance was kept below 5 KΩ. Electrooculographic (EOG) signals were recorded in order to monitor for vertical (VEOG) and horizontal (HEOG) eye movement. The EOG recordings were subsequently used for EEG artifact removal.

6.4.5 Data analysis

A validated workflow for hdEEG analysis recorded during task execution and resting state recording was used (Marino et al., 2019; Samogin et al., 2019; Zhao et al., 2019). The aforementioned workflow included different steps such as data preprocessing, head modelling based on MRI template and source-space activity reconstruction for ERP and ERD/ERS analysis.

6.4.6 Data Pre-processing

Data pre-processing implied corrections for bad channels, artifact removal EEG data re-referencing. Bad channels detection was performed via a validated procedure which included a Pearson correlation analysis of each channel against the others in a fixed frequency band ranging from 1 to 50 Hz and a 200-250 Hz frequency band noise variance (Liu et al., 2017; Zhao et al., 2019). Bad channels were then reconstructed starting from neighboring channels (Oostenveld et al.,

2011). EEG signals were then band-passed (1-80 Hz) and artefact removal was performed via a fast independent component analysis (ICA) (Zhao et al., 2019). Lastly, averaged re-referencing was run on EEG data (Liu et al., 2015).

6.4.7 ERP Analysis

EEG data for ERP analysis were band-pass filtered (1-40 Hz). Recorded data were segmented into epochs of 500ms, starting 200ms before the presentation of the visual stimulus until 300ms after stimulus onset. The time window from -200ms to 0ms served as baseline. Epochs with relevant artefactual activity derived from ocular movements or environmental noise were excluded from the analysis. Fronto-central P100 EEG component was studied. Fronto-central P100 was defined as the maximal negative deflection on channels C3, C4, Fc3 and Fc4 in a time window from 80 to 150 ms (Markus Kiefer et al., 2011; Sim et al., 2015). The amplitudes for the P100 component were quantified as the mean amplitudes in a time range going from 80 to 150 ms after stimulus presentation.

Statistical analysis on ERP data was performed via a two-way analysis of variance (ANOVA) with EMOTION (Fear and Neutral) and CHANNEL (C3, C4, Fc3, Fc4) as within factors. Before running the ANOVA, all data were checked for normality (Shapiro–Wilk test) and sphericity (Mauchly test). Greenhouse-Geisser's correction was applied whenever needed. Post-hoc analysis was performed by means of Bonferroni correction method for multiple comparison. Statistical analysis on ERP data was run via SPSS Statistics 23.0 (IBM, Somers, United States). P-values of 0.05 were considered as threshold for statistical significance.

6.4.8 Source-space activity reconstruction

Source localization of EEG signals was based on MRI templates taken from previous studies (for more details see Liu et al., 2017). Brain activity related to source space was reconstructed via the exact low-resolution brain electromagnetic tomography (eLORETA) method (Cao et al., 2018; Zhao et al., 2019). Considering previous studies (Botta et al., 2022; Sim et al., 2015) and the main research question we aimed to explore, the ROIs taken for the time-frequency analysis were the left and right primary motor cortex (lM1 and rM1, respectively) and the left and right primary somatosensory cortex (lS1 and rS1). Coordinates for each ROI were taken from the Neurosynth website (https://neurosynth.org/) on the basis of the paper of Mayka and colleagues for the motor area (Mayka et al., 2006) and the one from Roux and colleagues for the somatosensory area (Roux et al., 2018). Coordinates for each area can be found in the legend of figure 5. MNI coordinates of each ROI were then transformed to individual space and the voxels within 6mm from the ROI were selected (Zhao et al., 2019).

6.4.9 ERD/ERS Analysis

Frequency-dependent modulations of brain regions were assessed by conducting an ERD/ERS analysis on reconstructed neural signals. We first performed an ERD/ERS analysis for selected ROIs, and we then calculated ERD/ERS spatial maps. Time frequency analysis was performed both at a whole-brain level and for the four ROIs (see section above) separately, by means of short-time Fourier transform, with a moving window of 1 second.

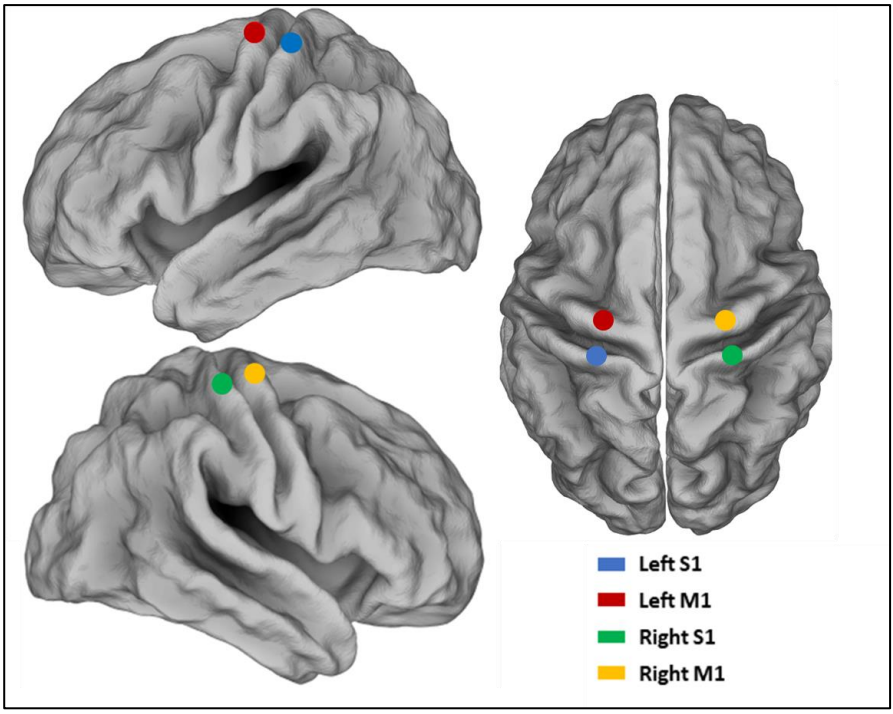

Figure 5. Regions of interest for the ERD/ERS analysis. Left M1: left primary motor cortex (MNI coordinates: [-37; -21; 58]); left S1: left somatosensory (MNI coordinates [-39; -27;60]); right M1: right primary motor cortex (MNI coordinates: [37; -21; 58]); right S1: right somatosensory cortex (MNI coordinates [[39; -27;60]). Coordinates are shown as [x; y; z].

The bands taken into account for the analysis were the μ-alpha band (8–13 Hz) (Debnath et al., 2019) and the lower β-band (13–20 Hz) (Siqi-Liu et al., 2018). The timeframes taken into account for the ERD/ERS analysis were from -500s to +1000ms from the stimulus onset. Activity in the firsts 500 milliseconds before the stimulus presentation was taken as baseline. More in details, a spectrogram in the aforesaid timeframe [-500ms, +1000ms] centered on the emotional stimulus presentation was computed for all the frequencies ranging

from 8-20Hz at steps of 1Hz and a time resolution of 10ms. The ERD/ERS intensity was then calculated via the following formula:

$$ERD/ERS(f,t) = \frac{P(f,t) - P_B(f)}{P_B(f)} \times 100\%$$

where *P(f, t)* is the power as a function of a given frequency and time and $P_B(f)$ is the average power in the [-500ms, 0ms] time window (i.e., baseline) (Zhao et al., 2019). Subsequently, we restricted the observation on the firsts 300ms after stimulus onset.

The same procedure was then used in order to perform a time-frequency analysis on all voxels included in the source space so that it was possible to obtain a spatial map of the time-frequency activity all over the brain by applying a non-rigid deformation using MRI templates (Zhao et al., 2019). Specifically, we reconstructed two spatial maps where we focused our attention on the differences of whole-brain activity in the μ-alpha and in the β bands between the 'Fear' and the 'Neutral' conditions at 200ms after stimulus onset.

To establish differences in ERD/ERS whole-brain activity between the 'Fear' and 'Neutral' conditions, we performed a one sample two-tailed t-test. We analyzed all voxels in the source space for the 'Fear' and the 'Neutral' conditions in the [0ms, 200ms] timeframe and computed the t-maps of the differences between the two experimental conditions separately for the frequency bands of interest.

Group-level analyses were performed on the ERD/ERS spatial maps by using a random-effect analysis. Specifically, a t-test across participants was

calculated for each of the two conditions in each frequency band of interest. Finally, a spatial map showing the significant differences between ERD/ERS activity between conditions was computed between conditions so to clarify which condition showed the highest activity in the ROIs.

Correction for multiple comparisons was performed via the false discovery rate (FDR) method for all analyses (Benjamini & Hochberg, 1995). The significance level for the t-test was set at $p < 0.05$ and so was the p-value after correction for multiple comparisons. All analyses were conducted with MATLAB (R2018a, Math-Works, Natick, MA, USA).

6.5 Results

6.5.1 ERP Analysis

Analysis on ERP data showed that all data were normally distributed and respected the sphericity assumption.

Statistical analysis on fronto-central ERP latencies showed no significant effects for EMOTION, CHANNEL and for the interaction effect EMOTION*CHANNEL (all $F < 1$ and all $p > 0.05$).

Statistical analysis on ERP amplitudes showed significant effects for EMOTION ($F(1,16) = 7.568$, $p = 0.01$, $p\eta^2 = 0.321$) and CHANNEL ($F(3,48) = 7.764$, $p < 0.01$, $p\eta^2 = 0.327$), while no significant effect was found in the interaction EMOTION*CHANNEL ($F < 1$; $p > 0.05$). Post-hoc analysis on EMOTION showed higher amplitudes recorded for neutral stimuli compared to

fearful on fronto-central channels (p = 0.01). Post-hoc analysis on CHANNEL showed an overall significant reduced amplitude recorded on channel C3 when compared to C4 (p < 0.01) and on channel C4 when compared to Fc3 (p < 0.01) and Fc4 (p < 0.01). ERP analysis results might be observed in figure 6.

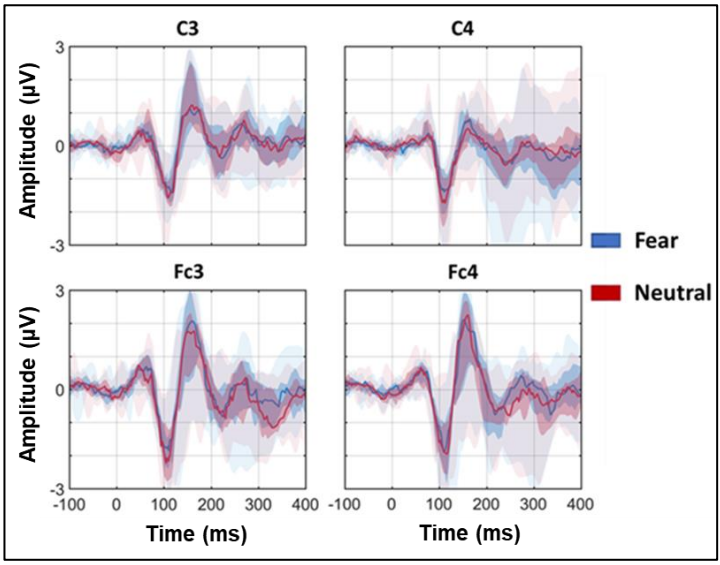

Figure 6: Fronto-central P100 over channels C3, C4, Fc3, Fc4. The picture shows the mean amplitudes recorded over fronto-central channels in the two experimental conditions 'Fear' (blue) and 'Neutral' (red). The more marked shaded areas represent the standard deviation, while the less marked shaded areas show the variability across participants.

6.5.2 ERD/ERS Analysis

Differences in whole-brain activity

This analysis was run in order to identify significant differences retrieved in whole brain activity. The differences observed in figure 7 do not show the

direction of the differences in terms of desynchronization/synchronization, which were subsequently analyzed in the ERD/ERS spatial maps (see section ROIs ERD/ERS maps).

Differences in whole-brain activity between 'Fear' and 'Neutral' conditions were analyzed in the timeframe [0ms, 200ms] after stimulus onset, in order to observe early brain activity (see fig. 7). All maps are corrected for multiple comparisons. The MNI coordinates of the cerebral areas showing significant differences in activity between conditions can be found in table 1. Coordinates were identified via the AICHA atlas (Joliot et al., 2015) included in the MRIcroGL software (Rorden & Brett, 2000).

Area	Frequency band	Side	X	Y	Z
Inferior occipital gyrus	μ-alpha	L	-40	-90	-12
Fusiform Gyrus	μ-alpha	L	-29	-76	-18
		R	35	-76	-18
Superior parietal lobule	μ-alpha	R	41	-37	50
Primary motor cortex (Hand)	β	L	-32	-23	64
Primary motor cortex (Leg)	β	L	-6	28	79
		R	5	-28	79
Primary somatosensory cortex (Hand)	β	L	-30	-32	64
Anterior prefrontal cortex	β	L	-33	61	2
Superior parietal Lobule	β	L	-16	-80	48
Insula	β	R	44	-8	48
Precuneus	β	L	-5	-78	46

Table 1: Brain areas showing significant activation differences between 'Fear' and 'Neutral'. The table shows all MNI coordinates of the brain areas whose differences in activity in the two investigated frequency bands resulted significant. MNI coordinated where extrapolated via the AICHA atlas included in the MRIcroGL software (Rorden & Brett, 2000).

- In the μ-alpha band, significant differences between 'Fear' and 'Neutral were found at the level of the left occipital cortex, the fusiform gyrus

(bilaterally) and in the right superior parietal lobule. No significant differences in activity between conditions were retrieved in the sensorimotor areas, specifically in the four ROIs object of this study (i.e., lM1, lS1, rM1, rS1).

- Regarding the β band, significant differences between conditions were found in the left PPC and in the precuneus, as well as in the left prefrontal cortex. Most importantly, significant differences between 'Fear' and 'Neutral' EBL were found in the selected ROIs. More specifically, differences were found in the lM1 and in the lS1, while on the right hemisphere, significant differences are observable in M1.

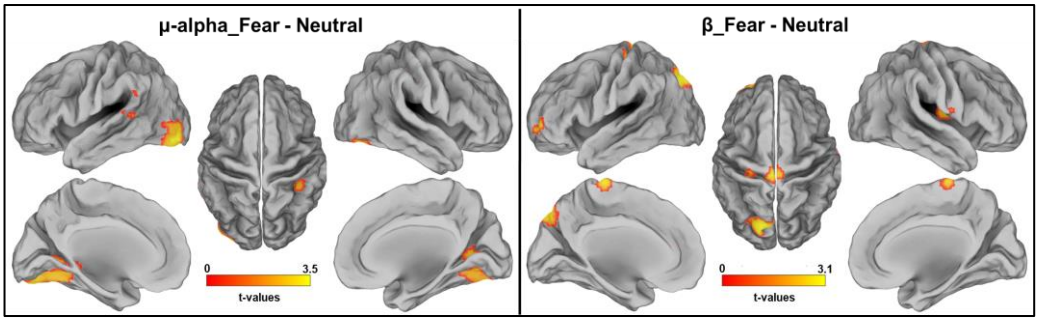

Figure 7: T-maps for whole-brain activity differences between 'Fear' and 'Neutral'. Brain maps depict the main differences retrieved in the μ-alpha and in the β frequency bands between the two experimental conditions. All highlighted areas represent significant differences in activity corrected for multiple comparison. The detailed MNI coordinates of the observable areas are retrievable in table 1. Maps were elaborated via the eLORETA method for source-space activity reconstruction (Zhao et al., 2019).

ROIs ERD/ERS maps

Time frequency analysis (ERD/ERS) was performed in the following ROIs selected *a priori*: left and right primary motor cortex (lM1 and rM1, respectively) and the left and right primary somatosensory cortex (lS1 and rS1).

Results of the group-analysis on the ERD/ERS spatial maps for ROIs might be observed in figure 8. Generally, as it is possible to observe in figure 8 (panels in rows A and B), there is a significant desynchronized activity in all ROIs for all experimental conditions. There is a consistent μ-alpha activity which generally arises around 50ms after stimulus onset, independently from the characteristics of the stimulus, which is prolonged all over the timeframe considered in the analysis. Lower β band, on the other hand, similarly shows an early activity onset (~50ms after stimulus onset) and a comparable duration, but in left and right M1 it does show desynchronized activity only in the 'Neutral' and not in the 'Fear' condition.

No significant differences were retrieved in the μ-alpha in all conditions (see figure 8, row C below the dashed line). On the other hand, for the β band the spatial maps 'Fear-Neutral' depicting the differences between conditions show a significant higher desynchronization for 'Neutral' compared to 'Fear' in the left M1 (fig. 8, row C, first map - dotted area above the dashed line), while the opposite trend might be observed in the left S1 where an higher desynchronization is retrieved for 'Fear' (fig. 8, row C, second map - dotted area above the dashed line). No significant differences resulted on the right hemisphere in the ROIs taken into consideration for this study in all frequency bands, but the same trend

is observable in the comparison between M1 and S1 (i.e., higher activation for ‘Neutral’ in rM1 and higher activation for ‘Fear’ in rS1).

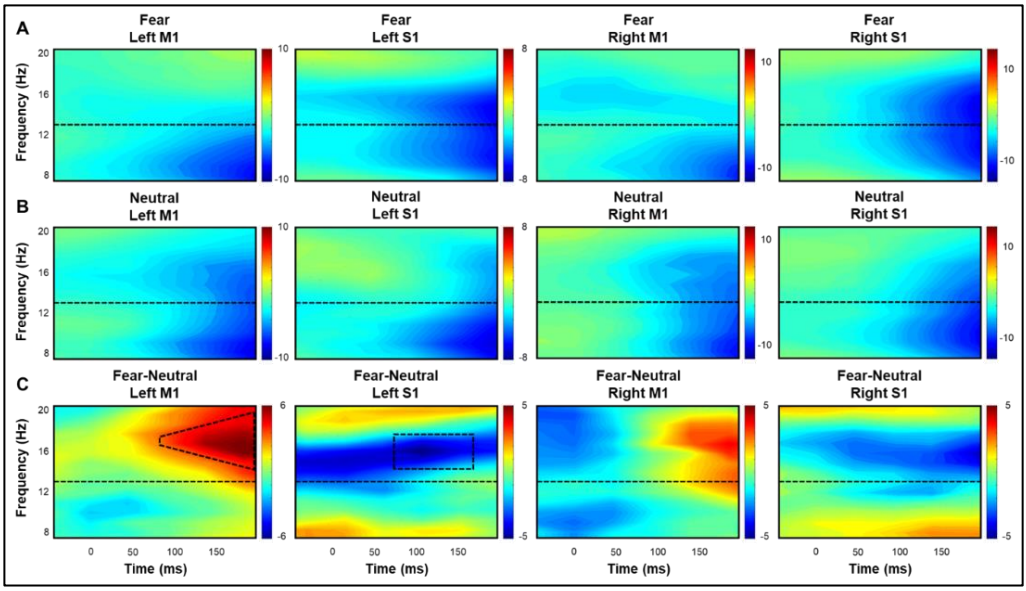

Figure 8: ROIs maps for ERD/ERS analysis. Rows A and B show the ERD/ERS maps of the experimental conditions (‘Fear’ and ‘Neutral’ respectively), while row C shows the map for the difference between conditions. The dashed line separates the μ-alpha band (below the dashed line) from the β band (above the dashed line). Significant differences are enclosed in the dotted lines observable in the first two maps from the left in row C.

6.6 Discussion

The aim of this study was to explore whether processing of EBL was able to modulate cortical activity in sensorimotor areas, hence ERPs and cortical oscillations in the μ-alpha and β frequency bands, at short latencies (around 100

ms after the onset of the EBL stimulus). This study was driven by our previous findings that sensorimotor integration, as tested in M1 with short-latency afferent inhibition (SAI) protocol, is modulated during fear-related EBL stimuli processing already at 120 ms from the stimulus onset (Botta et al., 2022). We hypothesized that modulation of sensorimotor integration was driven by increased activity in the primary sensory cortex, exerting an inhibitory effect on primary motor cortex excitability. By using high-density electroencephalography (hdEEG) coupled to source activity reconstruction we had the chance to obtain information on spatial distribution and temporal dynamics of neural oscillations during EBL processing at early latencies.

The main findings of our study are the following: (i) ERP analysis showed a lower P100 amplitude in motor and premotor channels for 'Fear' EBL stimuli compared to 'Neutral'; (ii) source-space reconstruction showed significant differences between 'Fear' and 'Neutral' EBL processing mainly retrievable in sensorimotor areas in the β band; (iii) ERD/ERS analysis showed decreased desynchronization in the β band in M1 for 'Fear' EBL with respect to 'Neutral' EBL stimuli; (iv) ERD/ERS analysis showed increased desynchronization in the β band in S1 for 'Fear' EBL with respect to 'Neutral' EBL stimuli.

In studying EBL, it is impossible to separate the emotional and the motor information expressed by the picture's subjects. By contrasting neural activity during 'Neutral' and 'Fear' EBL processing, we aimed to assess whether the emotional content was able to modulate sensorimotor areas or if the neural response was mainly driven by phenomena such as motor mimicry and/or motor resonance independently from emotions. Related to ERP analysis, our results

showed an overall lower amplitude in fronto-central channels (i.e., C3, C4, Fc3, Fc4) for fearful EBL compared to neutral EBL and no differences in terms of latencies. First, our study revealed by means of ERP source analyses the presence of early activity (around 100 ms) in fronto-central channels during EBL processing. This result is consistent with what observed by van Heijnsbergen et al. who, while studying the vertex positive potential (i.e., an ERP component retrievable in fronto-central areas of the brain at ~170 ms from stimulus onset) recorded a negative deflection around the firsts 100 ms from stimulus onset that was not further investigated or explained because considered to be not relevant for the aim of their study (van Heijnsbergen et al., 2007). Furthermore, in previous studies, ERP source analyses revealed early activity (120–150 ms) in pre- and post-central cortex, typically associated with action processing (Hauk et al., 2004; Hoenig et al., 2008; Marcus Kiefer, 2001; Markus Kiefer, 2005). Going further, Sim et al demonstrated that that early activity in central areas at about 120 ms from the onset of an affordable object image was modulated by the content (congruent vs incongruent) of a priming movie showing hands performing an action with the object being erased (Sim et al., 2015). The action-priming effect was significant over the fronto-central scalp: ERPs were more negative in the congruent than in the incongruent condition (Sim et al., 2015).

Our results also showed a modulation of fronto-central P100 activity, but in relation to the emotional content of EBL; P100 amplitude was smaller in motor and premotor channels for 'Fear' compared to 'Neutral' EBL stimuli. Considering the fact that the fronto-central P100 component has been shown to be mainly linked to action-processing processes and that the amount of motor information depicted in the used stimuli was comparable (Borgomaneri et al., 2012;

Borgomaneri, Vitale, Gazzola, et al., 2015), it is plausible to infer that 'Fear' EBL processing exerted an effect in terms of cortical modulation, resulting in a reduced activity in motor and premotor areas in comparison to the one evoked by non-emotional stimuli.

Taking advantage of hdEEG that provides us the possibility to gain information on the sources of the neural oscillations with an optimal temporal resolution and an improved spatial resolution with respect to standard EEG (Michel & Murray, 2012), we conducted a source-space reconstruction analysis to localize, at early latencies, the neural areas with significant differences in activity in μ-alpha and β bands between 'Neutral' and 'Fear' EBL. To this end, we used a custom developed pipeline for performing source localization from hdEEG data. This pipeline is able to detect multiple brain networks that are spatially similar to those obtained from fMRI data (Liu et al., 2017; Zhao et al., 2019). Source-space reconstruction analysis on whole brain activity showed that at early latencies significant differences were detectable between 'Fear' and 'Neutral' EBL processing in sensorimotor areas only in the β band. Indeed, in the μ-alpha band, significant differences between 'Fear' and 'Neutral were detectable mainly in the posterior cortex implicated in visual processing and embodiment (de Echegaray & Moratti, 2021) and particularly at the level of the left occipital cortex, the fusiform gyrus, the inferior temporal gyrus and the right posterior parietal lobe.

Going further, ERS/ERD analysis over fronto-central ROIs showed an overall desynchronization in μ-alpha and β bands in the four ROIs, consistent with what reported in the literature as neurophysiological marker of motor mimicry

during action processing (Hobson & Bishop, 2016; Schubring & Schupp, 2019; Siqi-Liu et al., 2018). Moreover, by computing the differences in frequency-bands activity between the fearful and the neutral EBL processing, we found a significant increased activation for neutral EBL in M1, while the opposite was found for fearful EBL, which showed a significant higher activity in S1, even though these differences resulted only for the lower β frequency band.

Borgomaneri et al. already showed a decreased activity in M1 during EBL processing in different studies by using transcranial magnetic stimulation. These authors showed that the observation of fearful EBL was associated with a transient reduced corticospinal excitability at short latency (from 70 to 90 ms after stimulus onset); of notice is the fact that this modulation was specific for fear, while no differences were found for joy or non-emotional body expressions (Borgomaneri et al., 2020; Borgomaneri, Vitale, & Avenanti, 2015). Furthermore, the influence of aversive EBL was also shown to modulate intracortical networks involved in facilitatory mechanisms of M1 such as the intracortical facilitation (ICF) network. In that case, Borgomaneri and her colleagues, observed a reduction in ICF in the firsts 120 ms during the observation of fearful EBL that they interpreted as a transient reduction in motor readiness and, hence, a 'freezing' of M1 (Borgomaneri, Vitale, Gazzola, et al., 2015).

Increased activity in S1 during fearful EBL processing may be explained considering the importance of the amygdala, the pivotal subcortical structure involved in Fear processing, in attention and vigilance. As shown by different experimental paradigms in animals (for a review see: Davis & Whalen, 2001; Whalen, 1998), the amygdala may be especially involved in increasing vigilance

by lowering neuronal threshold of widespread sensory cortical areas through the modulation of the release of acetylcholine from the basal forebrain (Bucci et al., 1998; Chiba et al., 1995). In addition, activation of cholinergic, dopaminergic, serotonergic, and noradrenergic neurons in the brainstem may have widespread influences on thalamic and subthalamic sensory as well as motor transmission. Furthermore, this mechanism is made stronger by the ambiguity of the stimulus proposed. It has been proposed that increased sensory vigilance is stronger during fearful faces processing with respect to angry faces because if both provide information about the presence of threat, the first gives less information about the source of that threat (Whalen, 1998). The same can be hypothesized for EBL processing. Indeed, as for facial expressions, processing of EBL activates brain regions involved in perceptual and affective processes such as the superior temporal sulcus, fusiform and postcentral gyrus, the amygdala, and medial prefrontal cortex (De Gelder, 2006, 2009; Downing & Kanwisher, 2001; Peelen et al., 2010; Peelen & Downing, 2005; Ross et al., 2020).

Increased activity in S1, by means of augmented release of acetylcholine from basal forebrain fits very well also with our recent finding on increased SAI at 120 ms after stimulus onset, observed specifically for fearful EBL (Botta et al., 2022). Although it could be argued that the mechanisms underpinning SAI are still not entirely understood, the activity exerted by the pyramidal neurons in the somatosensory cortex engaged by the processing of afferent peripheral stimuli are likely to inhibit motor output by increasing the GABAergic tone in the cortex (for a review see Turco et al., 2018). Furthermore, this inhibitory intracortical network is not mediated only by GABA, but also by cholinergic activity (V. Di Lazzaro et al., 2005a; Vincenzo Di Lazzaro et al., 2005b), Considering this pieces of

evidence, it seems plausible to infer that the results we found for the lower β band might be related to an increased, instinctual, attentional load exerted by fearful EBL over S1, which, by its increased activity, is able to tune down the activity of M1 through the activation of intracortical inhibitory networks.

Anyhow, differences in activity were only retrieved in the lower β band, and not in the μ-alpha band. In fact, no significant differences between conditions were found for the μ-alpha band and this might be related to the fact that this rhythm is mainly linked to action observation and to action execution (Debnath et al., 2019; Hobson & Bishop, 2016; Pineda, 2005). Body expression pictures inevitably contain motor information, being them embedded with emotional or non-emotional contents. Apparently, our results are going on the opposite direction of the (few) evidence available where it has been shown that the emotional content of EBL is associated with a higher μ-alpha suppression (i.e., a reduced power in the μ-alpha band indicating stronger desynchronization in the central brain areas) when compared to non-emotional body expressions (Schubring & Schupp, 2019; Siqi-Liu et al., 2018). This apparent contradiction might be explained by the time window used in our experiment for ERD/ERS activity observation. The μ-alpha suppression associated to non-emotional action observation has been shown to appear more or less at 600 ms after stimulus onset (Babiloni et al., 2002; Hobson & Bishop, 2016), while latencies of ERD response to EBL was found between 1 and 2 seconds after stimulus onset for point-light display stimuli (Siqi-Liu et al., 2018) and between 700 and 1000 ms in the case of erotic pictures (Schubring & Schupp, 2019). As it may be noted, although evidence shows that EBL has an early processing (van Heijnsbergen et al., 2007) and is able to modulate intracortical networks at short latency (Borgomaneri et

al., 2020; Borgomaneri, Vitale, Gazzola, et al., 2015; Botta et al., 2022), to our knowledge no studies have ever focused their attention on early modulations of cortical oscillations during processing of emotional body expressions. Moreover, no ERD/ERS studies investigating fearful EBL were performed since now, but only studies involving happiness, anger and/or sadness (Schubring & Schupp, 2019; Siqi-Liu et al., 2018). Our results might indicate that the μ-alpha suppression at short latencies is not sensitive for emotional content but only to motor information carried out by EBL stimuli, hence resulting in a lack of differences in ERD activity between emotional and non-emotional conditions that appears only at longer latencies. Anyway, in order to properly address this issue, further study will be necessary to explore the temporal dynamics of the μ-alpha band modulation during emotional processing, comparing also other emotions in order to understand whether the modulation of this rhythm is influenced by emotional valence.

In conclusion, results of the present study show evidence of an early modulation of central cortical activity and cortical oscillation in the frequency range of the lower β rhythm, indicating a higher activity in the somatosensory areas which in turn is responsible for a decreased activation of the motor cortex when observing fearful EBL. On the other hand, no differences were retrieved in the sensorimotor areas at short latency for the μ-alpha band, possibly showing that early suppression is not sensitive to emotions but only to motion. These results support the idea that fearful EBL is rapidly processed at a subcortical level and that this elaboration shows an early modulatory effect on the sensorimotor system, probably related to an augmented sensory arousal in presence of potential threats.

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7. Experiment 4: Modulation of response times in Parkinson's disease during emotional processing

7.1 Rationale

Patients with Parkinson's disease (PD) frequently experience non-motor symptoms, including emotional-processing impairments. Difficulty in recognizing emotions from faces has been reported in PD, even if results were controversial (for a review see Argaud et al., 2018). Data on emotion discrimination, rather than emotion recognition, are also reported in literature. Indeed, emotion processing involves multiple stages, which can be measured by different tasks: sensitivity in discriminating between emotions depends on sensory and visuo-spatial processes and it is assessed by forced-choice tasks, whereas recognition further requires to identify the correct emotion label among several alternatives as assessed by identification tasks (Haxby et al., 2000).

Recently, emotion discrimination was evaluated in a large sample of people with PD (Mattavelli et al., 2021), using a backward masking paradigm with different presentation durations and Ekman's facial expressions as set of stimuli. This paradigm allows assessing sensitivity for stimuli processed with full awareness, but also for stimuli automatically processed, i.e., presented below the threshold for conscious perception (Pessoa et al., 2005). Crucially, the authors did not find significant differences between PD and control groups in the emotion discrimination task. This result is consistent with a neurophysiological study showing, via EEG recording, no diminished early activity in posterior

occipitotemporal regions, in patients with PD, during facial expression recognition (Wieser et al., 2006).

The investigation of how humans perceive and respond to emotional signals conveyed by body expressions has been for a long time secondary compared with research addressing the recognition of emotional faces or emotional scenes (Beatrice de Gelder et al., 2010; Beatrice De Gelder, 2009). Only in the last decades, an increased interest in whole-body expressions and their emotional correlates has started to emerge, showing that EBL is able to convey as much information as facial expressions (B. de Gelder et al., 2015; Beatrice de Gelder et al., 2010; van de Riet et al., 2009) and activates similar cortical and subcortical structures in comparable time windows (Meeren et al., 2005, 2013; Peelen et al., 2010; Ross & Atkinson, 2020; van Heijnsbergen et al., 2007). Additionally, EBL strongly activate subcortical motor areas such as the caudate nucleus and the putamen and several regions of the cortical motor system, with stronger (Borgomaneri et al., 2017; Beatrice de Gelder et al., 2010; Beatrice De Gelder et al., 2004) and faster (Borgomaneri, Vitale, & Avenanti, 2015; Borgomaneri, Vitale, Gazzola, et al., 2015) response to threatening expressions. Such motor activations may reflect sensorimotor simulation and/or the activation of motivational tendencies which facilitate emotionally congruent behavior, with positive stimuli activating the approach tendencies and negative stimuli activating the avoidance tendencies (Ekman & Davidson, 1995; Lang et al., 1990; Lang & Bradley, 2010).

Recently, we showed significantly lower RTs for pictures depicting fearful body postures when compared with happy or neutral postures, suggesting

a faster processing of fearful body language in young healthy subjects and an effect of aversive EBL on motor readiness (Botta et al., 2021). EBL has the advantage of conveying not only emotional information, but also motor information from limbs and axial muscles, hence implicating mechanisms of motor mimicry and embodiment (Ross & Atkinson, 2020). Evidence on imitative mechanisms of PD patients are nowadays inconclusive, showing contrasting results indicating on one hand preserved motor mimicry mechanisms (as an example see Bek et al., 2018) and on the other deficits in engaging the motor system during action observation (Tremblay et al., 2008). Moreover, also the processing of EBL shows contradictory evidence in PD patients, especially in terms of emotional valence evaluation: in fact, if on one side the evaluation of EBL showed a reduced perceived valence of positive emotions and a higher scoring for the negative ones in patients compared to healthy controls (Bellot et al., 2021), on the other it appears that PD patients are more able to correctly identify the emotional content of body expressions compared to age-matched controls (Eriksson et al., 2022).

7.2 Aim of study

Based on this piece of evidence, emotional processing in PD appears to be a controversial topic, being it referred to facial expressions or to EBL. For this reason, the first aim of the present study was to investigate if there was a specific modulation of motor response during processing of EBL by assessing RTs in a two-alternative forced choice task using the set of visual stimuli adopted by

Borgomaneri and colleagues (Borgomaneri et al., 2012). Of notice is the fact that, by using RTs, we wanted to observe not only the ability of PD patients in discriminating emotions in whole body expressions, but also how EBL, and emotional language more in general, influences motor readiness and behavior.

Furthermore, in trying to understand whether emotional discrimination and motor response is influenced by the type of information depicted in the stimuli, we compared RTs recorded in the EBL task with those obtained from the observation of two other set of stimuli: the first was the set of facial expressions from the Facial Action Coding System (FACS) by Ekman and Rosenberg (Ekman & Rosenberg, 2005), while the second was taken from the International Affective Picture System (IAPS) database (Lang Bradley, M.M., & Cuthbert, B.N., 2008). By doing so, we aimed to understand whether the type of information depicted in the picture (i.e., bodies, faces, or scenes) and the amount of motor information included in emotionally enriched visual stimuli might influence the emotion discrimination task or the motor performance.

7.3 Experimental Procedure

7.3.1 Participants

Twenty-five patients with PD (11 female, mean age: 65.31 ± 8.44; H&Y: 1.81 ± 0.52) and 25 age matched healthy participants (HC; 10 females, mean age: 63.78 ± 5.61) were enrolled in the experiment. All participants reported to be right-handed. PD patients were enrolled from the Center for Parkinson's Disease

of the IRCCS San Martino of Genoa (Italy). All PD patients had to meet the following inclusion criteria: i) diagnosis of idiopathic PD (according to the United Kingdom Parkinson's Disease Society Brain Bank criteria), ii) Hoehn and Yahr stage < 2.5 and iii) Mini Mental State Examination (MMSE) score > 24. Patients were excluded from the study in presence of i) history of other neurological disorders (except PD) and (ii) visual or orthopedic impairments of the upper limbs that could hamper task performance. Disease severity was assessed with the MDS- Unified Parkinson Disease Rating Scale, part III (MDS-UPDRS III) and cognitive functions were evaluated via the PD-Cognitive Rating Scale (PD-CRS). The affective status of patients was assessed via the Hamilton scales for depression (HAM-D) and anxiety (HAM-A). All patients were under dopaminergic therapy, and they were asked to perform the experiment in a time window of one-to-two hours after antiparkinsonian therapy intake. All participants gave answered to a digital informed consent. The experimental protocol was approved by the ethics committee of the University of Genoa and was carried out in agreement with the declaration of Helsinki for experiments involving human participants (World Medical Association, 2001).

7.3.2 Visual Stimuli

Emotional pictures were taken from three different databases: EBL from the experiments of Borgomaneri and colleagues (Borgomaneri et al., 2012), emotional scenes from the IAPS database (Lang Bradley, M.M., & Cuthbert, B.N., 2008) and facial expressions from the Ekman's Facial Action Coding System (FACS) test (Ekman & Rosenberg, 2005).

A total of 90 slides were used in the experimental sessions, divided as follow: 30 for EBL, 30 for IAPS and 30 for FACS. Each slide was structures to include two images, one emotional (fearful or happy) and one non-emotional, and of the 30 slides used in the experiments 15 were depicting fear and 15 were depicting happiness. Emotional pictures were randomly allocated on the left or on the right of the slide, as for the order of slide presentation.

7.3.2 Tasks and experimental procedure

The experiment implied three home-based sessions, one for each of the set of pictures, where all participants had to download the task from the E-Prime Go online platform (Psychology Software Tools, Pittsburgh, PA, United States) and perform it in a comfortable and silent location on their personal computer. The experiment was programmed on E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA, United States).

In each session the slide appeared on the screen for 2000 ms, with a fixation cross in between visual stimuli lasting for 1500 ms. The order of the three sessions was randomized. Participants were asked to complete a two-alternative forced choice discrimination task in which they had to press as fast as possible the key (left arrow key or right arrow key) corresponding to the emotional (i.e., fearful or happy) visual stimulus respect to the non-emotional (i.e. neutral), in order to estimate response times (RTs). RTs were taken as the difference in milliseconds between the onset of the visual stimulus and the pressing of the key on the keyboard.

After the completion of the three experimental sessions, an online questionnaire evaluating the valence and the arousal of the visual stimuli was submitted to all participants. Valence and arousal were evaluated on a Liker scale ranging from 1 to 9, where 1 stood for 'absolutely unpleasant' and 9 corresponded to 'absolutely pleasant for valence, while 1 corresponded to 'no arousal' and 9 stood for 'high arousal' in the scale for arousal evaluation.

7.3.3 Data Analysis

Response time data

Analysis on RT data was performed via a repeated measures analysis of variance (RM ANOVA) with GROUP (PD and HC) as between factor and PICTURE (EBL, IAPS and FACS) as within subjects. All analysis were performed separately for 'Fear' and 'Happiness' visual stimuli. RTs were considered as outliers if they fell outside the interval of two standard deviations from their respective means.

Correlation analysis

We also investigated whether RTs in the discrimination task was influenced by dopaminergic therapy (Levodopa Equivalent Daily Dose – LEDD), disease duration (years), disease severity (H&Y stage and MDS-UPDRS III), cortical and subcortical dysfunction (PD-CRS subcortical and cortical sub-scores), anxiety (HAM-A) and depression (HAM-D). Non-parametric correlation analysis was run via the Spearman R method.

Valence and arousal

Data on valence and arousal were analyzed via a RM ANOVA performed separately for each emotion with GROUP as between-subjects factor and PICTURE as within-subjects main effect. Post hoc analysis was performed via Bonferroni correction of significance.

7.4 Results

7.4.1 Participants

Twenty-two patients with PD and twenty-two HC entered in the analysis. Three patients per group were excluded, since RT data were outliers in one of the experimental conditions. The two groups were matched for age ($p = 0.45$) and gender ($p = 0.65$). Participants' characteristics are reported in Table 1.

7.4.2 Response time data

Related to RTs in the discrimination task, results are shown in Figure 1.

For fearful stimuli, RM ANOVA showed a significant effect of GROUP ($F_{1, 42} = 15.252$; $p < 0.001$), PICTURE ($F_{2, 84} = 35.724$; $p < 0.001$) and a significant PICTURE*EMOTION interaction ($F_{2, 84} = 5.749$; $p = 0.048$). Post hoc analysis of the main factor GROUP showed that PD were generally slower than controls in the RT discrimination task ($p < 0.001$).

Demographic, clinical and neuropsychological characteristics.

	PD	HC	P value
Number of subjects	(11M, 11F)	(12M, 10F)	p = 0.64
	Mean ± SD	**Mean ± SD**	
Age (years)	65.31 ± 8.44	63.78 ± 5.61	p = 0.45
Disease duration (years)	8.29 ± 4.07	-	-
Hohen & Yahr (stage)	1.79 ± 0.47	-	-
LEDD (mg)	541.19 ± 228.10	-	-
UPDRS part III (score)	23.19 ±11.78	-	-
PD-CRS TOT (score)	99.10 ± 25.52	-	-
PD-CRS cortical (score)	31.39 ± 11.20	-	-
PD-CRS cortical (score)	72.43 ± 17.83	-	-
HAM-A	10.00 ± 6.86	-	-
HAM-D	12.95 ± 8.27	-	-

PD, Parkinson's Disease; HC, Healthy Control; UPDRS, motor section of the Movement Disorder Society Unified Parkinson's Disease Rating Scale; LEDD, levodopa equivalent daily dose; PD-CRS, Parkinson's Disease Cognitive Rating Scale; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale.

Table 1: Demographic, clinical and neuropsychological data of all participants.

Post hoc analysis of the main factor PICTURE showed that RTs were longer when the discrimination task included EBL stimuli compared to when it implied FACS ($p < 0.001$) and IAPS ($p < 0.001$), with no difference between FACS and IAPS ($p > 0.05$). However, the post hoc analysis on the interaction effect showed that this RTs were longer for EBL with respect to FACS and IAPS only in HC group (EBL vs FACS, $p < 0.001$; EBL vs IAPS, $p < 0.001$), but not in

PD patients' group. Indeed, in PD patients significant shorter RTs were observed for FACS in the comparison with EBL ($p < 0.001$) and IAPS ($p < 0.001$), but no differences were found between EBL and IAPS ($p > 0.05$).

For happy stimuli, RM ANOVA showed a significant effect of GROUP ($F_{1, 42} = 20.789$; $p < 0.001$) and PICTURE ($F_{2, 84} = 84.681$; $p < 0.001$) and no significant PICTURE*EMOTION interaction ($F_{2, 84} = 0.250$; $p = 0.779$).

Post hoc analysis of the main factor GROUP showed that PD were slower than controls in the RT discrimination task ($p < 0.001$). Post hoc analysis of the main factor PICTURE showed that RTs were longer when discrimination task included EBL respect to FACS ($p < 0.001$) and IAPS ($p < 0.001$).

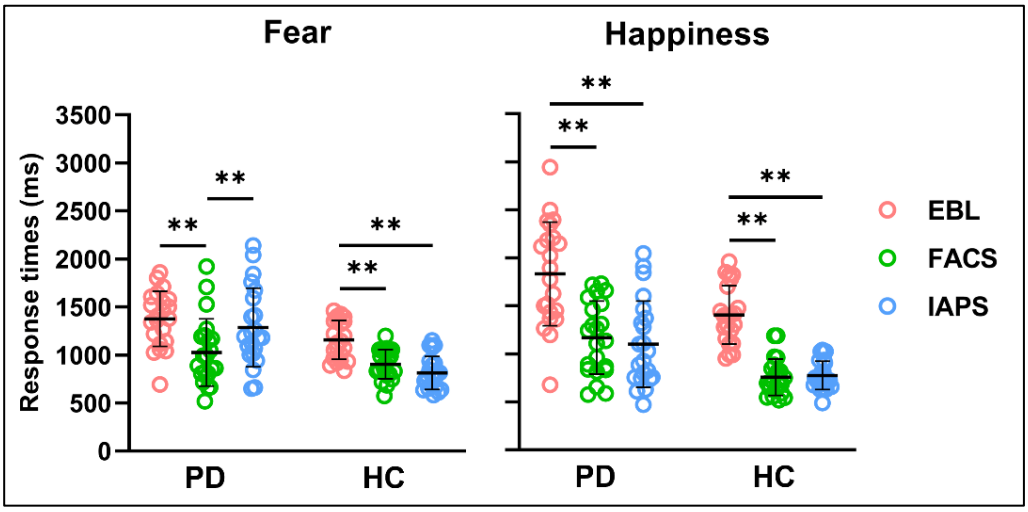

*Figure 5: Response times. The picture shows the mean RTs for both groups (PD and HC) for 'Fear' (upper panel) and 'happiness' (lower panel). The continuous light-grey line indicates significant differences between EBL and FACS, the dotted light-grey line indicates significant differences between FACS and IAPS and the black line highlights differences between EBL and FACS. ** = p < 0.01.*

7.4.3 Correlation analysis

Correlation analysis showed a significant correlation between RTs when discriminating EBL and disease duration (Fear; Rho = 0.45, p = 0.03; Happiness; Rho = 0.56, p = 0.008) and a significant correlation between RTs when discriminating EBL and HY stage (Fear; Rho = 0.40,

p = 0.04; Happiness; Rho = 0.41, p=0.04). RTs when discriminating EBL did not correlate with the other variables considered. No significant correlations were found for RTs when discriminating IAPS and FACS and clinical and demographic characteristics.

7.4.4 Valence and arousal scores

Related to valence and arousal scoring, results are reported in Table 2.

Twenty PD patients and 16 healthy controls regularly completed the online questionnaire.

Statistical analysis for valence in the 'Fear' condition showed a significant effect for PICTURE ($F_{2, 68}$ = 16,695; $p < 0.001$) but not for GROUP and for the interaction effect ($F < 1$; $p > 0.05$). Specifically, valence scores were lower for IAPS stimuli compared to EBL ($p < 0.001$) and FACS ($p < 0.001$), while no differences were found between EBL and FACS. Analysis on arousal for 'Fear' showed a significant effect for GROUP ($F_{2, 36}$ = 8.779; p = 0.006) and PICTURE ($F_{2, 68}$ = 32.252; $p < 0.001$), but not in the interaction effect. Post hoc analysis on GROUP showed lower arousal for PDs compared to HCs, while post hoc analysis

on PICTURE showed higher arousal for IAPS stimuli compared to EBL ($p = 0.001$) and FACS ($p = 0.006$).

Regarding the 'happiness' condition, statistical analysis on valence showed significant results for PICTURE ($F_{2, 68} = 22.225$; $p < 0.001$) but no significant effect was retrieved for GROUP and for the interaction effect ($F < 1$; $p > 0.05$). Post hoc analysis revealed higher valence for IAPS stimuli compared to EBL ($p < 0.001$) and FACS ($p < 0.001$). Arousal data analysis showed a significant effect for GROUP ($F_{2, 68} = 8.240$; $p = 0.007$) and PICTURE ($F_{2, 68} = 11.514$; $p < 0.001$) and no significant interaction effect. Also, in the 'happiness' condition, PD patients scored lower arousal compared to HC. Post hoc analysis on PICTURE showed higher arousal for IAPS pictures respect to EBL ($p < 0.001$) and FACS ($p < 0.001$).

Valence and arousal scores.

		FEAR			HAPPY		
		EBL	**IAPS**	**FACS**	**EBL**	**IAPS**	**FACS**
PD	*Valence*	3.03 ± 1.24	1.72 ± 0.65	2.86 ± 1.03	6.45 ± 1.28	8.03 ± 0.75	6.94 ± 1.23
	Arousal	4.14 ± 2.31	6.04 ± 2.26	4.42 ± 2.22	4.42 ± 2.26	6.99 ± 1.61	5.59 ± 2.15
HC	*Valence*	2.37 ± 0.60	1.89 ± 0.52	2.63 ± 0.94	6.92 ± 1.11	7.86 ± 0.71	7.21 ± 1.02
	Arousal	5.35 ± 1.56	7.18 ± 1.24	6.15 ± 1.69	6.30 ± 1.52	7.39 ± 0.82	6.60 ± 1.02

Table 2: Valence and arousals scores for Emotional Body Language (EBL), emotional scenes (IAPS) and emotional facial expressions (FACS) stimuli, for patients with Parkinson's disease (PD) and healthy controls (HC).

7.5 Discussion

The aim of this study was to explore whether the response time in discriminating fearful and happy stimuli differed among different types of stimuli, characterized by the presence or not of embodiment in patients with PD. Particularly, we focused for the first time on emotional body language (EBL) stimuli, and compared data to another set of embodied stimuli (i.e., FACS and IAPS).

The main results are the following: (i) in general response time in PD were longer than HC for all the three sets of stimuli when discriminating both 'Fear' and 'happiness' stimuli; (ii) in PD, as in HC, when discriminating 'happiness' with respect to 'Neutral' stimuli RTs were longer when discriminating EBL with respect to FACS and IAPS; (iii) in HC also when discriminating 'Fear' respect to 'Neutral' stimuli RTs were longer when discriminating EBL with respect to FACS and IAPS; (iv) in PD, when discriminating 'Fear' respect to 'Neutral' stimuli, RTs were longer when discriminating EBL only with respect to FACS but not with respect to IAPS; (v) in PD, when discriminating 'Fear' respect to 'Neutral' stimuli, RTs were shorter when discriminating FACS with respect to IAPS; (v) RTS in discriminating EBL stimuli correlated with disease duration and HY stage in PD.

Longer response times in PD for all the set of stimuli, for both 'Fear' and 'happiness' stimuli, with respect to control may be ascribed to bradykinesia, one of the cardinal symptoms in PD. Related to the emotional content of the stimuli, PD and HC showed a similar behavior for 'happiness' stimuli with both groups

presenting longer RTs when discriminating EBL with respect to both IAPS and FACS and no difference between the latter two. This result is consistent with that of our previous study, comparing RTs in discriminating EBL with respect to IAPS (Botta et al., 2021) and can be ascribed to the properties of the stimuli. A possible explanation for this finding might be that the information conveyed by the arms and hands in EBL, that are crucial to correctly process some specific emotions (Dael et al., 2012; Ross & Flack, 2020), are ambiguous. A closed fist, that is often present in 'happiness' EBL stimuli might be an indication of anger (Calbi et al., 2020; Dael et al., 2012), and consequently, it might need more time to correctly interpret the whole-body posture observed. Since the hands draw attentional resources in interpreting the mood expressed in body language and that our happy stimuli depicted mainly the actor with closed fists but in pleasant postures (e.g., jubilation or exultation), it might be plausible to infer that the longer RTs retrieved in the happy postures condition are a result of this mismatch between the whole-body posture and the hands.

Differences between PD and HC emerged in the discrimination task involving 'Fear' stimuli. Our results showed that motor responses are speeded when observing a potential threat ('Fear' stimuli), particularly for the embodied set of stimuli. The speeding up of RTs in PD resulted in no significant differences in RTs when discriminating 'Fear' EBL with respect to 'Fear' IAPS and in faster response when discriminating 'Fear' FACS with respect to 'Fear' IAPS. Alternatively, we can also interpret our data as a difficulty for PD in discriminating 'Fear' IAPS stimuli, with RTs that resulted longer than FACS RTs and like EBL RTs. However, this explanation is unlikely, since analysis on

valence scores did not show any PICTURE*GROUP interaction, suggesting a similar ability for PD and HC in emotion recognition in the three sets of stimuli.

It is noteworthy that, as in our previous study (Botta et al., 2021), we selected the IAPS stimuli for the fear condition from a restricted sample of pictures that have been reported to mainly evoke fear (e.g., human attacks and accident-depicting pictures) (Barke et al., 2012). Secondly, to exclude most of the body movement information, we decided to exclude all IAPS pictures that depicted whole human bodies involved in actions. This allowed us to have an appropriate set to compare with the "embodied" set of stimuli, namely FACS and EBL.

Our result can be discussed in relation to the "Kinesia paradoxa" phenomenon, defined as "the sudden transient ability of a patient with PD to perform a task he or she was previously unable to perform" (Glickstein & Stein, 1991).

Three mechanisms are generally proposed for "Kinesia paradoxa": (a) life-threatening events (b) external stimuli, and (c) medication (Banou, 2015). This phenomenon may have multiple explanations (Duysens & Nonnekes, 2021). One interesting explanation relies on the organization of the multiple and segregated cortico-subcortical-cortical pathways involving the basal ganglia (Alexander & Crutcher, 1990). Indeed, it has been pointed out that patients with PD tend to have the greatest deficit in the posterior putamen (linked to automatic movements), while keeping the potential to use the rostro-medial striatum, a structure known to be concerned with goal directed movements (as reviewed in Redgrave and colleagues) (Redgrave et al., 2010). Interestingly, Redgrave and

colleagues also pointed out the relative sparing of a third modular part of the basal ganglia, the limbic territories (next to the automatic and the goal-directed section) (Redgrave et al., 2010). Activation of these limbic parts (fed by the mesolimbic dopamine system and the limbic cortex) could "energize" the emotional motor system, as is indeed observed in "Kinesia paradoxa". Hence, an emotional stimulus may induce a switch to the emotional basal ganglion module, making motor response (here, response time) faster. It can be speculated that this emotional alternative motor pathway can play an important compensatory role in PD, to counteract dysfunction in other basal ganglia motor pathways, because of dopaminergic dysfunction, making the motor aspects in PD so sensible to emotional distress (Berlot et al., 2020). With disease progression, this compensatory mechanism may gradually worsen, as suggested by the positive correlation between RTs in discriminating 'Fear' EBL and disease duration and HY stage. Noteworthy, significant correlation emerged only for EBL, making this set of stimuli particularly sensible to test motor readiness in response to emotional stimuli, possibly because of the amount of motor information conveyed by this set of stimuli. Following this line of reasoning we can also speculate that lost in this compensation may be responsible of worsening of motor symptoms as in the case of freezing of gait (FOG). FOG is defined as a "brief, episodic absence or marked reduction of forward progression of the feet despite having the intention to walk" (Giladi & Nieuwboer, 2008). In the early stage of the disease, about 20% of patients report experiencing FOG, and this percentage raises up to 80% in the later stages, linking FOG with disease progression. It has been suggested that dysfunctional emotional processing may play a role in pathophysiology of freezing of gait (FOG) (Avanzino et al., 2018).This line of research started from

the observation that FOG precipitates in crowded places or when PD patients are under pressure. PD patients with FOG showed reduced step length and increased reaction time in a gait initiation task in response to negative valence emotional stimuli (Lagravinese et al., 2018). Here we did not recruit patients with FOG but following our hypothesis, RTs when discriminating 'Fear' EBL and FACS stimuli are not supposed to be speeded up in FOG patients, due to the loss of compensatory activity in the emotional alternative pathway. It would be interesting address this hypothesis in future studies.

In accordance with what observed in our previous study RTs when discriminating fearful EBL were lower compared with happy (i.e., positive) postures in both HC and PD, suggesting a faster processing of fearful body language. However, differently from what observed in our previous study, this did not result in non-significant difference between EBL and IAPS RTs in HC: indeed, in controls, RTs when discriminating fearful EBL were longer than when discriminating fearful IAPs, as it happened also for happy pictures.

This discrepancy with our former study may be due to the different population selected (younger people in our previous study, age-matched controls to PD patients in the present study). It is to consider that age may be a factor potentially influencing emotional processing and discrimination. Several neuroimaging studies involving processing of emotional facial expressions in young versus adults have shown that, during passive viewing of fearful faces, the young population showed an increased activation of the amygdala, the fusiform gyrus, the orbitofrontal cortex and the anterior cingulate cortex, while in adults it was retrieved an higher connectivity between the amygdala and the hippocampus

(Guyer et al., 2008; Monk et al., 2003, 2008). These different patterns of activation suggest that healthy young subjects exhibit greater emotion-based modulation of brain structures, while in adults this modulation is more related to attentional demands (Monk et al., 2003). If we consider as ascertained that facial expressions convey similar embodied information as EBL (Beatrice De Gelder, 2009), the discrepancies reported in relation to our previous study (Botta et al., 2021), might be related to the fact that the young participants enrolled in our previous behavioral study paid more attention to the emotional content of the pictures with respect to adults, hence showing differences in terms of RTs between EBL and IAPS.

Some limitations of the present study should be considered. First, the experiment was conducted on-line and no direct monitoring from the experimenter was available. Second, this study included only patients in the early-mid stage of the disease and to include patients in a more advanced stage (also with FOG) will be mandatory to confirm our hypothesis on the relation between RTs modulation with EBL and disease progression.

In conclusion, we showed that motor responses were faster in PD patients when the discrimination task involved 'Fear' embodied stimuli (FACS and EBL). This is supported by shorter RTs for FACS with respect to IAPS and by no difference (despite the difference in complexity) in RTs for EBL with respect to IAPS. We speculated mechanisms for the involvement of an alternative emotional motor pathway for motor performance improvement under 'Fear' processing.

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8. Conclusions and future directions

The aim of this thesis was to explore the relationship between emotional processing and the sensorimotor system, mainly focusing on one information source derived from emotional body language.

In the first study, the main aims were to investigate motor correlates of emotional body language processing and to assess whether processes underlying motor response relied mostly on the emotional content of the picture observed or if they were driven by motor resonance. We found that fearful body language is rapidly recognized and processed, leading to faster motor response when compared to happy and non-emotional body language probably because of a rapid and instinctual activation of several brain structures involved in defensive reactions.

The second experiment wanted to explore the effects of emotional language on a specific phenomenon linked to the sensorimotor system that is short-latency afferent inhibition, but in an earlier timeframe. Our results showed that sensorimotor inhibition in the first 120 ms after stimulus onset is increased during processing of fearful emotional stimuli, reflecting the fact that automatic processing of threatening information which in turn activates a series of subcortical structures able to modulate attentional resources and cholinergic activity in the cerebral cortex may consequently modulate sensorimotor inhibition. Moreover, we also saw that at longer latencies (i.e., 300 ms after stimulus onset), sensorimotor inhibition is significantly reduced, probably because of the uprise of cognitive processes related to image complexity.

In our third experiment, we aimed to further investigate such a complex mechanism that is sensorimotor inhibition during emotional processing by recording cortical activity via electroencephalography. In this experiment we always focused on an early time window (i.e., 0-200 ms after stimulus onset) and we directed our attention only on emotional body language. What we found was that during processing of fearful body expressions there was increased activity in the β frequency band in the somatosensory cortex which in turn may be one of the factors responsible for reducing the activation of motor related areas. These findings support the idea that fear thanks to a rapid involvement of subcortical structure may in turn induce an early modulation of cortical areas, resulting in sensory arousal and motor inhibition, as suggested by our results on short-latency afferent inhibition. Moreover, we also observed that a brain rhythm usually associated to action observation and motor performance such as the μ-alpha rhythm, probably is not sensitive to pictures' emotional content in the first 200 ms after stimulus processing, but only to motor related information.

Finally, in our fourth experiment, we investigated investigate motor correlates of emotional body language processing and facial expressions in patients with Parkinson's disease. By doing so, we aimed to explore if and how emotional information processing might be impaired in patients affected by Parkinson's disease a neurodegenerative disease characterized by basal ganglia dysfunction, a subcortical structure largely implicated in emotional processing. Our results showed that motor responses in PD patients are speeded when observing a potential threat ('Fear' stimuli), for both the embodied set of stimuli (EBL and facial expressions). We discussed this finding in relation to the "Kinesia

paradoxa" phenomenon, defined as "the sudden transient ability of a patient with PD to perform a task he or she was previously unable to perform".

Although the results shown in this thesis allow a better comprehension of the underlying mechanisms involved in emotion processing, there are still several unanswered questions arising.

Firstly, attentional and sensorimotor processes should be further explored with different neurophysiological methodologies. It is true that the TMS protocol exploring short-latency afferent inhibition has been shown to be most probably related to the cholinergic system, but there are still no definite evidence showing a correlation between SAI and cholinergic activity. Following this partially unresolved issue, it might be of interest to investigate the integrity (or deficit) of the cholinergic system via neuroimaging techniques coupled with specific cholinergic radioligand, and the relationship between the aforementioned system and the sensorimotor network inspected via SAI, both at rest and during emotional processing.

Secondly, in order to better understand the influence of Parkinson's disease on emotion processing, the neurophysiological protocols described in this thesis should be adapted to a pathological population to explore SAI and neural oscillations in the early and late phases of EBL processing and emotional processing more in general.

Lastly, one of the limitations often reported in our studies was the one related to the fact that neurophysiological modulation was mainly retrieved during processing of threatening visual stimuli (with no difference in terms of the type of stimulus submitted to the participants). Since there are several negative

emotions besides fear (e.g., anger, sadness, disgust), it would certainly be of interest to investigate if there are similar modulatory effects of other emotions on the sensorimotor system, or if the results we observed in our studies are exclusively linked to fear.