

## Research Paper

## Relational Impact of Emotional Stimuli on Putative Mirror Neuron Activity: A Transcranial Magnetic Stimulation Study



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## ABSTRACT

**Introduction:** Mirror neurons' function is thought to be enhanced by emotion processing. There is some evidence that the valence of an emotional presentation (positive or negative) can influence subsequent mirror neuron activity differently. Additionally, mirror neurons are claimed to provide the mechanism necessary for the embodied simulation of others' mental states. Therefore, there is an assumption that relational emotion processing may manipulate mirror neuron functions.

**Methods:** Via transcranial magnetic stimulation (TMS) of the primary motor cortex (PMC) and electromyography recording from contralateral hand muscles, 20 participants viewed videos of either a static hand or a transitive hand action preceded by either neutral or general (negative and positive) and relational (negative and positive) images.

**Results:** Corticospinal excitability facilitation during action observation was significantly greater following the positive general than negative general emotion stimuli. Regarding relational emotions, we observed an increased mirror neuron system (MNS) activity following relational negative compared to relational positive, relational negative compared to general negative, and general positive compared to relational positive stimuli.

**Conclusion:** This finding supports the assumption that relational content interferes with mentalizing capacity.

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## Highlights

- Corticospinal excitability (CSE) was significantly greater following the presentation of the general positive stimuli than the general negative stimuli.
- There is increased mirror neuron system activity following the presentation of relational negative stimuli than relational positive stimuli.
- There is increased mirror neuron system activity following the presentation of relational negative stimuli than general negative stimuli.
- There is increased mirror neuron system activity following the presentation of general positive stimuli than relational positive stimuli.

## Plain Language Summary

Mirror neurons are the neurons that fire during the observation and execution of actions. The valence of an emotional presentation (positive or negative) can influence subsequent mirror neuron activity. The mirror neuron system can facilitate social cognition (ability to process social stimuli which is called mentalization capacity). When children or adolescents are exposed to situations triggering general emotions versus relational emotions, they show different mentalization capacity. Transcranial magnetic stimulation (TMS) is a common method for studying the mirror neuron system. The present study investigated the response of this system to relational vs general stimuli (images). Participants were 28 female adults. We delivered TMS pulses during both active and static hand observations. According to the results, corticospinal excitability was significantly greater following the presentation of the general positive stimuli than the general negative stimuli. There was increased mirror neuron system activity following the presentation of relational negative stimuli than relational positive stimuli, relational negative stimuli than general negative stimuli, and general positive stimuli than relational positive stimuli.

## 1. Introduction

**M**irror neurons are the neurons that fire during the observation and execution of actions. The mirror neuron system (MNS) was first observed in the frontal cortex of macaque monkeys (Di Pellegrino et al., 1992). Later, human MNS was discovered in the motor cortex, which occupies the posterior precentral gyrus in the frontal area (Fadiga et al., 1995; Kilner et al., 2009). Although MNS has been described initially in imitating actions (Jeannerod & Decety, 1995), further research illustrates that it is also essential for perceiving others' mental states (Gallese & Goldman, 1998; Luyten & Fonagy, 2015). This finding suggests that a fundamental process allowing us to appreciate the actions and emotions of others involves the activation of the MNS (Fonagy & Luyten, 2016). MNS is also valence-sensitive; the valence of an emotional presentation (positive or negative) can influence subsequent mirror neuron activity (Enticott et al., 2012), so it has higher excitability to negative emotional stimuli (Schmidt et al., 2020). Therefore, MNS, as a funda-

mental base for understanding others, facilitates "social cognition" in a healthy brain. Impaired MNS has been described in the pathophysiology of several psychiatric conditions, such as autism spectrum disorder (Hadjikhani et al., 2006), schizophrenia (Mehta et al., 2014), depression (Nejati, 2018; Nejati et al., 2012), and psychopath (Fecteau et al., 2008).

Social cognition is the ability to process social stimuli characterized by a variety of interpersonal skills such as self-perception (Nejati et al., 2012), the theory of mind (Mier et al., 2009), empathy (Corradini & Antonietti, 2013), intention understanding (Catmur, 2015), facial (Enticott et al., 2008) and emotion recognition (Nejati et al., 2022). These skills are gathered under an umbrella concept called mentalization capacity (Luyten & Fonagy, 2015). As a form of social cognition, mentalization enables us to perceive and interpret human behavior through intentional mental states, concluding needs, desires, feelings, beliefs, and goals (Fonagy & Luyten, 2009). The acquisition of this capacity depends on the quality of early bonding with the mother or other attachment figures (Fonagy, 2011). Therefore, the

child's early attachment is the primary foundation for developing social cognition (Ziv & Arbel, 2020). Furthermore, mentalization is likely to vary greatly among specific relationships instead of general ones. General circumstances in this setting represent individuals' general emotions, whereas relational conditions raise mental representations specific to each individual's primary attachments (Fonagy & Luyten, 2009; Overall et al., 2003). Although overlapping, general and relational mentalization appears distinct (Happe & Frith, 1996; Humfress et al., 2002; O'Connor & Hirsch, 1999). It has been observed that when children (Humfress et al., 2002) or adolescents (O'Connor & Hirsch, 1999) are exposed to situations triggering general emotions vs relational emotions, they show different mentalizing capacity. Children with avoidant attachment styles had greater difficulty mentalizing relational stimuli than general stimuli due to their lower mentalization capacity. However, this difficulty was less in the secure attachment group. Based on these findings, it can be assumed that mentalizing relational stimuli requires a higher level of mentalization (Humfress et al., 2002; O'Connor & Hirsch, 1999; Repacholi & Trapolini, 2004). Accordingly, the within-person variation in the capacity to mentalize others supports this capacity, which contains both general and relational representations (Fonagy & Luyten, 2009; Overall et al., 2003). This fact raises the possibility that relational content requires a higher mentalization capacity than general emotional content. To put it another way, there is an assumption that MNS activity might be manipulated differently by relational vs general emotions triggered by social situations.

Transcranial magnetic stimulation (TMS), a non-invasive brain stimulation technique, is a well-documented tool for studying the MNS. TMS applies a brief magnetic pulse to the underlying brain structure through surface scalp coils (Terao & Ugawa, 2002). In social cognition studies, the peripheral muscle activity, measured by electromyography after applying TMS pulses over the primary motor cortex (PMC), has been described as an indicator of the MNS function (Maeda et al., 2002). Altogether, both action observation and facing situations that require understanding other's mental states activate mirror neurons, thus increasing the PMC excitability, which results in an enhanced motor evoked potential (MEP) amplitude (Enticott et al., 2008; Maeda et al., 2002).

The present study investigated the MNS responses to relational vs general content. We hypothesize that PMS excitability will be different in these two distinct conditions. To do so, we delivered TMS pulses during both active and static hand observations. At the same time,

participants were exposed to images with relational vs general stimuli of both negative and positive valence. Based on previous findings (Enticott et al., 2012; Hill et al., 2013), it was hypothesized that exposure to negative compared to positive stimuli enhances MNS activity in the general context. Also, there might be a difference between MNS activity when presenting relational stimuli.

## 2. Materials and Methods

### Study participants

The sample consisted of 28 female adults (age: Mean $\pm$ SD 39.4 $\pm$ 13.1, range=21-61 years) selected by voluntary response sampling method. The inclusion criteria include right-handedness, as assessed by the Edinburgh-Handedness Inventory (Oldfield, 1971), an age range of 18 to 40 years, and no history of neurological or psychiatric disorders. As previous studies stimulated each cortical hemisphere separately and found larger MEP amplitude in negative and positive trials in the left hemisphere (Aziz-Zadeh et al., 2004; Hill et al., 2013), we decided only to stimulate the left M1 and, therefore, excluded left-handed participants.

All stages of the research were carried out between October and March 2021-2022. Before participating, the candidates were screened concerning TMS safety criteria (Rossi et al., 2009). The Ethics Committees of Iran University of Medical Sciences granted ethical clearance for the project, which was carried out in the Nasional Brain Mapping Lab (NBML). Participants had normal or corrected-to-normal vision. Each participant gave written informed consent, and at the end, they were honored with cash and non-cash gifts.

### Study materials

#### Visual stimuli

Participants are shown five blocks: a) General emotion, including negative and positive; b) Relational emotion, including negative and positive; and c) Neutral stimuli. Each block contains:

1. Videos are 3-second long and feature either 1- a right hand performing a transitive movement (picking up a mug, Figure 1) or 2- a static right hand next to a mug. Consistent with previous research, the transitive hand movements are used to elicit an MNS response, whereas the static hands are employed as a control condition and have been previously shown not to activate the MNS (Enticott et al., 2012; Enticott et al., 2008)

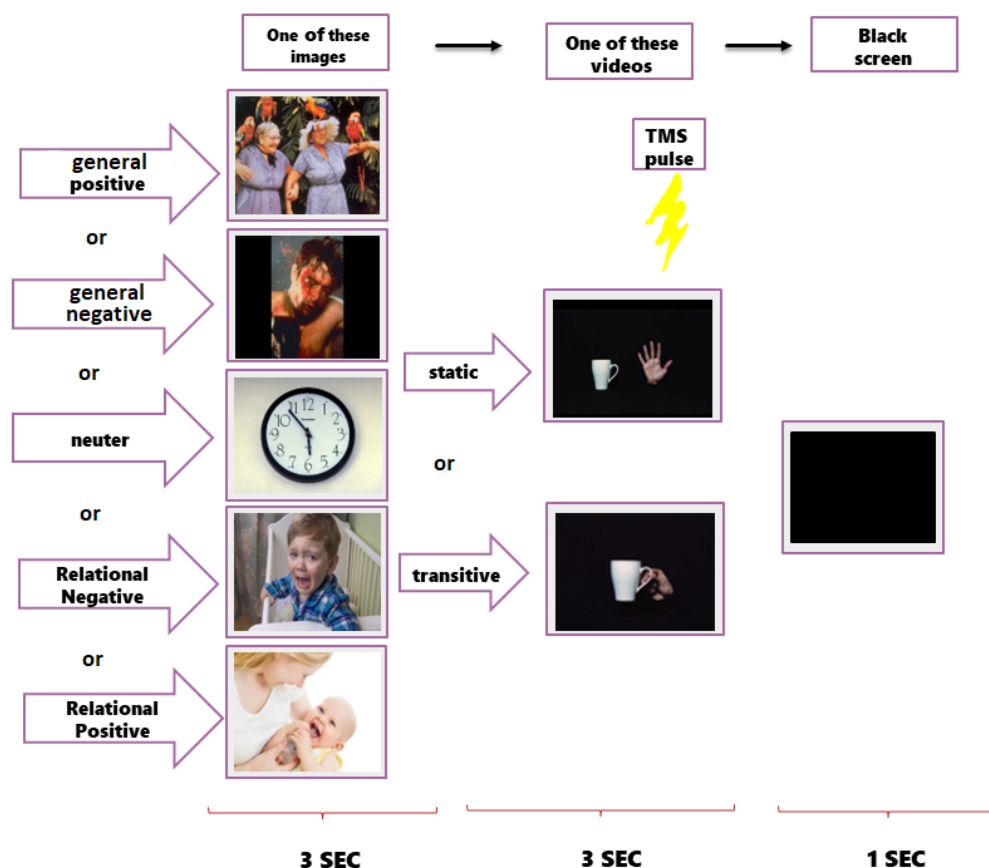


Figure 1. Summary of the protocol for a single TMS trial

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## II. Images

1. Images for general emotion (positive and negative) and neutral blocks were taken from the international affective picture system (IAPS). The IAPS contains a large bank of emotionally evocative images, which have been rated for emotional valence and arousal by a large American cohort (Lang, 2005). These images were selected based on their valence and arousal properties as rated by this cohort. As IAPS images are rated differently by males and females, we only selected from the images related to the females:-

2- Images for the relational emotion (positive and negative) blocks were taken from the attachment-related picture set (ARPS). The ARPS contains a bank of emotional attachment and pictures rated by 310 individuals for valence and arousal (Maleki et al., 2021).

We had 5 emotional blocks of 40 trials each. Each trial contains an image displayed for 3 s, followed by the video clip (3 s) of the static or transitive hand in a quasi-random order, followed by 1 s of black screen (a total of 7s: Figure 1).

## Study procedure

Participants were comfortably seated on a recliner chair 60 cm from a 22-LCD monitor. EMG electrodes are placed over the first dorsal interosseous (FDI), as mug grasping involves activating the right hand's FDI muscle and abductor digiti minimi (ADM) muscles as a control site. To ensure low skin impedance, the electrode sites were cleaned with alcohol. The EMG signal was amplified using an MA300 system (Motion Lab System, CO) with low/high pass filtering set at 500 Hz and 10 Hz, respectively.

A single TMS pulse at 100% resting motor threshold (RMT) was delivered over the left M1 through a 70 mm figure-of-eight coil powered by a MagPro X100 stimulator (Magventure company, USA). When stimulated, the M1 area was defined as the site that produced the largest MEP in the FDI muscle. The RMT was defined as the lowest stimulus intensity required to produce a reliable peak-to-peak MEP amplitude of approximately 1 mv in the FDI muscle (Mean±SD RMT=57±9.7). The decision to stimulate the left hemispheres was made based on the participants' handedness.

During the video clip presentations, a jittered TMS pulse occurred in the video frame right before the hand grasps the mug (corticospinal excitability [CSE] is maximal immediately before an object is grasped with the hand) (Gangitano et al., 2001), and, for the static hand video, TMS pulses occurred the same second into the video clip.

### Statistical analysis

The peak-to-peak MEP amplitude was calculated with Matlab software (R2019b, MathWorks Inc., Natick, MA). To correct muscle contraction-contaminate EMG activity, we mean substitute trials in which an EMG activity within 200 ms of the TMS pulses was evident (0.1% of all trials). Moreover, an outlier removal using a performance package (Lüdtke et al., 2021) was conducted to remove the effect of influential trials (involuntary movements, sensor drift, etc.). As previously suggested, to control the inflation of the MEP responses (Enticott et al., 2012), median peak-to-peak amplitudes for each of the static and transitive hand videos were employed to compute the MEP log ratio (MEP-LR). MEP-LR was calculated using the Equation 1:

$$1. \text{MEP-LR} = 10(\log \text{MEP}_{\text{transitive}} / \text{MEP}_{\text{static}})$$

This estimation provides a relative index of putative mirror neuron activity, in which larger and positive MEP-LRs values show greater MNS responses.

All statistical analyses were carried out in the R (version 4.1.2) environment (Team, 2013) using afex (Singmann et al., 2015) and ggstatsplot (Patil, 2021) packages. The normality and homogeneity of variance were assured by the Shapiro-Wilk and Levene's tests, respectively. Repeated measures ANOVA were conducted for the dependent variable (MEP-LR), with Image types (relational positive, general positive, relational negative, general negative, nature) and muscle (FDI, ADM) as the within-subject factors. Mauchly's test of sphericity was conducted, and the Greenhouse-Geisser correction was applied when necessary. Post hoc analyses were calculated using false discovery rate (FDR)-corrected pairwise student t-tests (two-tailed).

## 3. Results

### MNS activity and muscle specificity

No significant image types  $\times$  muscle interaction was found ( $F_{(4, 108)} = 0.26$ ,  $P = 0.90$ ,  $\eta_p^2 = 0.01$ ; 95% CI, 0.00%, 1.00%), indicating that MEP-LR values in response to the stimuli were not muscle specific. However, subse-

quent FDR-corrected paired sample t-test revealed a significant difference between positive and negative emotional stimuli in general image type in FDI muscle ( $t_{27} = 3.12$ ,  $P = 0.04$ ,  $d_{\text{cohen}} = 0.59$ ) (Figure 1).

No main effect of Muscle was found ( $F_{(1, 27)} = 0.93$ ;  $P = 0.34$ ,  $\eta_p^2 = 0.03$ ; 95% CI, 0.00%, 1.00%). However, as was expected, the overall MEP-LR value was higher in the FDI muscle compared to ADM (Mean $\pm$ SE FDI:  $0.35 \pm 0.12$ , ADM:  $0.24 \pm 0.13$ ) (Figure 2).

### MNS activity and emotional valence

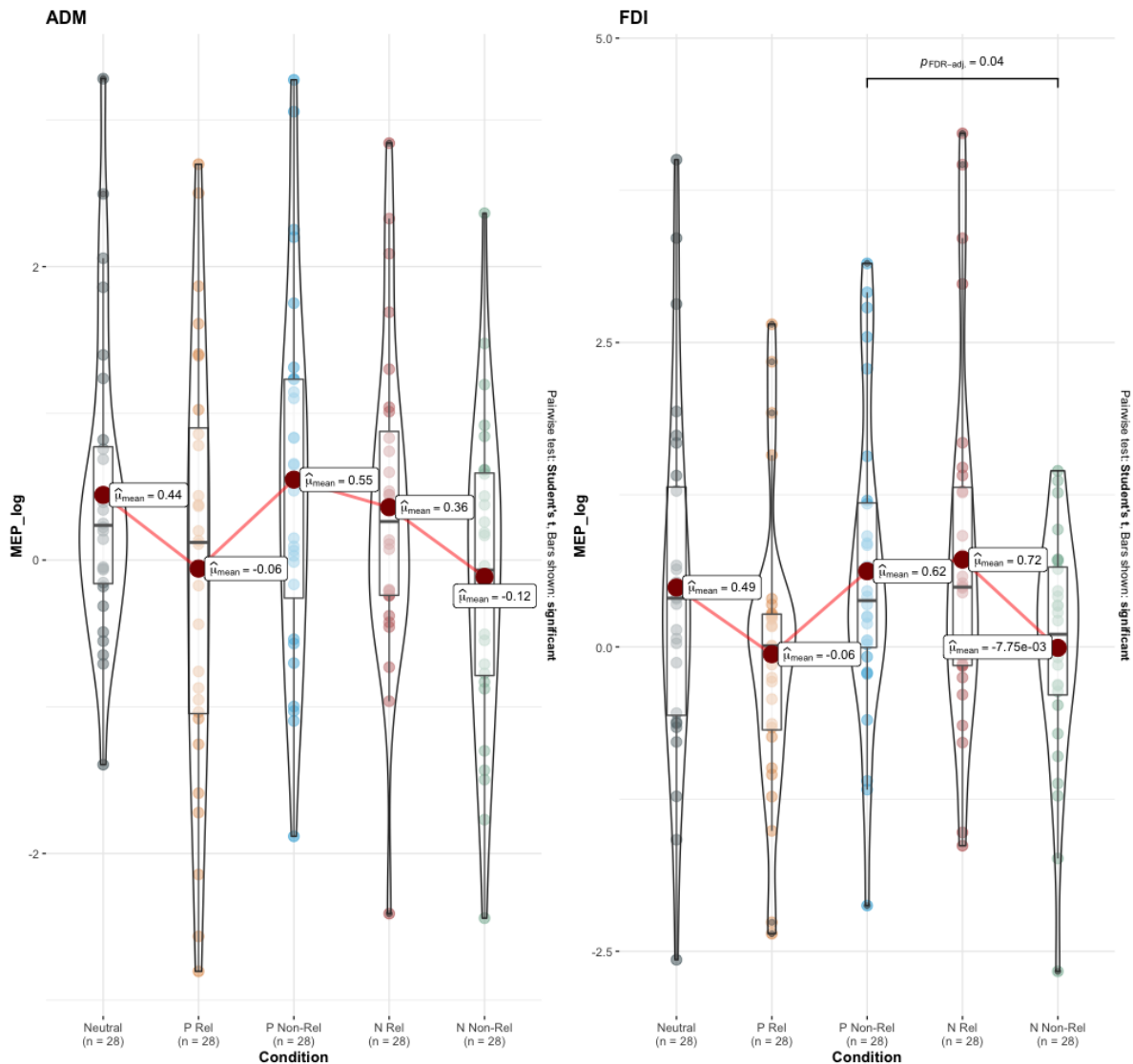
A significant medium main effect of image types was observed ( $F_{(4, 108)} = 3.65$ ;  $P = 0.008$ ,  $\eta_p^2 = 0.119$ ; 95% CI, 0.02%, 1.00%). Given that, no muscle-specific effect on image types was found. We performed an FDR-corrected paired sample t-test to investigate the overall impact of image types across collapsed FDI and ADM muscles. The Post hoc comparisons revealed a significant effect between neutral and negative emotion in general image type ( $t_{27} = 2.81$ ,  $P = 0.02$ ,  $d_{\text{cohen}} = 0.38$ ). In addition, a significant difference between positive and negative emotional stimuli was observed both in relational ( $t_{27} = 2.48$ ,  $P = 0.03$ ,  $d_{\text{cohen}} = 0.33$ ) and general ( $t_{27} = 3.75$ ,  $P = 0.004$ ,  $d_{\text{cohen}} = 0.50$ ) image types. Furthermore, a difference between relational and general image types was also evident in positive ( $t_{27} = 2.79$ ,  $P = 0.02$ ,  $d_{\text{cohen}} = 0.37$ ) and negative ( $t_{27} = 2.99$ ,  $P = 0.02$ ,  $d_{\text{cohen}} = 0.40$ ) emotions (Figure 3).

## 4. Discussion

This study set out to test the modulation of emotion processing valence and relationality on MNS activity. This approach was achieved via a TMS paradigm during action observation relative to the observation of static control, indicating putative MNS activity (Enticott et al., 2012; Hill et al., 2013; Strafella & Paus, 2000). Concerning the first hypothesis, which addresses the effect of general emotion processing on MNS activity, we found an increased MEP amplitude in FDI muscle (but not in ADM muscle) following positive compared to negative images in general conditions. In addition, we observed increased MEP amplitude in neutral relative to general negative images across collapsed FDI and ADM muscles. Interestingly, regarding the second hypothesis considering the effect of relational emotion on MNS activity, this study revealed a difference between positive and negative emotions in both general and relational conditions. However, this finding was only significant across collapsed FDI and ADM muscles, and no muscle specificity was observed. Therefore, we conclude that (1) General positive vs general negative emotion, (2)



## Differences between conditions

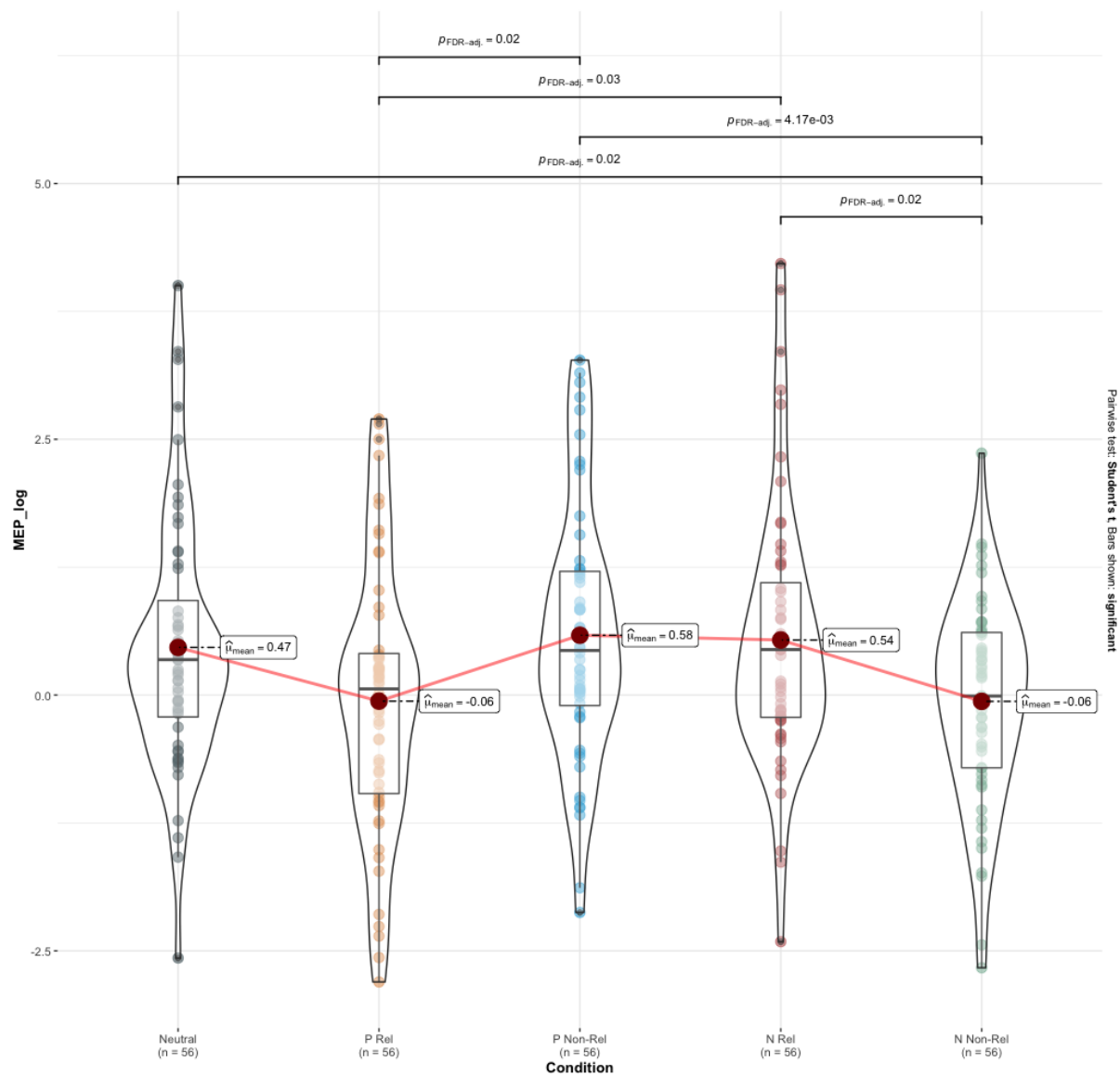


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**Figure 2.** A combination of raw data points with box and violin plots of MEP\_log values for the ADM (left) and FDI (right) muscle following neutral, relational (negative and positive), and general (positive and negative) stimuli

Relational negative vs relational positive, (3) Relational negative vs general negative, and (4) General positive vs relational positive modulate MNS activity. Our finding supports the evidence for the modulatory effect of emotions with different valences on the MNS activity (Enticott et al., 2012; Hill et al., 2013) by showing the impact of processing emotions with different valences on mirror neuron MEP amplitude. However, unlike Enticott et al. (2012), who observed an increased MNS activity following the negative images and not the positive ones, we replicated Hill et al. (2013). We observed this increased activity following positive versus negative images in the general condition in FDI muscle.

Furthermore, in the current study for the first time, in addition to valence, we also investigated the effect of relational emotion processing on MNS function. Surprisingly, our results showed that MNS activity across positive and negative emotions was altered under both relational and general conditions. This finding supports the assumption that relational content interferes with mentalizing capacity.



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**Figure 3.** A combination of raw data points with box and violin plots of MEP\_log values following neutral, relational (negative and positive), and general (positive and negative) stimuli, collapsing across FDI and ADM muscles

### MNS activity and emotional valence

Compared to increased MNS activity following exposure to negative stimuli, it is less clear why positive emotions might induce an augmented MNS response. There are more assumptions about the involvement of negative stimuli in MNS activity, including the likelihood of their occurrence in situations that threaten one's survival, which associate them with fast and decisive actions to help protect an individual from physical harm (Fredrickson, 2001). However, taking the social cognitive perspective into account, positive interactions with others could increase MNS function as well, which, in turn,

would enhance social cognitive abilities such as emotional engagement and empathic understanding (Gallese & Goldman, 1998). The advantages of improved social cognitive skills can be seen in positive situations, for example, when one can engage more strongly with the environment and other people (Fredrickson, 2001). Therefore, the current result that shows general positive emotion exerts a more facilitatory effect on MNS than general negative emotion can be argued for from a social cognitive perspective. However, besides the difference between positive and negative emotions, we observed an increased MEP amplitude following neutral compared to negative emotions. The facilitatory effect of neutral

emotion on MNS activity was unexpected. However, considering the cultural differences, previous studies show that continuous exposure to violent content in the media eventually causes desensitization (Tarabah et al., 2016). Images being rated as negative in the IAPS bank are evaluated from an American perspective, while they are often seen in the Iranian culture and cause a kind of desensitization in the audience (for example images related to war and traumas such as torture). In addition, the American people's images rated as neutral in IAPS bank might not be completely neutral in our participants' culture. The most promising example is the image of an airplane, which is generally a neutral image. In Iranian culture, it might, however, remind people of the tragic experience of Ukraine international airlines flight 752. Undoubtedly, this issue needs further investigation.

### MNS activity and relational emotion

The novelty of our finding is consistent with data showing that relational emotion influences mentalization capacity. It has been observed that when children (Humfress et al., 2002) or adolescents (O'Connor & Hirsch, 1999) are exposed to situations triggering general emotions relative to relational emotions, they show different mentalizing capacities. More specifically, as reported by Repacholi and Trapolini (2004), children with high scores on the avoidance dimension of the separation anxiety test show less mentalizing capacity in the case of a mother-child relationship. Humfress et al. (2002) consistently reported that children who exhibited a less coherent attachment model were more likely to be rated as exhibiting a dismissing/avoidant style in the attachment interview. Accordingly, attachment representations characterized by high levels of avoidance appear to interfere with children's ability to fully engage their social-cognitive skills when reasoning about maternal mental states (Repacholi & Trapolini, 2004). These findings align with studies suggesting the involvement of MNS in understanding others' mental states (Gallese & Goldman, 1998; Schmidt et al., 2020) and raise the possibility that relational content requires a higher mentalization capacity than general emotional content. Fonagy and Loyten (2009) took it one step further and discussed it in the context of more insecure patients, e.g. individuals diagnosed with a borderline personality disorder. They claimed that the stronger the attachment in a particular relationship at a specific moment, the more likely that anomalies in mentalization will emerge in bipolar personality disorder patients. Our TMS-EMG result confirms this assumption, which adds to Repacholi and Trapolini's (2004) findings and reveals an altered MNS activity following relational emotion relative to general emotion. They

observed that relational stimuli were more difficult for avoidant children. Because they demanded more mentalization capacity, they observed that relational stimuli were more difficult for avoidant children. As a result, it demanded more mentalization capacity, and these children could not meet that demand due to their avoidant attachment style. However, our result reveal that the processing of relational negative emotion compared to relational positive one and relational negative compared to the general negative one exerts an upregulation effect on the MNS function of our participants. This outcome could be because mentalizing relational stimuli requires a higher capacity for mentalization, which can be shown by more MNS activity in a relational negative situation. However, the augmented MNS activity following general positive relative to relational positive images could be explained by the circle of security model (Fonagy et al., 2018; Maxwell et al., 2021), arguing that in the first place, children need more mentalizing capacity for processing negative emotions, which enable them to process negative encounters (during coordination with their mother). In parallel, experiencing positive emotions would pave their way towards tolerating some distance from the mother and socializing. We have also seen that images related to primary negative relationships, require more mentalizing capacity, indicating more MNS activity. On the other hand, experiencing positive emotion in a general condition, which triggers the second half of the circle of the security model, initiates the socialization process and therefore increases the MNS activity as well.

In short, an individual needs a higher mentalization capacity during relational negative emotions (initial emotions) and when experiencing general positive emotions (not necessarily relational ones).

### MNS activity and muscle specificity

The result shows a clear pattern of muscle specificity of the MNS response following positive relative to negative images in general conditions but not in the ADM muscle. This finding aligns with a previous TMS study on mirror neurons (Enticott et al., 2012). In the current study, we employed a hand action designed to primarily activate the FDI (picking up a cup by the handle), and participants' recorded MEP-log values for the FDI muscle were larger than those for the ADM when participants viewed transitive hand movement. However, this muscle specificity has not been found in relational trials.



## 5. Conclusion

The current study supports the literature on the modulatory effect of emotions with different valences on MNS activity by reporting the augmented mirror neuron's MEP amplitude following the processing of general positive emotions compared to general negative emotions. Additionally, for the first time, we investigated the effect of relational emotion processing on MNS function. We observed an altered MNS activity following relational compared to general stimuli, which supports the assumption that relation-based content interferes with mentalizing capacity.

## Study limitations and future direction

Some limitations should be taken into account in the present study. First, this study is exploratory, with a relatively limited number of healthy participants and some caution in clinical application. Furthermore, given the valence theory of emotion, the right and left hemispheres are involved in negative and positive emotional processing, respectively and it would be compared to both hemispheres in the study.

In addition, using a condition including the mug without the presence of the hand helps to control "the expectation effect" in the subject, which was not considered in this research due to the large number of trials.

## Ethical Considerations

### Compliance with ethical guidelines

The Ethics Committees of [Iran University of Medical Sciences](#), Tehran, Iran, granted ethical clearance for the project.

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### Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

## Conflict of interest

The authors declared no conflict of interest.

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