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Title: Relational Impact of Emotional Stimuli on Putative Mirror Neuron Activity: A TMS Study

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Abstract

Intro: 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 might manipulate mirror neuron functions.

Method: Via Transcranial Magnetic Stimulation of the primary motor cortex 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 compared to negative general emotion stimuli. Regarding relational emotions, we observed an increased 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 of relational content interferes with mentalizing capacity.

Keywords: Mirror neuron system, Emotion processing, Relational emotions, Mentalization, TMS

1. Introduction

Mirror 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 on, 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 imitation of actions, (Jeannerod & Decety, 1995) further research illustrated that it is also essential for perceiving others' mental states (Gallese & Goldman, 1998; Luyten & Fonagy, 2015). This suggests that a fundamental process allowing us to appreciate the actions and emotions of others involves the activation of the mirror neuron system (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 fundamental base for understanding others, facilitates *social cognition* in a healthy brain, and 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; Vahid 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 (Vahid 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). All of these skills are gathered under an *umbrella concept* named Mentalization capacity (Luyten & Fonagy, 2015). Mentalization as a form of social cognition enables us to perceive and interpret human behavior in terms of 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 can be considered the primary foundation for developing social (Ziv & Arbel, 2020). Furthermore, mentalization is likely to vary greatly among specific relationships as opposed to general ones. Individuals' general emotions are represented by general circumstances in this setting,

whereas relational conditions raise mental representations specific to each individual's primary attachments (Fonagy & Luyten, 2009; Overall et al., 2003). Interestingly, although overlapping, general and relational mentalization appear to be distinct (Happé & 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) were exposed to situations triggering general emotions vs. relational emotions, they showed different mentalizing capacity; children with avoidant attachment style had greater difficulty in mentalizing relational stimuli compared to general stimuli due to their lower mentalization capacity; However, this difficulty was less in the secure attachment group. From these findings, it can be assumed that mentalizing relational stimuli require 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 a view of this capacity which contain 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 (NIBS) technique, is a well-documented tool for studying the MNS. TMS applies a brief magnetic pulse to the underneath 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 MEP amplitude (Enticott et al., 2008; Maeda et al., 2002).

In the present study, we investigated the MNS responses to relational vs. general content. We hypothesize that PMS excitability will be different in these two different 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

2.1. Participants

The sample consisted of 28 female adults (age: $M = 39.4$, $SD = 13.1$, $Range = 21-61$ years) which were selected by voluntary response sampling method. Inclusion criteria include right-handedness, as assessed by the Edinburgh-Handedness Inventory (Oldfield, 1971), age range of 18 to 40 years, and with 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 to only stimulate the left M1 and therefore, excluded left-hander 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 science granted ethical clearance for the project and the project 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.

2.2. Materials

3.2.1 visual stimuli

Participants were shown five separate blocks: a) general emotion including negative and Positive, b) Relational emotion including negative and positive c) Neutral stimuli. Each block contains:

- I. 3-second videos featuring either 1- a right hand performing a transitive movement (picking up a mug: see fig 1) or 2- a static right hand next to a mug. Consistent with previous research the transitive hand movements were used to

elicit an MNS response, whereas the static hands were employed as a control condition and have been previously shown not to activate the MNS (Enticott et al., 2012; Enticott et al., 2008)

II. images

1- Images for general emotion (positive and negative), and neutral blocks were taken from 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' table¹. see the average image valence and arousal ratings in table 1.

Table 1: Summary of the Mean (SD) IAPS Image ratings for Females.

Note: Valence and arousal ratings were scored from 1 to 9. A lower valence score indicates more negative content, while a lower arousal score indicates less arousing content.

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 attachment emotionally pictures which have been rated by 310 individuals for both valence and arousal (Maleki et al., 2021).

¹ Positive images: 1340, 1463, 1710, 1811, 1999, 2300, 2303, 2352.1, 2550, 4532, 4599, 4601, 4603, 4610, 4614, 4628, 5260, 5301, 5470, 5480, 5623, 5626, 5628, 5825, 5833, 5910, 7260, 7330, 7430, 7461, 7508, 8033, 8034, 8162, 8170, 8350, 8380, 8420, 8496, 8540.

Negative images: 2276, 2375.1, 2456, 2694, 2710, 2717, 2799, 3185, 3220, 6020, 6555, 6561, 6562, 6825, 6836, 6940, 9007, 9145, 9332, 9403, 9409, 9415, 9421, 9426, 9427, 9428, 9430, 9435, 9470, 9495, 9561, 9584, 9590, 9592, 9610, 9630, 9902, 9920, 9925, 9930

Neutral images: 1560, 1908, 2122, 2210, 2211, 2220, 2372, 2780, 3005.2, 3550.2, 5535, 5970, 6314, 7077, 7081, 7137, 7211, 7240, 7365, 7402, 7476, 7487, 7496, 7497, 7504, 7560, 7595, 7600, 7632, 7640, 8065, 8117, 8192, 8232, 8250, 8251, 8260, 9080, 9090, 9468

We had 5 emotional blocks of 40 trials each. Each trial contains an image displayed for 3s, followed by the video clip (3s) either of the static or transitive hand in a quasi-random order, followed by 1s of black screen (A total of 7s: see fig1).

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

2.3. Procedure

Participants were comfortably seated on a recliner chair 60cm away from a 22-LCD monitor. EMG electrodes were placed over the first dorsal interosseous (FDI), as mug grasping involves activation of the FDI muscle, and abductor digiti minimi (ADM) muscles of the right hand, 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% RMT was delivered over left M1 through a 70 mm figure-of-eight coil powered by a MagPro X100 stimulator (Magventure company, USA). The M1 area was defined as the site that, when stimulated, produced the largest MEP in the FDI muscle. The resting motor threshold (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 RMT = 57, SD: 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 (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.

3.4 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 200ms of the TMS pulses was evident (0.1% of all trials). Moreover, an outlier removal using a performance package (Lüdecke 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 computed using the below formula:

$$\text{the MEP-LR} = 10 * \left(\log \frac{\text{MEP}_{\text{transitive}}}{\text{MEP}_{\text{static}}} \right)$$

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

All of the 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 Shapiro-Wilk and Levin 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 FDR-corrected pairwise Student's t-tests (two-tailed).

3. Results

4.1. MNS activity and muscle specificity

No significant Image types \times Muscle interaction was found, $F_{4,108} = .26$, $p = .90$, $\eta_p^2 = .01$ 95% CI [0.00, 1.00], indicating that MEP-LR values in response to the stimuli were not muscle specific. However, subsequent FDR-corrected paired-sample t-test revealed a significant difference between positive and negative emotional stimuli in general image type in FDI muscle ($t_{27} = 3.12$, $p = 0.04$, $d_{\text{Cohen}} = 0.59$) (Fig X).

No main effect of Muscle was found $F_{1,27} = .93$, $p = .34$, $\eta_p^2 = .03$ 95% CI [0.00, 1.00]. although, as it was expected the overall MEP-LR value was higher in the FDI muscle compared to ADM (FDI: $.35 \pm .12$, ADM: $.24 \pm .13$; Mean \pm SE) (Fig 2).

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.

4.2. MNS activity and emotional valence

A significant medium main effect of image types was observed $F_{4,108} = 3.65$, $p = .008$, $\eta_p^2 = .119$ 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 effect 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 in negative ($t_{27} = 2.99$, $p = 0.02$, $d_{\text{cohen}} = 0.40$) emotions (Fig 3).

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.

4. Discussion

This study set out to test the modulation of emotion processing valence and relationality on MNS activity. This 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) 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 found for the modulatory effect of emotions with different valences on the MNS activity (Enticott et al., 2012; Hill et al., 2013) by showing the effect of processing emotions with different valences on mirror neuron MEP amplitude. However, in contrast to 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) and observed this increased activity following positive compared to 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 both positive and negative emotions was altered under both relational and general conditions. Overall, this finding supports the assumption that relational content interferes with mentalizing capacity.

4.1. 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 pose a threat to 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 serve to 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 abilities can be seen in positive situations, for example, when one is able to 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 relative to 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 of them (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 images that American people have 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.

4.2. 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) were exposed to situations triggering general emotions relative to relational emotions, they showed different mentalizing capacities. More specifically, as reported by Repacholi & Trapolini (2004) children with high scores on the avoidance dimension of the separation anxiety test showed less mentalizing capacity in the case of a mother-child relationship. Consistently, Humfress et al (2002) reported that children who exhibited a less coherent model of attachment were more likely to be rated as exhibiting a dismissing/avoidant style in the attachment interview. Accordingly, attachment representations that are 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 are in line 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 capacity for mentalization compared to general emotional content. Fonagy and Loyten

(2009) took it one step further and discussed it in the content of more insecure patients e.g., individuals diagnosed with borderline personality disorder. They claimed that the stronger the attachment in a particular relationship at a particular moment, the more likely that anomalies in mentalization will emerge in BPD patients. This assumption is confirmed by our TMS-EMG result which adds to Repacholi and Trapolini (2004) and reveals an altered MNS activity following relational emotion relative to general emotion. They observed that relational stimuli are more difficult for avoidant children. Because they demanded more mentalization capacity and they observed that relational stimuli are more difficult for avoidant children. As a result, it demanded more mentalization capacity, and these children were not able to meet that demand due to their avoidant attachment style. However, our result revealed that the processing of relational negative emotion compared to relational positive one, as well as relational negative compared to the general negative one, exerted an upregulation effect on the MNS function of our participants. This could be due to the fact that mentalizing relational stimuli require 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. Here too, we have 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).

4.3. 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 is in line with a previous TMS study on the mirror neurons (Enticott et al., 2012). In the current study, we employed a hand action that is 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 hasn't been found in relational trials.

4.4. Limitation and future direction

Some limitations should be taken into account in the present study. First, this study is an exploratory study 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 involve 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 mag 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.

5. Conclusion

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

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Table 2: Summary of the Mean (SD) IAPS Image ratings for Females.

| <i>Image type</i> | <i>Valence</i> | <i>arousal</i> |
|-------------------|-------------------|-------------------|
| <i>Positive</i> | 7.50(1.57) | 5.41(2.33) |
| <i>Negative</i> | 2.56(1.45) | 5.49(2.15) |
| <i>Neutral</i> | 5.04(1.72) | 4.89(2.15) |

Figure 4 Summary of the protocol for a single TMS trial.

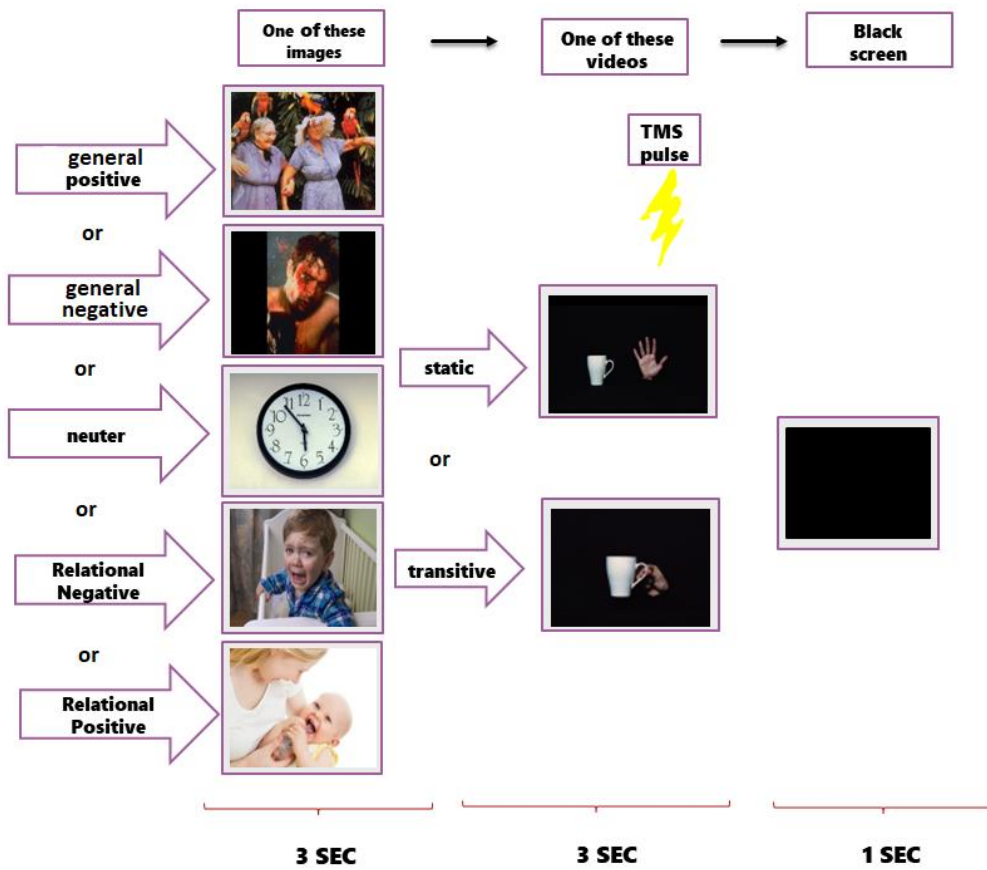
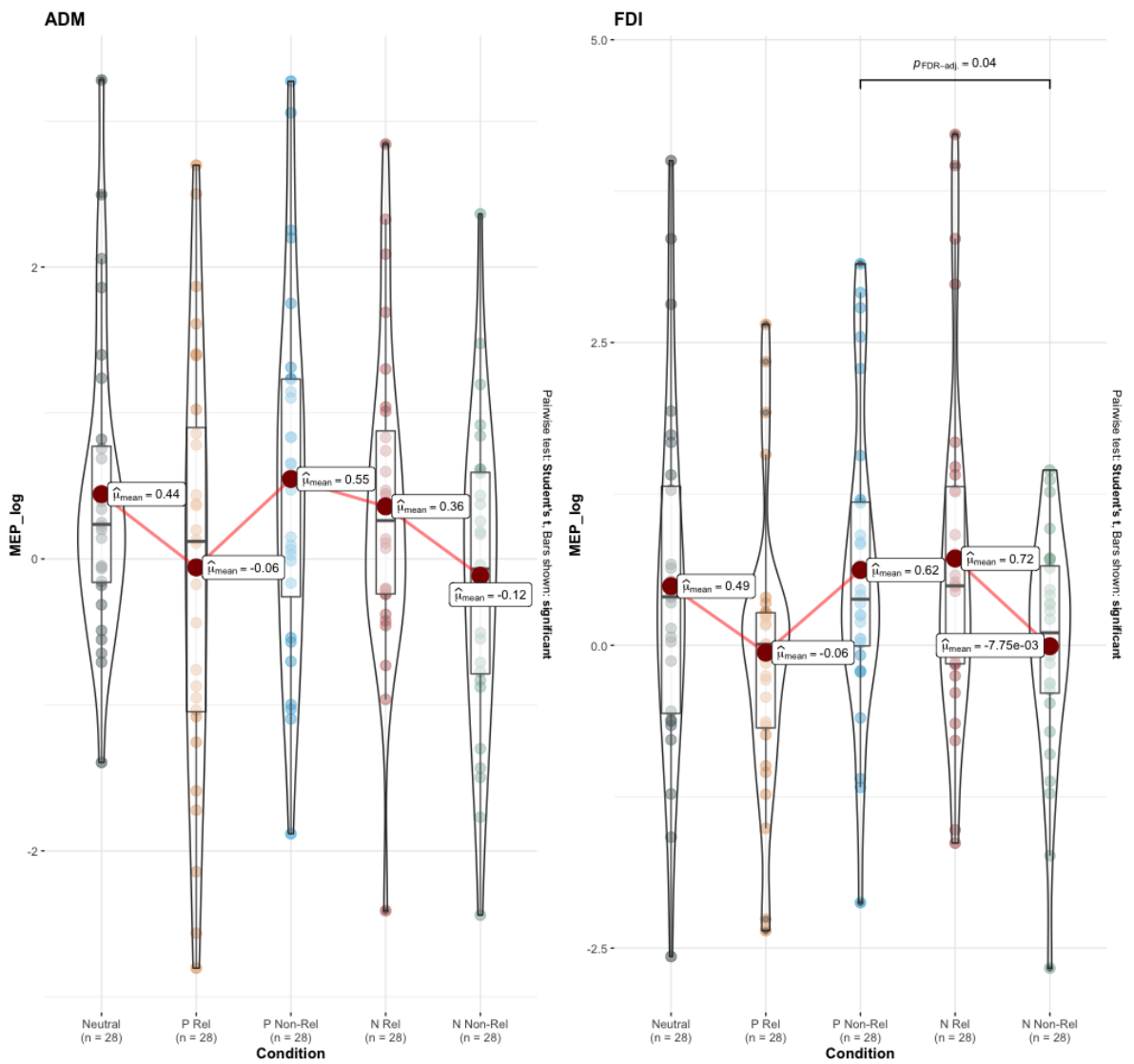


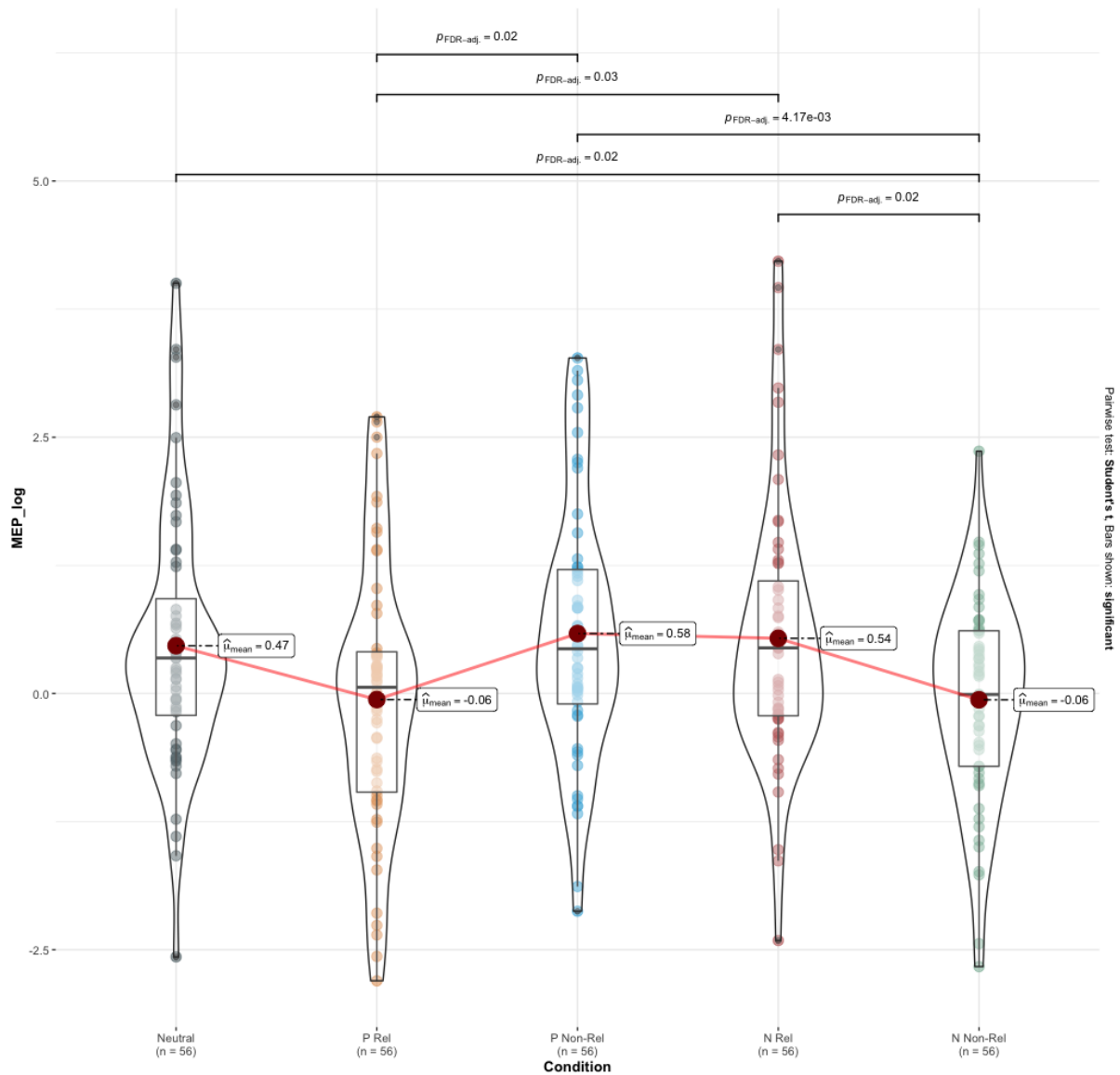
Figure 5: A combination of raw data points with box and violin plots of MEP_log values for the ADM (left) and FDI (right) muscle following Neutral, Relational (Negative and Positive), And General (Positive and Negative) stimuli.

Differences between conditions



Accepted

Figure 6: 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.



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