SUMMARY

Background: The mu rhythm as an 8–13 Hz component generated by the primary sensorimotor cortex, has been noted as a promising marker of the human mirror neuron system (MNS). The MNS discharge both when a movement is executed and when that same movement is observed and imagine, has been considered to facilitate emotion processing. But whether emotional stimulus can influence the ensuing MNS is unclear. The purpose of this study is to understand emotional valence among various factors that can influence the activation of mirror neurons.

Material/Methods: Fifteen healthy female subjects viewed videos of either transitive hand action scenes or still objects for imagery, both preceded by either a neutral, positive, or negative stimulus from the International Affective Picture System. The EEG activity in the Mu rhythms was evaluated by a method which segregated the time-locked for each block.

Results: Our results showed Mu suppression increased during observation and imagery following a negative emotional valence.

Conclusions: These data support the notion that the human mu rhythm is suppressed by action observation and imagery, emotional valence can influence the mu suppression. That results are expected to provide information on the neuropsychology related to the intervention of cognitive rehabilitation (observation or imaging).

Key word: action observation, mu-rhythm, emotional valence, mirror neuron system
INTRODUCTION

The mu rhythm is an 8–13 Hz component (Chatrian, Petersen, & Lazarte, 1959; Gastaut & Bert, 1954; Sabate, Llanos, Enriquez, & Rodriguez, 2012), known as a central, rolandic, sensorimotor, wicket, or arceau rhythm, generated by the primary sensorimotor cortex (Francuz & Zapala, 2011; Hari, 2006); it is most prominent when subjects are resting. The suppression of mu rhythms is considered to reflect an event-related desynchronization (ERD) caused by execution, observation and imagery (Babiloni et al., 2002; Muthukumaraswamy & Johnson, 2004; Perry & Bentin, 2009). These suggests that mu suppression may index the increased activity of the sensory motor cortex. Some researchers link this phenomenon with the operation of the mirror neuron system (MNS).

Due to the ease of registering mu rhythms with Electroencephalography (EEG), the mu rhythm has been noted as a promising marker of human MNS (Francuz & Zapala, 2011). Mirror neurons are groups of perceptual-motor nerve cells (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996) that were discovered when one performs an action and when one observes the same action performed in the premotor cortex of macaque monkeys (Francuz & Zapala, 2011). These mirror neurons have been shown to be related to motor preparation, including in the precentral gyrus of the cortex, inferior parietal lobule, and intraparietal sulcus (Rizzolatti & Craighero, 2004).

Various studies have been conducted on the factors that can modulate the MNS. Differences have been reported according to age (Derambure et al., 1993), sex (Cheng, Yang, Lin, Lee, & Decety, 2008), the observer’s dominant hand (Gardner & Potts, 2010), points of view (Marzoli, Mitaritonna, Moretto, Carluccio, & Tommasi, 2011), motor repertoire (Rizzolatti & Sinigaglia, 2010), the number of stimuli (Dinstein, Thomas, Behrmann, & Heeger, 2008), motion types (Ewan, Kimmond, & Holmes, 2010; Puzzo, Cooper, Cantarella, & Russo, 2011), and the observer’s intention and purpose (Hesse & Fink, 2007; Koch et al., 2010).

Questions have been raised on whether the motor control system is influenced by the emotion control system. Therefore, in the domain of neuroscience, studies have been performed on the functional connection. The MNS is a part of humans’ extensive network; it is presumed to functionally influence behavior, especially from the organization of goal-oriented behavior to emotional processing (Gallese, Keysers, & Rizzolatti, 2004; Tsao, 2009). In terms of neurophysiology, perception reflects the connection between the cortex-limbic areas related to the cortex, including mirror neurons (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003). The emotional network is directly connected to the brain structure that intermediates motor responses (Lang, Davis, & Öhman, 2000). Emotional stimuli has been reported to increases the excitability of the motor cortex (Rossi, Pasqualetti, Tecchio, Pauri, & Rossini, 1998; Pórla, Kaczmarek, Góral-Pórla, Kropotov, Sucharska, & Pachalska 2016) and to modulate voluntary actions (Coombes, Cauraugh, & Janelle, 2006), and suggests that this can influence the activation of the MNS. Enticott et al. (Enticott et al., 2012) performed a study in which partic-
Participants were instructed to observe static or transitive hands along with positive or negative emotional stimuli by using the Transcranial Magnetic Stimulation (TMS). They showed that corticospinal excitability was facilitated during the observation of transitive hands. In particular, during the presentation of negative emotional stimuli, the activation of the dorsal interosseous muscle was found to greatly increase.

Despite the evidence that emotional stimulus elements can modulate the motor control system, most studies have neglected relevant topics. Moreover, studies on correlations between the two factors have been inadequate. The purpose of this study is to understand the process how emotional stimulus can influence the activation of mirror neurons and in particular examine the effects of valence through mu suppression. This study also aims to provide information on the physiological psychology related to the intervention of cognitive rehabilitation treatment (observation or imaging).

**METHODS**

**Participants**

We considered the subjects age, sex, dominant hand; fifteen healthy adult women in their twenties participated in this study. All subjects were physically healthy, had no neurological diseases, had not taken medicines for the purpose of treatment, had normal or correct normal vision, and were right-handed based on the Edinburgh Handedness Inventory. All subjects understood the purpose of this study and submitted written consent prior to their participation in accordance with the ethical standards of the Declaration of Helsinki.

**Materials**

Stimuli hand action consisted of different video clips (3s duration); put the pen in the desk pen pots, fill a cup with water, open bottle caps, grip a cup, squeeze toothpaste, turn over a page on the calendar, put a straw into spray water, glass, attaching sheets of paper with stapler, stir coffee with a stick, write with a pen, cutting a piece of paper with scissors, put small items (eg. bars) into a box, etc. Thirty actions were used, but divided randomly into hand action or target objects.

All this was recorded using a high-resolution digital camcorder and edited with the International Affective Picture System (IAPS). The number of the positive image is 1463, 1999, 2040, 2091, 2391, 4603, 5020, 8190, 8210, 8835; the negative image is 2120, 2661, 2800, 3010, 3060, 3140, 3350, 3400, 6550, 8230.

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1 Valence is a psychological phenomenon that simultaneously causes physiological responses and cognitive behavioral changes; it is a short and subjective feeling. Basic emotion can be divided into positive emotion, which provides stable feelings, and negative emotion, which provides uncomfortable or uneasy feeling. (Enticott et al., 2012).

2 International affective picture system is a standard emotion inducement stimulus set for experimental studies on emotions using slide stimuli. It consists of about 956 slides or digital-format color images and provides visual stimuli, such as situations, incidents, people, animals, and nature, and includes an range of emotional interpretations (Lang et al., 2000).
A 32-channel computerized measurement device WEEG-32 (LXE3232-RF; Laxtha Inc., Korea) set was used. The electrode was attached according to the international 10–20 electrode system, average activity of the linked right and left mastoids was used as a reference; recorded in the mono-polar mode. Electrode impedances were below 10kΩ (usually below 5kΩ) both before and after testing. EEG was sampled at 256 Hz with a 0.5–50Hz analogue bandpass filter, and converted to 12-bit AD using Telescan 2.8 (Laxtha Inc.). Figure 1. shows an example of the order and organization of suggested stimuli.

Three blocks of videos (Neutral, Positive or Negative) were employed. Within each block of videos the two video types were each presented five times. Each image was displayed for 3 s, which was followed by a 1 s action cue screen, then the 3 s observe or imagery video clip, then another black screen.

The emotional valence was verified using the Likert Scale based on subjective evaluation. The Likert scale is a 9-point scale: -4 is very unpleasant (negative valence), 0 is marked with a neutral, 4 is very pleasant (positive valence) (Cuthbert, Bradley, & Lang, 1996). When processing the data, these were converted to 9-points.

**Procedure**

Before the experiment, all subjects answered questions to evaluate their dominant hand. The experiment took place in a dimly-lit, acoustically and electrically isolated booth with a 22-inch monitor about 70 cm in front of them; they were instructed to sit in the chair in a comfortable manner with their hands on the armrest. During an action trial presentation, they were instructed to keep their eyes open and fixated on the computer monitor and to refrain from any movement whatsoever. They were given about 10 minutes to adapt to the test environment.

Each subject was randomly presented to both two different conditions (observation and imagery) in order to exclude the effects of their test order. Each condition was divided into three blocks (neutral, positive, negative) by considering the carry-over effects between consecutive tests. The test continued for 170 seconds including breaks. Each block consisted of a black-background instruction phrase, mutually different positive or negative images selected from the IAPS. A randomised one 7-second set as a video for observation or imagery (see Figure 1); each block was repeated five times, and thus all subjects were provided with 30 sets of stimuli in total. After all the stimuli were finished, the partic-

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**Figure 1. Examples of video stimuli and instructions**

<table>
<thead>
<tr>
<th>Black Screen</th>
<th>Neutral or Positive or Negative</th>
<th>Action cue</th>
<th>Observe or Imagery</th>
<th>Black screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>3s (^{\circ} )</td>
<td>3s (^{\circ} )</td>
<td>1s (^{\circ} )</td>
<td>3s (^{\circ} )</td>
<td>Repeat (^{\circ} )</td>
</tr>
</tbody>
</table>

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Participants expressed the resulting valance by looking at the presented slides again (mean positive: 7.03 [SD = 1.25]; mean negative: 2.25 [SD = 1.36]).

Data analyses

The EEG was time-locked by each block when the visual stimuli (observation or Imagery) presented itself, which was segmented into epochs of 2s beginning at the start of the stimuli; the mu rhythms oscillate for short periods (from .5s to 2s) (Francuz & ZapaBa, 2011). The study, based on IAPS indicate a late positive potential for both positive and negative images lasting beyond 1500 ms, and for as long as 6000 ms; suggesting that the emotion processing component overlapped with the hand observation or imagery (Enticott et al., 2012).

EEG artefacts during the visual stimuli were removed prior to analysis using a cosine window: the eye blink and eye-and-head movements were inspected visually to eliminate epochs with artefacts. The 135 epochs to be analysed for each condition, segmented in an integrated power of the range 8–13 Hz was computed using a fast fourier transform (Kropotov 2009).

Mu suppression is calculated by differences in power ratios between the sub-conditions and baseline conditions (Bernier, Dawson, Webb, & Murias, 2007; Pineda & Hecht, 2009). A ratio was used to control for variability in mu power as a result of individual differences as opposed to mirror neuron activity. However, as ratio data are inherently non-normal such as scalp thickness and electrode impedance, a log transformation was applied for the analyses. A log ratio of less than zero indicates mu suppression (e.g., the activation of the respective brain area), whereas a value of zero indicates no suppression and a value greater than zero indicates enhancement.

Although data were obtained from electrodes across the scalp, mu rhythm is defined as oscillations measured over the sensorimotor cortex, thus only data from C3 and C4 are presented (Bernier et al., 2007; Lepage, Saint-Amour, & Theoret, 2008; Oberman, McCleery, Ramachandran, & Pineda, 2007), these data were compared in repeated ANOVA measures.

All analyses used the statistical analysis program SPSS for Windows 18.0. The statistical significance level was set at p=.05.

RESULTS

The data collected in the experiment was submitted to an analysis of the ANOVA variance (3×2×2) with the within-subject factors of BLOCKS (“Neutral”, “Positive”, “Negative”), CONDITIONS (“Observation”, “Imagery”), and LOCATION (C3, C4). It was found that the variable BLOCKS (“Neutral”, “Positive”, “Negative”) on the suppression of mu rhythms were significantly enhanced (F(2,28)=12.184, p=.001, partial η² = .465) following the presentation of a negative stimulus (“Neutral” vs. “Positive”, p=.872; “Positive” vs. “Negative”, p=.002; “Neutral” vs. “Negative”, p=.000) (See Figure 2).
The effect of the variable CONDITIONS ("Observation", "Imagery") on the suppression of mu rhythms within the test group was not statistically significant ($F(1,14)=1.692, p=.214, \eta^2=.108$). The variable LOCATION affects the level of suppression of mu rhythms ($F(1,14)=3.637, p=.077, \eta^2=.206$), although we found that C3 was a little stronger ($M=-1.358, SD=.018$) than C4 ($M=-1.315, SD=.026$).

DISCUSSION

Studies are being conducted on observation and imagery to maximize motor learning skills. From the current studies it may be suggested that EEG mu power is suppressed by action observation and imagery. Shahid, Sinha, and Prasad (Shahid, Sinha, & Prasad, 2010) found mu suppression in the sensory motor cortex opposite the observed body. Based on Sabate et al. (Sabate et al., 2012), mu desynchronization is regarded as the indicator of motor preparation that went beyond the primary motor area: it was exhibited on the opposite side before the actual execution because programming of action causes an initial activation in the sensory motor area. They also reported that this effect is identically exhibited in the imagery group only if there is intention, and noted that observers expressed more sensitive responses when they previously had the same motor knowledge (Calvo & Castillo, 2005).

We found the significance of emotional valence, it may be that modulate MNS during action observations: emotional processing (especially, Negative) is to increase the activity of MNS. James (James, 1994) reported that if emotional stimuli are sensed in the brain’s sensory area and physical responses occur in the motor cortex, the corresponding feedback is sent to the brain cells, and then
generates emotional experiences. He argued that specific brain regions responsible for the emotions are not present. Cognitive processes are determined based on the initial activation of the sensory cortex and are modulated by attention, memory, and emotion (Damasio AR, 2000). Enticott et al. (Enticott et al., 2012) the valence can influence subsequent mirror neuron activity, which may reflect an adaptive mechanism. While the present study showed the same results, its mechanism was not clarified.

From neurophysiology, cortical and subcortical loops interact between the emotion system and the motor control system through overlapping. Researchers, Stins and Beek (Stins & Beek, 2007), have argued that motor plans are influenced by the information stimuli mediated by emotions, Nummenmaa et al. (Nummenmaa, Hirvonen, Parkkola, & Hietanen, 2008) showed that functional coupling increased by emotional stimuli in the thalamus through the somato sensory cortex, and then the premotor cortex. In particular, they reported that activation further increased while the subjects were observing threatening or harmful scenes: elements that cause negative emotions. In addition, Morgane, Galler, and Mokler (Morgane, Galler, & Mokler, 2005) have reported that stimulus information projected from limbic and sensory areas to the limbic association cortex passes the prefrontal cortex; that these mechanisms promote the emotional and motivational effects of the action. Oliveri et al. (Oliveri et al., 2003) suggest that those interactions are generated between the amygdale, anterior cingulate cortex, and supplementary motor areas. In particular, the amygdaloid body, which is the first subcortical circuitry that has a strong two-way connection with the sensory area: a critical mechanism in the activity of mirror neurons, triggered by negative emotions (Hajcak et al., 2007). This has been described as top-down modulation related to emotions (Brass & Rüschemeyer, 2010).

In the positive blocks, we did not find significant changes in comparison to the neutral blocks. Enticott et al. (Enticott et al., 2012) showed that positive valence can decrease the activation of mirror neurons or can be an obstacle to the subsequent information processing (Calvo & Castillo, 2005; Ode, Winters, & Robinson, 2012; Yuan, Lu, Yang, & Li, 2011). Our findings are in agreement with these studies.

Our study confirmed that the sensorimotor area is activated during action observation and imagery, and is modulated by emotional valence. However, we could not control the subjects internal emotional conditions, did not consider the individual differences and detailed sentiments in response to emotional valence. Therefore, it is difficult to identify which factors caused changes in the activation of mirror neurons.

Such studies could be useful for patients who cannot perform properly during action learning due to environmental and physical limitations, and who can be helped by organizing observation training programmes: a cognitive intervention programme to enhance their actual movement. Moreover, our findings suggest that the application of emotional valence will facilitate the motor learning through action. Therefore, our findings should serve as an appropriate indicator to develop effective observational training programmes.
REFERENCES


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