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Resting-state EEG microstates predict mentalizing ability as assessed by the Reading the Mind in the Eyes test

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ABSTRACT

Microstates analysis of electroencephalography (EEG) has gained increasing attention among researchers and clinicians as a valid tool for investigating temporal dynamics of large-scale brain networks with a millisecond time resolution. Although microstates analysis has been widely applied to elucidate the neurophysiological basis of various cognitive functions in both clinical and non-clinical samples, its application in relation to socio-affective processing has been relatively under-researched. Therefore, the main aim of the current study was to investigate the relationship between EEG microstates and mentalizing (i.e., the ability to understand the mental states of others). Eighty-two participants (thirty-six men; mean age: 24.28 ± 7.35 years; mean years of education: 15.82 ± 1.77) underwent a resting-state EEG recording and performed the Reading the Mind in the Eyes Test (RMET). The parameters of the microstates were then calculated using Cartool v. 4.09 software. Our results showed that the occurrence of microstate map C was independently and positively associated with the RMET total score and contributed to the prediction of mentalizing performance, even when controlling for potential confounding variables (i.e., age, sex, education level, tobacco and alcohol use). Since microstate C is involved in self-related processes, our findings may reflect the link between self-awareness of one's own thoughts/feelings and the enhanced ability to recognize the mental states of others at the neurophysiological level. This finding extends the functions traditionally attributed to microstate C, i.e. mind-wandering, self-related thoughts, prosociality, and emotional and interoceptive processing, to include mentalizing ability.

1. Introduction

Over the past years, the use of electroencephalography (EEG) techniques has improved our knowledge of the neurophysiological basis of both cognitive functions and neuropsychiatric disorders (Khanna et al., 2015; Zhang et al., 2023). Among these techniques, EEG microstates analysis has gained increasing attention among researchers and clinicians as a valid tool for investigating the temporal dynamics of large-scale brain networks with a millisecond time resolution (Kleinert et al., 2024; Schiller et al., 2023; Tarailis et al., 2024).

EEG microstates reflect global patterns of electrocortical events that change dynamically over time in an organized manner (Lehmann et al., 1987; Michel and Koenig, 2018, p. 2). More specifically, brain activity can be represented by specific configurations of scalp field maps (Schiller et al., 2023). These maps remain stable for a short period of time (i.e., approximately 60–120 ms) before rapidly transitioning to a new topographic configuration, which in turn remains stable for a short period of time (Michel and Koenig, 2018; Schiller et al., 2023). Such field maps are thought to reflect the rapidly changing synchronization of certain large-scale brain networks in the resting-state (RS) condition as well as during the performance of a specific cognitive task (Michel and Koenig, 2018; Schiller et al., 2023; Tarailis et al., 2024).

According to the literature (Koenig et al., 1999; Michel and Koenig, 2018), analysis of EEG microstates has identified different classes of brain configurations, typically labelled A to D (although occasionally other maps can be found), each associated with different cognitive and perceptual processes. These microstates are thought to serve as building blocks of spontaneous brain activity, enabling efficient switching between different mental states and cognitive functions (Michel and Koenig, 2018). In addition, changes in the properties of microstates, such as their duration or frequency, have been associated with various neuropsychiatric conditions and altered states of consciousness and thus offer

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potential biomarkers for the diagnosis and understanding of these phenomena (Artoni et al., 2022; Tarailis et al., 2024).

Although microstates analysis has been widely applied to elucidate the neurophysiological basis of various cognitive functions in both healthy subjects and patients with certain neuropsychiatric disorders (Asha et al., 2024; Khanna et al., 2015), its application in relation to socio-affective processing has been relatively understudied (Schiller et al., 2023) even though this approach "*offers a powerful tool for opening the 'black box' of neurophysiological processing underlying our socio-affective mind*" and it is a valuable complementary source of information for researchers and clinicians (Schiller et al., 2023).

Among socio-affective processes, the ability to recognize the mental states of others (e.g., intentions and feelings) from facial expressions, known as "mentalizing" (Frith and Frith, 2006; Kliemann and Adolphs, 2018) or "Theory of Mind" (Enrici et al., 2019; Premack and Woodruff, 1978), is crucial for initiating and maintaining social and affective relationships (Engel et al., 2014). Accordingly, this ability is severely impaired in various psychiatric and neurodegenerative conditions (Johnson et al., 2022; Poletti et al., 2012; Stafford et al., 2023). Traditionally, mentalizing has been studied using the Reading the Mind in the Eyes Test (RMET; Baron-Cohen et al., 2001), a task requiring the recognition of complex affective mental states as expressed by human eyes (Di Tella et al., 2020; Eddy and Hansen, 2020; Pavlova and Sokolov, 2022).

Although some previous studies have investigated the relationship between EEG microstates and specific socio-affective states or traits (for a review see, Schiller et al., 2023), to our knowledge, there are no reports that have investigated the relationship between this type of EEG data and a mentalizing task. Therefore, with the aim of extending previous findings, we investigated whether analyzing RS-EEG microstates can predict performance on the RMET. Recent findings (Pavlova and Sokolov, 2022) show that a network of brain areas is involved in RMET performance, consisting mainly of the dorsomedial prefrontal cortex, the inferior parietal lobule, the precuneus and the temporoparietal junction (TPJ). These areas are recognized to be part of the Default Mode Network (DMN), a large-scale network involved in several integrative higher-order mental functions, such as self-referential processing and mentalization (Andrews-Hanna, 2012; Andrews-Hanna et al., 2014; Buckner et al., 2008). Accordingly, we hypothesized that performance on the RMET would be positively associated with the map most spatially and functionally related to the DMN, namely map C (Michel and Koenig, 2018).

2. Materials and method

2.1. Participants

An a priori power analysis was performed by means of G*Power 3.1 software (Faul et al., 2009) using the following indices: statistical power ($1 - \beta = 80$ %) with an α-error probability of 0.05 and an effect size of $r = 0.30$. According to the power analysis, a sample of at least 82 participants in a two-sided test correlational model was required to achieve satisfactory statistical power. This sample size was also suitable for performing a linear regression analysis considering an effect size of $f^2 = 0.15$, one tested predictor and six predictors in total.

Participants were recruited on the campus of the University of Turin using advertising material. Recruitment lasted from May 2023 to February 2024. Age $\geq$ 18 years, normal or corrected-to-normal vision, Italian nationality and a good understanding of Italian were the only inclusion criteria. Exclusion criteria were: i) left-handedness [i.e., Laterality Quotient < of 61 according to the Edinburgh Handedness Inventory – short form (EHI – SF; Veale, 2014)], ii) self-reported current or lifetime diagnosis of a neurological and/or psychiatric disorder (including intellectual disability and head injury in the month prior to the experiment), iii) use of illicit psychoactive and/or psychotropic drugs in the 2 weeks prior to the EEG recordings.

A checklist with dichotomous items was used to assess the inclusion/exclusion criteria. In addition, data were collected on chronological age, education level (i.e., years of education), sex, ethnicity, and tobacco and alcohol consumption. One-hundred individuals were screened for eligibility. Eighty-two Caucasian participants (thirty-six men; mean age: 24.28 $\pm$ 7.35 years; mean years of education: 15.82 $\pm$ 1.77) met the inclusion criteria and were included in the present study.

2.2. EEG recordings and processing

All EEG recordings lasted 5 min and were performed during eyes-closed RS condition in a semi-dark room. Participants were asked to abstain from alcohol, caffeine and nicotine in the 4 h prior to the recording. EEG acquisitions were performed using a 62-channel headset with ground and reference placed at electrode positions AFz and FCz, respectively (i.e., BrainAmp DC by Brain Products) and impedances were kept below 5 kΩ, see supplementary Fig. 1. Post-processing of the EEG signals was performed using the EEGLAB toolbox for MatLab version 2022.1 (Delorme and Makeig, 2004). Firstly, a downsample from 1000 Hz to 256 Hz was applied, then, a visual inspection was performed to identify evident artifacts. Secondly, a passband filter of 1–40 Hz was used and the average reference was calculated. Thus, the main electrical, muscular, and visual artifacts were removed using Independent Component Analysis (ICA) based on the infomax decomposition algorithm applied to all EEG channels ("runica" tool of EEGLAB). Finally, a three-dimensional spherical spline interpolation was performed on most artifact channels (Ferree, 2006).

2.3. Microstates analysis

In the current study, a k-means clustering approach was applied to determine the optimal set of topographies explaining the EEG signal without considering the polarity of the maps (Brunet et al., 2011; Pascual-Marqui et al., 1995). To determine the optimal number of clusters, a meta-criterion combining different independent optimization criteria was used [Gamma (GAMMA), Point-Biserial (BISERIAL), Davies-Bouldin (DB), Dunn Robust (DUNNR), Krzanowski - Lai (KL), Silhouettes] (Bréchet et al., 2019).

The clustering analysis was performed exclusively on data at time points where the local maximum of the Global Field Power (GFP) occurred, which improved the signal-to-noise ratio (Koenig et al., 2002; Pascual-Marqui et al., 1995). The GFP is a scalar measure of the field strength of the scalp potential and is calculated as the standard deviation of all electrodes at a given time point (Michel et al., 1993). The clustering analysis was performed on a global level and included all study participants. The template maps derived from the cluster analysis across all subjects and recordings were back-fitted to the original data of each individual using a winner-takes all spatial correlation (Koenig et al., 2002). The following temporal smoothing parameters were used to prevent noise during low GFP from interrupting temporal segments of stable topography: window half size = 6, strength (Besag Factor) = 10 (Brunet et al., 2011).

Three temporal parameters were then calculated for each cluster map (Michel and Koenig, 2018; Schiller et al., 2023) according to previous reports (Carbone et al., 2024; Damborská et al., 2019): occurrence (i.e., the frequency of occurrence of a given map within a second regardless of its duration), coverage (i.e., the percentage of total time spent in a given microstate class), and mean duration (i.e., the stability of a given microstate measured in milliseconds). Finally, according to the literature (e.g., Gärtner et al., 2015; Murphy et al., 2020), the expected and observed transition between fitted microstates was computed using the Markov matrix. All analyses were performed using Cartool v 4.09 Software (Brunet et al., 2011).

2.4. Reading Mind in the Eyes Test

Approximately five minutes after the RS-EEG recording, each participant underwent the RMET. Participants were seated in a testing room and looked at a monitor approximately 60 cm from their head. To avoid any possible compilation bias, all participants performed the task in front of the same monitor with the same brightness characteristics (i.e., 60 Hz, 15.6″, 1920 × 1080 FDH resolution). After participants had received the instructions and completed a few practice trials, the main experiment began.

The RMET (Baron-Cohen et al., 2001) consists of 36 black and white photographs of the eyes region on the face. Each photo is presented together with four words describing affective mental states (e.g., item #34: "*aghast*", "*baffled*", "*distrustful*", "*terrified*"). During the task, participants can refer to a glossary to better understand the meaning of the words. Participants must indicate which word (only one is correct) best matches the affective mental state depicted. The total score ranges from 0 to 36, with higher scores indicating better mentalizing performance (for further information about the task see Baron-Cohen et al., 2001). Although some authors reported a multidimensional factor structure of the RMET (Higgins et al., 2024), most studies, including those conducted in Italian samples (Preti et al., 2017; Vellante et al., 2013), supported a unidimensional mode.

2.5. Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences 25 (IBM, Armonk, NY, USA). The relationships between the RMET and the EEG microstates were assessed using Spearman's *rho* correlation coefficients due to the non-normality [i.e., absolute values of kurtosis and skewness >2.0 (George and Mallery, 2010)] of several variables. According to a previous study on EEG microstates (Damborska et al., 2019), a formal Bonferroni correction was applied to each family of comparisons to effectively address the problem of multiple testing.

To evaluate the independent predictive role of EEG microstate parameters on RMET performance, a multiple linear regression analysis was performed. Specifically, the RMET total score was set as the dependent variable and all significant EEG data detected at the bivariate level (i.e., in the correlation analysis) were set as the independent variables. Potential confounding variables related to RMET total score [i.e., age, sex, education level, tobacco and alcohol consumption (Greenberg et al., 2023; Gutierrez-Cobo et al., 2023; Nandrino et al., 2014; Ospina et al., 2016)] were also included in the model. Multiple regression assumptions were tested in accordance with Williams et al. (2013). Multicollinearity was analyzed by calculating the tolerance value and the Variance Inflation Factor (VIF) for each variable. Influential data points were determined using Cook's distances. The results were expressed as standardized beta coefficients (β) and their corresponding *p*-values.

3. Results

A qualitative visual inspection of the EEG signal revealed no relevant evidence of drowsiness and/or unusual patterns (i.e., epileptic discharges) during the recordings. In the current sample, the mean RMET total score was 27.37 ± 2.91. EEG microstates clustering analysis using the meta-criterion detected five distinct maps (i.e., A, B, C, D, F) as shown in Fig. 1. To effectively address the multiple testing issue, a formal Bonferroni correction was applied for each parameter (i.e., occurrence, coverage, and mean duration). Thus, the threshold for significance was $p = 0.01$ (i.e., 0.05/5, the number of maps detected).

The RMET total score was negatively correlated with the duration of Map A (*rho* = −0.226, $p = 0.041$) and the duration of Map F (*rho* = −0.224, $p = 0.043$). However, these correlations were no longer significant after the Bonferroni multiple testing correction (Table 1). No significant correlation was found between RMET performance and the coverage index (Table 2). Finally, a significant positive correlation (*rho* = 0.380; $p < 0.001$) was found between the RMET total score and the occurrence of Map C (Table 3).

Assumptions of multiple regression were respected except for Homoscedasticity (i.e., Breusch-Pagan test $F_{6;75} = 2.255$; $p = 0.047$). Thus, the wild bootstrap with 5000 permuted samples was performed. The model explained 19 % of the RMET variance ($F_{6;75} = 2.856$; $p = 0.015$). Map C occurrence was independently associated (Table 4 and Fig. 2) with RMET total score ($\beta = 0.297$; $p = 0.022$; BCa = [0.115; 0.805]). The statistical factor of tolerance and VIF showed that there were no interfering interactions between the variables (i.e., tolerance values > 0.10 and VIF < 5), and Cook's distances were also adequate (i.e., max value = 0.135). Finally, the transitions between the microstates are shown in Table 5. Specifically, "the expected probabilities are theoretical values based on the count of segments for each template map, while the observed probabilities come from actually scanning the transitions from each and every segment to all the others" (Brunet et al., 2011). In this case, the transitions between microstates are not random because, compared to what would be expected in a random model, some transitions are observed particularly frequently and others less frequently. In this line, a general positive pattern of transitions to the microstates of map A was observed, except for microstates C. In contrast, a repeated and significant negative pattern of transitions to microstate D was reported for all maps except for microstate F. In addition, positive transition values from C to B and negative transitions from C to D are reported. All comparisons between observed and expected probabilities were corrected for 20 transitions (i.e., 5 microstates × 4 transitions) using the Bonferroni correction and no significant correlation was found

Table 1
Association between Reading Mind in the Eyes Test (RMET) total score and EEG microstates duration in all sample ($N = 82$).

		Map A duration	Map B duration	Map C duration	Map D duration	Map F duration
RMET	M ± SD	23.47 ± 5.57	25.46 ± 5.97	32.81 ± 8.50	21.18 ± 5.72	21.40 ± 5.25
	rho	−0.226	−0.184	−0.078	−0.176	−0.224
	p value	0.041	0.098	0.487	0.113	0.043

Abbreviation: M = mean; SD = standard deviation.

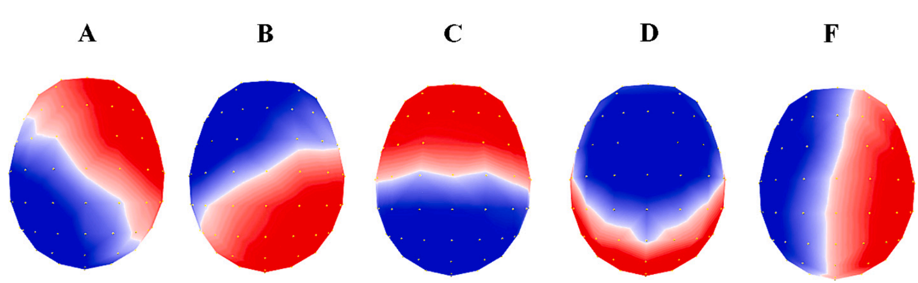

Fig. 1. EEG Microstates maps at global level.

Table 2
Association between Reading Mind in the Eyes Test (RMET) total score and EEG microstates coverage in all sample (N = 82).

		Map A coverage	Map B coverage	Map C coverage	Map D coverage	Map F coverage
RMET	M ± SD	16.86 ± 7.03	22.16 ± 6.59	39.21 ± 12.13	10.18 ± 7.03	11.59 ± 5.91
	rho	0.021	−0.050	0.192	0.001	−0.113
	p value	0.885	0.657	0.085	0.997	0.313

Abbreviation: M = mean; SD = standard deviation.

between the transition values and the RMET total score.

4. Discussion

The main aim of the current study was to investigate the relationship between RS-EEG microstates and the ability to recognize mental states of others, i.e. mentalizing. Consistent with our hypothesis, the results showed that the microstate C was positively associated with the RMET total score. Specifically, the occurrence of microstates C (i.e., the frequency of occurrence of this map within one second regardless of its duration) contributes to the prediction of RMET performance, even when controlling for potential confounding variables (i.e., age, sex, education level, tobacco and alcohol use).

The RS microstate C is thought to be associated with task-negative thoughts, mind-wandering, self-related thoughts, and emotional and interoceptive processing (Tarailis et al., 2024). From a topographical perspective, microstate C is spatially correlated with the synchronization and activity of the DMN (Tarailis et al., 2024), particularly with some of its nodes, including the dorsal medial frontal cortex, and posterior cingulate/precuneus (Michel and Koenig, 2018). The DMN has been theorized as a distributed, large-scale neural system consisting of a number of functionally specialized subsystems involved in several self-referential processes, such as self-consciousness and autobiographical memory (Andrews-Hanna, 2012; Andrews-Hanna et al., 2014). It has been hypothesized that the DMN is also significantly involved in the processing of social information in relation to others, e.g. in mentalizing (Mars et al., 2012; Spreng et al., 2009). Accordingly, a common neurocognitive pathway has been recently identified for the ability to process the mental states of self and others. Indeed, in a recent electrocorticographic study, Tan et al. (2022) showed that self- and other-mentalizing recruit nearly identical cortical areas within the DMN in a common spatiotemporal sequence. Interestingly, the only difference between the processing of self-mentalizing and other-mentalizing was that the mentalizing of others' mental states elicited slower and longer neural activity in the DMN regions than self-mentalizing. The authors suggest that this may be due to the fact that we know ourselves better than others, and that other-mentalizing may therefore require longer processing at more abstract and inferential levels of representation. In light of the above, the relationship we found between a high RS occurrence of microstate C and RMET performance could indicate that the better the ability to self-awareness one's own thoughts and feelings, the better one can recognize the mental states of others. Accordingly, a recent RS study (Schiller et al., 2020) found a link between the trait prosociality (i.e., a personality disposition that partially overlaps with mentalizing ability; Bellucci et al., 2020) and microstate C (more specifically, the transitions from microstate C to A). Thus, our findings extend the functions traditionally attributed to microstate C, i.e. mind-

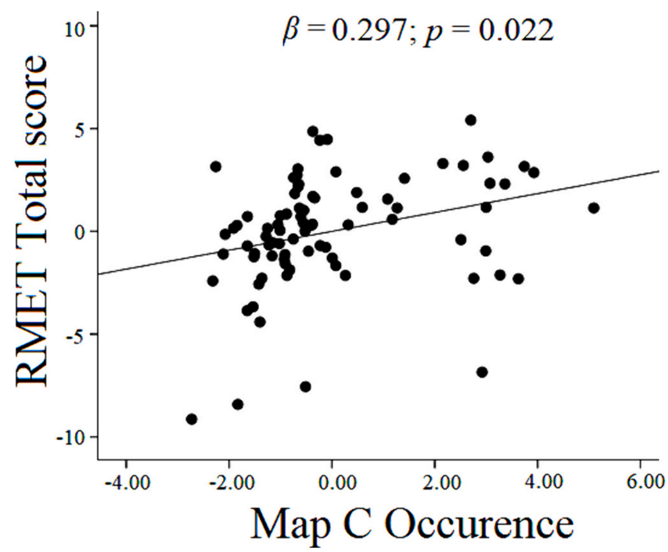

Fig. 2. Scatterplot of the association between Reading Mind in the Eyes Test (RMET) total score and map C occurrence, controlling for confounding variables (i.e., age, sex, education level, tobacco and alcohol use).

Table 3
Association between Reading Mind in the Eyes Test (RMET) total score and EEG microstates occurrence in all sample (N = 82).

		Map A occurrence	Map B occurrence	Map C occurrence	Map D occurrence	Map F occurrence
RMET	M ± SD	5.54 ± 1.92	6.72 ± 1.68	8.48 ± 1.80	3.50 ± 2.04	4.19 ± 1.80
	rho	−0.208	−0.186	0.380	0.066	−0.018
	p value	0.061	0.095	**< 0.001**	0.557	0.873

Abbreviation: M = mean; SD = standard deviation.
In bold significant variables associated with RMET total score.

Table 4
Wild bootstrap linear regression analysis with 5000 permuted samples (*N* = 82).

Dependent variable	R^2	$F_{6;75}$	Independent variables	β	*p*	[95 % BCa]
RMET total score	0.19	2.86			**0.015**	
			Age	−0.042	0.664	[−0.084;0.059]
			Sex	0.195	0.088	[−0.146.2.565]
			Education level	0.174	0.169	[−0.090;0.693]
			Tobacco use	−0.146	0.152	[−1.880;0.111]
			Alcohol use	−0.067	0.204	[−2.141; 0.318]
			Map C occurrence	**0.297**	**0.022**	**[0.115;0.805]**

Note: In bold significant values.
Abbreviation: RMET = Reading Mind in the Eyes Test; BCa = Bias corrected and accelerated.

Table 5
Microstates transition probabilities.

FROM \ TO	A	B	C	D	F
A		0,0150	-0,0506	-0,0035	0,0452
B	0,0765		0,0119	-0,0167	-0,0681
C	-0,0293	0,0288		-0,0198	0,0219
D	0,0565	-0,0173	-0,0213		-0,0183
F	0,0930	-0,0668	-0,0013	-0,0204	

Note. The Matrix represents the observed transition values minus the expected probabilities. Positive values mean more transitions than expected, negative values less. Finally, the colored boxes (i.e., red = less transition; green = more transition) represent the statistically significant differences in the Wilcoxon *z*-test between the observed and expected transition after Bonferroni correction.

wandering, self-related thoughts, prosociality, and emotional and interoceptive processing, to include mentalizing ability.

Finally, a positive transition trend to microstate A and a negative one to microstate D were generally observed, with the exception of transitions from microstate F. Moreover, a positive transition from microstate C to B was reported, while a negative transition from C to D was observed. Despite the observed patterns, there was no significant correlation between the transition probabilities and the RMET total scores, suggesting that although the microstate transitions exhibit distinct and specific RS dynamics, they may not be directly related to performance on the RMET task in this sample. Accordingly, our findings are consistent with previous literature reporting non-random transitions in healthy participants (Murphy et al., 2020), but emphasize the need for further research to investigate the functional relevance of this pattern in different cognitive and emotional tasks.

Although the current findings may be of interest, several limitations should be considered. First, although participants self-reported having no previous and/or current neuropsychiatric disorders, no formal structured clinical interview was conducted. Second, this is a cross-sectional study, so a causal relationship between the associated variables cannot be established and should be investigated by longitudinal studies. Third, we investigated the EEG microstates during a task-free condition, so our results are limited to the RS eyes-closed condition. Therefore, further studies should also be conducted during RMET performance. Finally, although in the present study the results were discussed relative to the current literature, it is important to consider the variability of different analytical approaches for EEG microstates. In this regard, future studies could replicate our findings by conducting an empirical comparison between the observed microstate maps and those in the literature using standardized tools and methods such as the "meta-microstates" available in new and open sources such as the MICROSTATELAB Toolbox (Koenig et al., 2024; Nagabhushan Kalburgi et al., 2024).

5. Conclusion

Despite these limitations, to our knowledge, this is the first RS study that has investigated the relationship between EEG microstates and the ability to recognize the complex affective mental states of others, providing some insights into the neurophysiological processing underlying mentalizing ability. Specifically, our results showed that a high occurrence of microstate C predicted performance in RMET, likely reflecting, from a neurophysiological perspective, the link between self-awareness of one's own thoughts/feelings and the increased ability to recognize mental states of others.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpsycho.2024.112440.

CRediT authorship contribution statement

Giuseppe A. Carbone: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Aurelia Lo Presti:** Writing – review & editing, Methodology, Investigation, Data curation. **Benedetto Farina:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Mauro Adenzato:** Writing – original draft, Supervision, Methodology, Conceptualization. **Rita B. Ardito:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization. **Claudio Imperatori:** Writing – original draft, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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