NeuroImage: Clinical 30 (2021) 102649

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Brain activity of the emotional circuit in Parkinson's disease patients with freezing of gait

Elisabetta Sarasso [a,f], Federica Agosta [a,e], Noemi Piramide [a,e], Elisa Canu [a], Maria Antonietta Volontè [b], Massimo Filippi [a,b,c,d,e,*]

[a] Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy
[b] Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[c] Neurorehablitation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[d] Neurophysiology Service, IRCCS San Raffaele Scientific Institute, Milan, Italy
[e] Vita-Salute San Raffaele University, Milan, Italy
[f] Laboratory of Movement Analysis, IRCCS San Raffaele Scientific Institute, Milan, Italy

ARTICLE INFO

Keywords:
Parkinson's disease
Freezing of gait
Emotional circuit
fMRI

ABSTRACT

Objective: Emotional processes might influence freezing of gait (FoG) in Parkinson's disease (PD) patients. We assessed brain functional MRI (fMRI) activity during a "FoG-observation-task" in PD-FoG patients relative to healthy controls.
Methods: Twenty-four PD-FoG patients and 18 age- and sex-matched healthy controls performed clinical and neuropsychological evaluations, and fMRI experiments including: i) "FoG-observation-task" consisting of watching a patient experiencing FoG during a walking task (usually evoking FoG); ii) "gait-observation-task" consisting of watching a healthy subject performing similar walking tasks without experiencing FoG.
Results: During both tasks, PD-FoG patients showed reduced activity of the fronto-parietal mirror neuron system (MNS) relative to controls. In the "FoG-observation-task" relative to the "gait-observation-task", PD-FoG patients revealed an increased recruitment of the anterior medial prefrontal cortex and a reduced recruitment of the dorsomedial prefrontal cortex and hippocampus relative to controls. Healthy controls in the "FoG-observation-task" relative to the "gait-observation-task" showed increased recruitment of cognitive empathy areas and decreased activity of the fronto-parietal MNS.
Conclusion: Our results suggest that when PD-FoG patients observe a subject experiencing FoG, there is an increased activity of brain areas involved in self-reflection emotional processes and a reduced activity of areas related to motor programming, executive functions and cognitive empathy. These findings support previous evidence on the critical role of the emotional circuit in the mechanisms underlying FoG.

1. Introduction

Freezing of gait (FoG) is a very common phenomenon that patients with Parkinson's disease (PD) usually described as the sensation to have the feet glued to the floor, often triggered by specific conditions such as high cognitive demanding or stressful situations (Nutt et al., 2011). Recent evidence suggests that dysfunctional emotional processing plays a key role in FoG, with this being often related to the experience of fear, anxiety, or proper panic attacks (Avanzino et al., 2018; Ehgoetz Martens et al., 2014, 2018; Gilat et al., 2018; Lagravinese et al., 2018; Lieberman, 2006). The emotional circuit involves an umbrella of brain regions that subtend the entire emotional phenomena, from automatic feelings to conscious experience (Dalgleish, 2004). Among these regions, the basal ganglia (particularly the ventral striatum) are part of the primitive emotional brain, while amygdala, thalamus, hippocampus, anterior cingulate cortex and prefrontal cortex are the proper limbic system characterized by a complex interaction with cognitive top-down control processes (Dalgleish, 2004). Both emotional and cognitive states can influence motor behaviours in healthy subjects and even more in PD patients because of the disrupted automatism of movement (Avanzino et al., 2018; Lagravinese et al., 2018).

The possible involvement of a non-motor system, such as the

* Corresponding author at: Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Via Olgettina, 60, 20132 Milan, Italy.
E-mail address: filippi.massimo@hsr.it (M. Filippi).

https://doi.org/10.1016/j.nicl.2021.102649
Received 13 January 2021; Received in revised form 24 March 2021; Accepted 25 March 2021
Available online 29 March 2021
2213-1582/© 2021 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

emotional circuit, further complicates an already challenging picture, suggesting a problematic interplay between motor, cognitive, and emotional circuits in the mechanisms underlying FoG in PD (Lewis and Barker, 2009). Several functional magnetic resonance imaging (fMRI) studies supported this hypothesis (Agosta et al., 2017; Canu et al., 2015; Ehgoetz Martens et al., 2014, 2018; Filippi et al., 2018, 2019; Gilat et al., 2018; Lewis and Barker, 2009; Piramide et al., 2020). It has been shown that not only the cognitive demand but also the parallel recruitment of limbic areas might interfere with the motor circuit activity, overloading the striatum capability and exacerbating FoG (Ehgoetz Martens et al., 2018; Gilat et al., 2018; Lewis and Barker, 2009). An increased striato-limbic connectivity might contribute to FoG together with the reduced connectivity within the motor circuit and the reduced interaction between the striatum and cortical cognitive areas (Ehgoetz Martens et al., 2018; Gilat et al., 2018; Lewis and Barker, 2009), with some authors observing a strong association between anxiety-eliciting situations and FoG severity (Ehgoetz Martens et al., 2014). However, to the best of our knowledge, no study has investigated the activity of the whole emotional circuit using specific task-based approaches in PD-FoG patients. The majority of fMRI studies have investigated the activity of brain circuits involved in FoG manifestation using motor tasks (sometimes evoking FoG). Alternated plantar/dorsal flexion of the feet or foot movements in a virtual reality environment using MRI-compatible pedals have been commonly used to mimic brain activity during gait or FoG (Agosta et al., 2017; Ehgoetz Martens et al., 2014, 2018; Piramide et al., 2020; Shine et al., 2013). However, these tasks are not specifically targeted to activate the emotional circuit.

A widely used approach to elicit limbic circuit activity during fMRI is the observation of a situation evoking emotional processes. It is well known that watching a person feeling positive or negative sensations or experiencing an emotional-driven situation can evoke individual psychological processes linked to affective ('emotional contagion') or cognitive ('perspective taking') empathy, or to self-reflective thoughts or feelings, with all of these states having specific limbic correlates (Adolphs, 2002; Fan et al., 2011; Johnson et al., 2002). Moreover, watching other's movements and/or sharing other's emotions activate brain loops involved in direct experiences, such as the fronto-parietal and medial frontal mirror neuron system (MNS) related to proper movement and emotion observation, respectively (Rizzolatti, 2005; Singer and Lamm, 2009). Thus, the observation of another person experiencing FoG ("FoG-observation-task") can be useful to activate the emotional circuit in PD-FoG patients because it might relieve a personal experience usually holding high emotional impact. In order to observe the specific effect of watching FoG episodes on the emotional circuit in PD-FoG patients, it is important to compare the "FoG-observation-task" with the observation of a normal gait pattern ("gait-observation-task"). Against this background, this study assessed the fMRI activity of the emotional brain circuit during a "FoG-observation-task" relative to a "gait-observation-task" in PD-FoG patients compared to healthy controls. We expected a reduced activity of the MNS during gait observation in PD-FoG patients relative to healthy controls, as previously suggested (Agosta et al., 2017). In addition, we hypothesized that, in the "FoG-observation-task", healthy subjects would feel empathy for the patient experiencing FoG and activate brain areas implicated in cognitive empathy such as the dorsomedial prefrontal cortex (Abu-Akel and Shamay-Tsoory, 2011; Denny et al., 2012; Iacoboni et al., 2004). On the other hand, in PD-FoG patients relative to healthy controls, we expected an increased activity of brain areas implicated in the elaboration of self-related emotions such as the ventromedial prefrontal cortex (Abu-Akel and Shamay-Tsoory, 2011; Denny et al., 2012; Iacoboni et al., 2004) likely linked to the evoked FoG experience.

2. Material and Methods

2.1. Participants

Twenty-four consecutive, right-handed outpatients with idiopathic PD with FoG were recruited at the Movement Disorders Unit, Department of Neurology, IRCCS San Raffaele Scientific Institute, Milan, Italy according to the following inclusion criteria: (1) occurrence of FoG [i.e., item 3 of the FoG Questionnaire (FoG-Q) ≥ 2] (Giladi et al., 2000); 2) at least two among observation of FoG by an experienced neurologist, the participant's verbal reporting about occurrence of FoG, the recognition of typical FoG in the patient's experience when this was explained to him/her by a physician; (3) no levodopa-induced FoG at the neurological evaluation (ON *vs* OFF); (4) Hoehn and Yahr scale (H&Y) ≤ 3 (Hoehn and Yahr, 1967); (5) stable dopaminergic medication regimen for at least 4 weeks; (6) no dementia (Mini-Mental Status Examination score [MMSE] > 24) (Folstein et al., 1975); and (7) no significant head tremor. Patients underwent clinical, motor, and neuropsychological evaluations, and MRI visits. Eighteen age- and sex-matched, right-handed, healthy controls were recruited by word of mouth among non-consanguineous relatives and institute personnel, and performed the same neuropsychological and MRI assessments. Patients and controls were excluded if they had: (1) medical illnesses or substance abuse that could interfere with cognition; (2) any (other) major systemic, psychiatric or neurological illnesses (including musculoskeletal and visual disturbances); (3) (other) causes of gait impairment such as severe arthrosis or neuropathy; (4) brain damage at routine MRI, including lacunae and extensive cerebrovascular disorders; and (5) contraindications to perform MRI.

The ethical standards committee on human experimentation of IRCCS San Raffaele Scientific Institute approved the study protocol in accordance with the declaration of Helsinki. All subjects provided written informed consent prior to study participation.

2.2. Clinical evaluation

An experienced neurologist performed clinical evaluation during the ON-medication state of patients. Levodopa equivalent daily dose (LEDD) was calculated. The following evaluations were performed: the Unified Parkinson's Disease Rating Scale (UPDRS) III to assess motor impairment (Fahn, 1987); Hoehn and Yahr (H&Y) scale to assess disease severity (Hoehn and Yahr, 1967); clinical history, direct observation during FoG eliciting tasks, UPDRS III FoG score and FoG-Q (Giladi et al., 2000) to assess FoG presence and severity; 39-item PD questionnaire (PDQ-39) to assess quality of life (Peto et al., 1995). UPDRS and H&Y scores were also obtained during OFF-medication state.

2.3. Neuropsychological assessment

A neuropsychological assessment was performed by an experienced neuropsychologist, specifically focusing on the evaluation of executive-attentive functions that are usually altered in PD-FoG patients. Executive functions were assessed with the Phonemic and Semantic Fluency (Novelli et al., 1986), Modified Card Sorting Test (MCST) categories (Caffarra et al., 2002), and Ten point clock test (Manos, 1999). Attention and working memory were evaluated with Trail Making Test part B-A (TMT-B-A) (Giovagnoli et al., 1996), digit span backward (Monaco et al., 2013) and attentive matrices (Spinnler, 1987). Global cognitive functioning was evaluated with the MMSE (Folstein et al., 1975). Scores on neuropsychological tests were age, sex, and education corrected using normative values. The Empathy Quotient (EQ) questionnaire was administered to each subject to assess empathy processing (Baron-Cohen and Wheelwright, 2004).

2.4. Clinical features

PD-FoG patients and healthy controls were similar for age, sex, education and the Empathy Quotient (EQ) questionnaire score (Table 1). Clinical characteristics of PD-FoG patients are shown in Table 1. PD-FoG patients compared to controls performed worse in both executive functions and attention domains, showing a significantly higher score at the TMT-B-A and a significant lower score at the MCST categories (Table 2).

2.5. MRI acquisition

Using a 3.0 Tesla Philips Intera scanner, MRI scans were obtained between 12 AM and 1 PM during OFF-medication state, i.e., at least 12 h after their regular evening dopaminergic therapy administration, to mitigate the pharmacological effects on neural activity. In the case of long-acting medications such as sustained-release dopamine agonists, the patients were asked to suspend their assumption at least 24 h before MRI. Participants, laying down in the MRI scanner couch, were asked to perform two different tasks: i) the "FoG-observation-task" consisting of watching a video in which a PD patient was experiencing FoG during a walking task (Fig. 1A); ii) the "gait-observation-task" consisting of watching a video of a healthy subject performing similar walking tasks (e.g., turning or walking through narrow spaces) without experiencing FoG to adjust for the mere effect of action observation and the relative involvement of the MNS (Fig. 1B). Specifically, in the "FoG-observation-task" the video represented a PD patient walking down a hallway, experiencing FoG while turning 180° right or left and then walking back after FoG has finished. The video lasted 20 s (10 s for left turning and 10 s for right turning) and FoG (shuffling forward type) occurred from second 3 to second 7 (during right turning) and from second 13 to second 17 (during left turning). The same paradigm was used for the "gait-observation task", the only difference being that the task was performed by an healthy subject without FoG during turning. In both the "FoG-observation-" and "gait-observation- tasks" a block design (ABAB) was used, in which the activation A (lasting 20 s) corresponded to the performance of the "FoG-observation-task" or the "gait-observation-task", while during the resting period B (lasting 15 s) subjects were asked to watch the first frame of the subsequent video. Each block (AB) has been repeated 6 times. The same videos were used across blocks. Cushions were used to avoid head motion. Before scanning, participants were familiarized with the experimental conditions and the different videos, and we asked them to focus their thoughts on the feelings induced by the situation. It is important to clarify that healthy controls were also emotively educated to FoG phenomenon before the fMRI scan watching a video of an actor explaining in detail what is FoG and patients' feelings during FoG episodes (*see* Supplemental information).

Table 1
Demographic and clinical features of PD-FoG patients and healthy controls.

	PD-FoG	HC	p
N	24	18	/
Age [years]	66.54 ± 8.13	65.46 ± 8.28	0.43
Sex	17 M / 7F	9 M/9F	0.17
Education [years]	11.44 ± 4.27	10.94 ± 3.70	0.72
FOG-Q	12.04 ± 3.34	/	/
H&Y-ON	2.25 ± 0.36	/	/
H&Y-OFF	2.33 ± 0.41	/	/
UPDRS III-ON	25.05 ± 8.82	/	/
UPDRS III-OFF	32.83 ± 8.74	/	/
PDQ-39	22.37 ± 11.59	/	/
EQ	42.52 ± 7.75	43.12 ± 9.53	0.60
LEDD	954.32 ± 427.83	/	/

Values are means ± standard deviations or frequencies. P values refer to *t*-test for independent groups or Chi-square test for categorical variables. Statistical significance was accepted for values of $p < 0.05$. ***Abbreviations:*** *EQ = Empathy Quotient; FoG-Q = FoG Questionnaire; HC = healthy controls; LEDD = Levodopa equivalent daily dose; PD-FoG = Parkinson's disease patients with freezing of gait; H&Y = Hoehn and Yahr; PDQ-39 = Parkinson's disease Questionnaire; UPDRS = Unified Parkinson's Disease Rating Scale.*

Table 2
Cognitive and behavioral variables in PD-FoG and healthy controls.

	PD-FoG	HC	p
Global cognition			
MMSE	27.71 ± 1.90	29.22 ± 1.00	0.004
Executive functions			
Phonemic Fluency	32.86 ± 8.99	37.89 ± 7.90	0.18
Semantic Fluency	42.14 ± 8.79	44.39 ± 6.25	0.16
MCST, categories	2.33 ± 1.66	3.56 ± 1.95	0.03
MCST, perseverations	14.33 ± 11.29	7.58 ± 9.53	0.48
Attention and working memory			
TMT-B-A	97.96 ± 68.73	36.44 ± 32.66	0.004
Digit span, backward	4.16 ± 0.88	4.64 ± 1.00	0.55
Attentive Matrices	40.73 ± 8.42	50.33 ± 7.18	0.39

Values are means ± standard deviations. For cognitive and behavioral variables, p referred to *t*-test for independent groups. Statistical significance was accepted for values of $p < 0.05$. ***Abbreviations:*** *HC = healthy controls; MCST = Modified Card Sorting Test; MMSE = Mini-mental State Examination; PD-FoG = Parkinson's disease patients with freezing of gait; RAVLT = Ray Auditory Verbal Learning Test; TMT = Trail Making Test.*

A T2*-weighted echo-planar imaging sequence was obtained for each fMRI task, with the following parameters: echo time (TE) = 30 ms, repetition time (TR) = 2500 ms, flip angle = 85°, field of view (FOV) = 240 × 240 mm^2, matrix = 128 × 128, 84 sets of 30 axial slices, slice thickness = 4 mm, acquisition time = 3.5 min for each task.

The following structural brain sequences were also acquired: T2-weighted spin echo (TR = 3500 ms; TE = 85 ms; echo train length = 15; flip angle = 90°; 22 contiguous, 5-mm thick, axial slices; matrix size = 512 × 512; FOV = 230 × 184 mm^2); fluid-attenuated inversion recovery (TR = 11 s; TE = 120 ms; flip angle = 90°; 22 contiguous, 5-mm thick, axial slices; matrix size = 512 × 512; FOV = 230 mm^2); and 3D T1-weighted fast field echo (TR = 25 ms; TE = 4.6 ms; flip angle = 30°; 220 contiguous, axial slices; voxel size = 0.89 × 0.89 × 0.8 mm^3; matrix size = 256 × 256; FOV = 230 × 182 mm^2). All slices were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum.

2.6. fMRI analysis

Changes in blood oxygenation level dependent (BOLD) contrast associated with the performance of the tasks were assessed on a pixel-by-pixel basis, using the general linear model and the theory of Gaussian fields (Worsley and Friston, 1995). FMRI data were analysed using the statistical parametric mapping (SPM12) software 12. Prior to statistical analysis, all images were realigned to the first one to correct for subject motion, spatially normalized into the standard space of SPM, and smoothed with a 10-mm, 3D-Gaussian filter. None of the study participants were excluded from analysis because of motion artefacts (i.e., more than 3 mm of maximum displacement in the x, y and z directions and 3 degrees of angular rotation along each axis). In each subject, a first-level design matrix, where motion parameters were used as regressors of non-interest, was built. Then, specific effects were tested applying appropriate linear contrasts (i.e., BOLD changes occurring during the "FoG-observation-task" and "gait-observation-task" relative to the rest condition was assessed in each subject). Significant hemodynamic changes for each contrast were assessed using t statistical parametric maps (SPMt). A second-level random effect analysis in SPM12 was performed to assess fMRI brain activity differences between groups during the fMRI tasks using an independent *t*-test to compare each task between groups and a paired-*t* test to compare the two tasks within PD-FoG patients and healthy controls, separately. The direct comparison between the "FoG-observation-task" and the "gait-

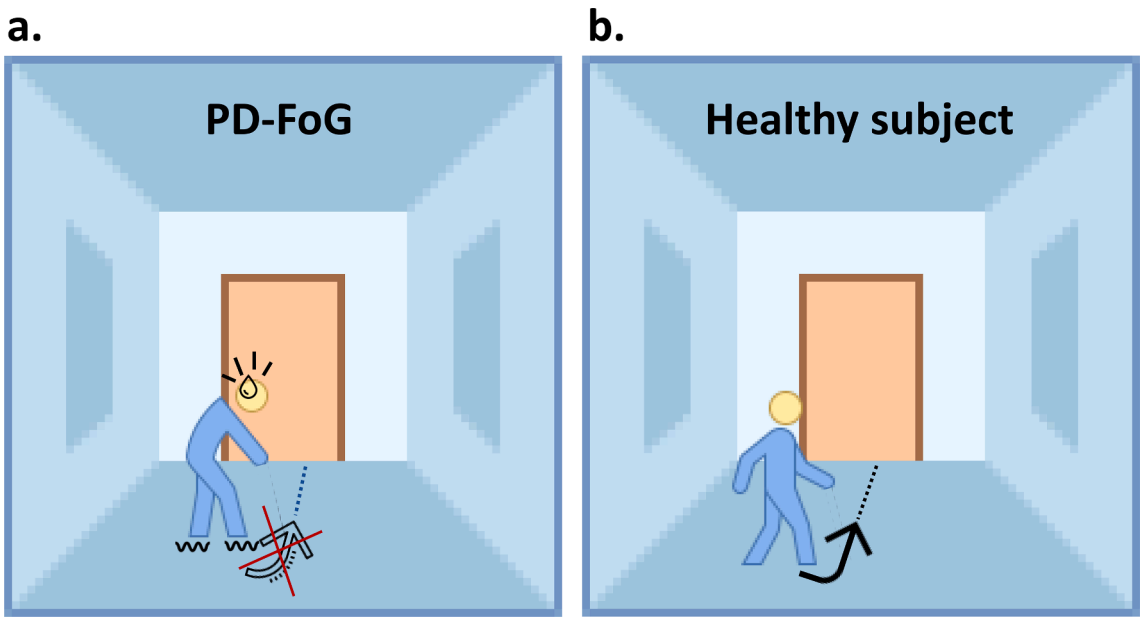

Fig. 1. Functional MRI tasks: A. The "FoG-observation-task" consisted of watching a video in which a patient was experiencing FoG during a walking task; B. The "Gait-observation-task" consisted of watching a video of a healthy subject performing similar walking tasks (e.g., turning or walking through narrow spaces) without experiencing FoG.

observation-task" allowed to evidence the effect of watching FoG excluding the mere effect of action observation (watching the movement of a person) and the involvement of the relative MNS. Differences between the two groups performing the "FoG-observation-task" *vs* the "gait-observation-task" were evaluated using a GLM model, in which group and condition were included as distinct factors (2 × 2 factorial design).

2.7. Statistical analysis

Demographic, clinical, and cognitive data were compared between groups using independent *t*-test for continuous variables or Chi-square test for categorical variables. The normal data distribution was assessed using the Q-Q plot and the Shapiro-Wilk test. All data were analysed using the software SPSS 21. Statistical significance was accepted for values of $p < 0.05$.

Multiple linear regression models were used to assess the correlation between fMRI activity in PD-FoG patients during the "FoG-observation task", FoG-Q values and those cognitive outcomes showing significant differences between PD-FoG and healthy controls. A single mask including areas involved in emotional processes (affective and cognitive empathy, self-reflective thought and fronto-parietal MNS) (Adolphs, 2002; Fan et al., 2011; Rizzolatti, 2005) was created from the AAL brain atlas (Tzourio-Mazoyer et al., 2002) and applied to the SPM dataset using WFU Pickatlas (Maldjian et al., 2003). The mask included the prefrontal cortex, cingulate cortex, premotor/supplementary motor area, inferior/superior parietal cortex, insula, hippocampus, amygdala, striatum and thalamus bilaterally. All findings are shown at $p < 0.001$ uncorrected at the voxel level but only clusters surviving a small volume correction for multiple comparisons, 10 mm radius, cut-off value for significance $p < 0.05$, were presented.

3. Results

3.1. fMRI findings

"FoG-observation-task": PD-FoG vs healthy controls. During the "FoG-observation-task" (Fig. 1A), PD-FoG patients relative to healthy controls showed a reduced activity of the dorsolateral prefrontal cortex including right inferior frontal gyrus pars triangularis and opercularis and of the bilateral supramarginal gyri and right SMA (Fig. 2A; Table 3).

"Gait-observation-task": PD-FoG vs healthy controls. During the "gait-observation-task", PD-FoG patients relative to healthy controls showed a reduced recruitment of the left supramarginal gyrus and an increased recruitment of the right superior frontal gyrus and bilateral hippocampus (Fig. 2B; Table 3).

"FoG-observation-task" vs "gait-observation-task": healthy controls. In the "FoG-observation-task" relative to the "gait-observation-task", healthy controls revealed an increased recruitment of the dorsomedial prefrontal cortex including the right middle frontal cortex and bilateral superior frontal gyri, right SMA and left hippocampus and a reduced activity of left orbitofrontal, inferior frontal and superior parietal cortices (Fig. 2C Table 3).

"FoG-observation-task" vs "gait-observation-task": PD-FoG. In the "FoG-observation-task" relative to the "gait-observation-task", FoG patients revealed an increased recruitment of the left anterior medial prefrontal cortex and a decreased activity of the left angular gyrus (Fig. 2D; Table 3).

"FoG-observation-task" vs "gait-observation-task": PD-FoG vs healthy controls. During the "*FoG-observation-task*" relative to the "*gait-observation-task*", PD-FoG patients relative to healthy controls showed a reduced recruitment of the dorsomedial prefrontal cortex, including the right superior frontal gyrus, and the bilateral hippocampus (Fig. 2E; Table 3).

Correlations. PD-FoG patients showed a correlation between a reduced recruitment of the medial superior frontal gyrus (BA 8) and the severity of FoG according to the FoG Questionnaire (FoG-Q) during the "FoG-observation-task" (Fig. 3A, Table 3). Moreover, PD-FoG patients showed also a correlation between the reduced activity of the right supramarginal gyrus during the "FoG-observation-task" and the altered performance at the TMT-B-A test assessing executive-attentive functions (Fig. 3B, Table 3).

4. Discussion

In this study, we investigated the neural correlates of FoG in the emotional brain circuit during an fMRI "FoG-observation-task" in PD-FoG patients. We asked our patients and healthy controls to watch a

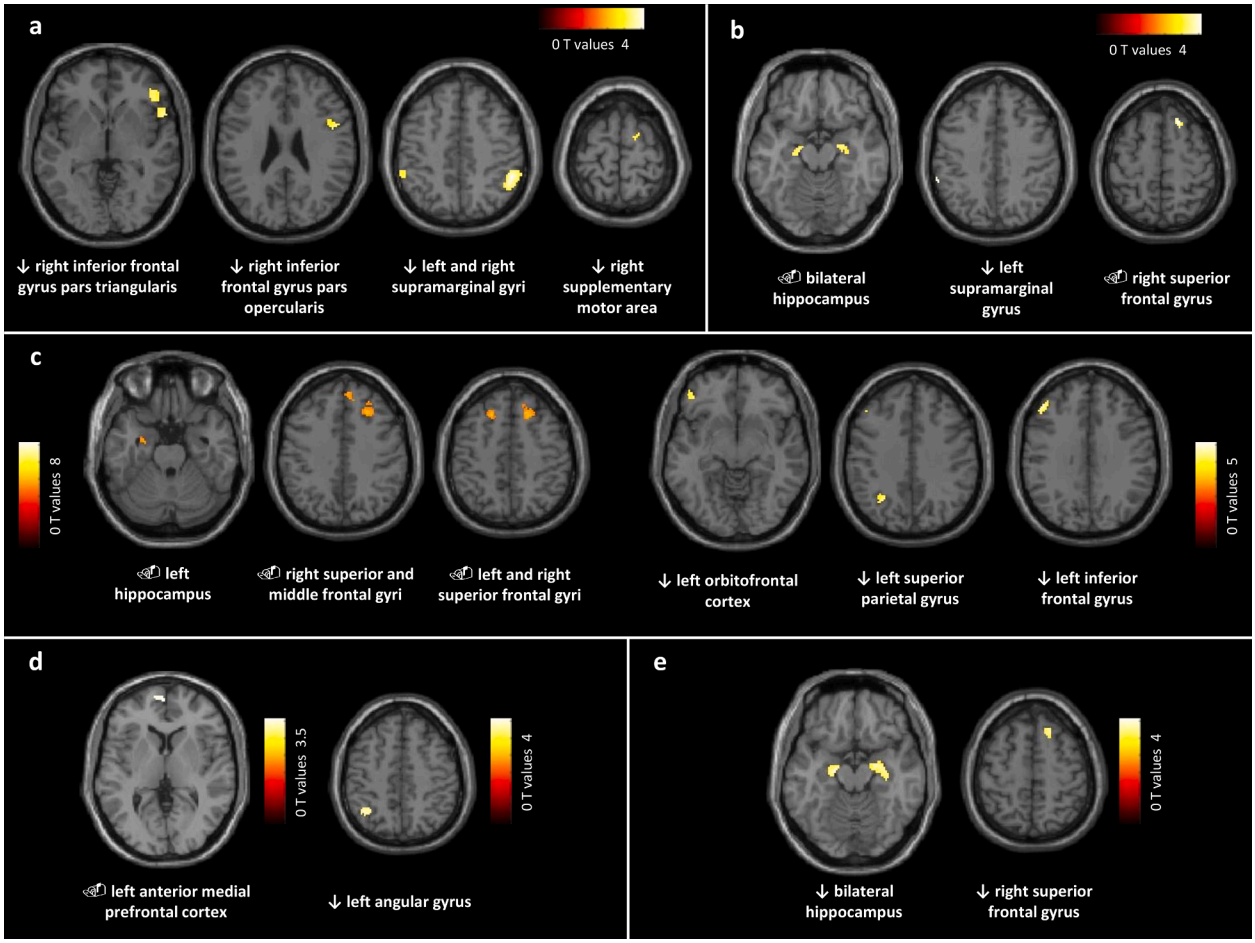

Fig. 2. Task-based functional MRI findings: A. Differences in fMRI patterns of activation between healthy controls and Parkinson's disease patients with freezing of gait (PD-FoG) during the execution of the "FoG-observation-task"; B. Differences in fMRI patterns of activation between healthy controls and PD-FoG during the execution of the "gait-observation-task"; C. Patterns of activation in healthy controls during the comparison between the "FoG-observation-task" and the "Gait-observation-task"; D. Patterns of activation in PD-FoG during the comparison between the "FoG-observation-task" and the "Gait-observation-task; E. Differences in fMRI patterns of activation in PD-FoG compared to healthy controls performing the "FoG-observation-task" relative to the "gait-observation-task". All findings are shown at $p < 0.001$ uncorrected at the voxel level but only clusters passing a small volume correction for multiple comparisons, 10 mm radius, cut-off value for significance $p < 0.05$ were presented. Results are shown on axial sections of the Montreal Neurological Institute standard brain. Colour bars denote T values.

video representing a subject experiencing FoG and to focus their thoughts on the feelings induced by the situation. Patients and controls had comparable empathic capabilities according to the EQ questionnaire, but different areas were recruited in the two groups performing the experimental task.

Healthy controls showed an increased recruitment of the dorsomedial prefrontal cortex, including superior and middle frontal areas (BA 8–9), SMA and hippocampus and a reduced recruitment of the fronto-parietal MNS during the "FoG-observation-task" relative to the "gait-observation-task" (i.e., excluding the mere effect of action observation-watching the movement of a person). As expected, this fMRI pattern suggests that healthy subjects activated areas involved in cognitive empathy to understand other's situations and emotional states (Fan et al., 2011). Particularly the dorsomedial prefrontal cortex is activated when subjects project themselves outside to focus on the perspective and feelings of other people (Abu-Akel and Shamay-Tsoory, 2011; Denny et al., 2012; Iacoboni et al., 2004). Subjects might empathize to such an extent that they might try to overcome FoG recruiting brain areas such as SMA and hippocampus that are usually involved in movement preparation and working memory. Although watching a person experiencing difficulties is expected to cause empathy (Moore et al., 2015) in the observer, we hypothesized that PD-FoG patients did not recruit areas of the empathy circuit as healthy controls during the "FoG-observation-task" because they are more prone to concentrate on to the elaboration of personal emotions likely linked to the evoked FoG experience. In line with this hypothesis, we know that for being involved in the elaboration of emotional circumstances experienced by other people it is necessary to inhibit the tendency to be self-focused (Moore et al., 2015; Singer and Lamm, 2009). Interestingly, during the "FoG-observation-task" relative to the "gait-observation-task", PD-FoG patients showed an increased recruitment of the anterior medial prefrontal cortex. Numerous studies found that the anterior medial prefrontal cortex is recruited during self-reflection, self-awareness, and self-monitoring situations regarding emotional states (Gusnard et al., 2001; Johnson et al., 2002; Lane et al., 1997). We hypothesised that PD-FoG patients are emotively involved during the observation of the FoG phenomenon in the video, reliving a personal experience that usually holds high emotional impact. The increased activity of the anterior medial prefrontal cortex supports this hypothesis. During "gait-observation-task" PD-FoG patients recruited the dorsomedial prefrontal cortex (BA 8) and hippocampus suggesting a preserved ability to project themselves outside and focus on things other than self and now (Abu-Akel and Shamay-Tsoory, 2011; Dalgleish, 2004; Denny et al., 2012; Iacoboni et al., 2004). However, the activity of these areas was reduced in PD-FoG patients compared to healthy controls during the "FoG-observation-task" relative to the "gait-observation-task". These findings supported the hypothesis that PD-FoG patients during the "FoG-observation-task" recruited self-related areas because of their personal emotional involvement and not because of the

Table 3
Regions of fMRI activity differences during the execution of the "FoG-observation-task" and "gait-observation-task" in patients with PD-FoG relative to healthy controls, and during the execution "FoG-observation-task" relative to the "gait-observation-task", respectively in patients with PD-FoG and in healthy controls.

	Area	BA	x	y	Z	T
"FoG-observation-task"						
PD-FoG vs HC	↓ R inferior frontal pars triangularis	45	48	34	0	4.21
	↓ R inferior frontal pars opercularis	44	50	6	26	4.16
	↓ R supramarginal	40	52	−44	46	4.77
	↓ L supramarginal	40	−52	−40	46	4.16
	↓ R SMA	6	18	0	68	3.41
"Gait-observation-task"						
PD-FoG vs HC	↓ L supramarginal	40	−60	−40	40	3.71
	↑ L hippocampus	54	−21	−13	−14	3.96
	↑ R hippocampus	54	24	−8	−20	3.86
	↑ R superior frontal	8	18	26	58	4.32
"FoG-observation-task" vs "Gait-observation-task"						
PD-FoG	↑ L anterior medial prefrontal cortex	10	−4	56	1	3.73
	↓ L angular	39	−38	−56	48	4.34
HC	↑ L hippocampus	54	−27	−12	−15	3.74
	↑ R superior frontal	9	8	46	38	4.62
	↑ R SMA	6	16	24	54	8.39
	↑ R middle frontal	8	26	28	40	4.62
	↑ L superior frontal	8	−18	26	48	5.08
	↓ L orbitofrontal	11	−44	43	11	4.29
	↓ L superior parietal	7	−28	−60	38	4.44
	↓ L inferior frontal	45	−46	34	28	5.27
"FoG-observation-task" vs "Gait-observation-task"						
PD-FoG vs HC	↓ L hippocampus	54	−16	−10	−15	4.40
	↓ R hippocampus	54	24	−9	−15	4.32
	↓ R superior frontal	8	18	25	56	3.96
"FoG-observation-task": negative correlation with FoG-Questionnaire						
PD-FoG	↓ L superior medial frontal	8	−2	28	42	4.25
"FoG-observation-task": negative correlation with TMT-B-A						
PD-FoG	↓ R supramarginal	40	48	−46	44	5.32

X, y, and z coordinates referred to the Montreal Neurological Institute (MNI) space.
Abbreviations: BA = Brodmann area; L = left; HC = healthy controls; PD-FoG = Parkinson's disease patients with freezing of gait; R = right; SMA = supplementary motor area; TMT-B-A = Trail Making Test B-A.

difficulty to experience cognitive empathy. We also found a correlation between a reduced activity of the dorsomedial prefrontal cortex and the severity of FoG in PD-FoG patients suggesting that during the "FoG-observation-task" subjects with more severe FoG experience had less cognitive empathy probably because they are more self-focused and personally involved (Abu-Akel and Shamay-Tsoory, 2011; Denny et al., 2012; Iacoboni et al., 2004).

Moreover, during both the "FoG-observation-task" and the "gait-observation-task" and when comparing the "FoG-observation-task" to the "gait-observation-task", PD-FoG patients showed a reduced activity of the fronto-parietal MNS relative to healthy subjects. The MNS includes different cerebral areas containing mirror neurons that can activate both during the observation of other's movements and during movement execution (Fan et al., 2011; Rizzolatti, 2005; Singer and Lamm, 2009). Our results are in line with previous findings reporting an impaired ability to recruit the fronto-parietal MNS in PD patients, particularly in those with FoG (Agosta et al., 2017). In addition, the fronto-parietal network in PD-FoG patients is usually less efficient both at rest and during gait-related motor task evoking FoG (Canu et al., 2015; Lewis and Barker, 2009; Piramide et al., 2020; Shine et al., 2013). During the observation of a patient experiencing FoG, PD-FoG patients showed an altered recruitment of the same areas (fronto-parietal cortices) involved during a FoG episode. The reduced recruitment of the dorsolateral fronto-parietal network could also be interpreted as an altered ability to activate areas involved in executive functions such as decision-making, conflict-resolution, action planning, working memory and attentive processes (Agosta et al., 2017). All these abilities are known to be affected in PD patients, particularly in PD-FoG subjects (Agosta et al., 2017). As expected, our patients showed altered executive-attentive abilities relative to healthy subjects. Interestingly, we found a correlation between the reduced activity of the right supramarginal gyrus during the "FoG-observation-task" and a lower TMT-B-A performance suggesting that the reduced recruitment of the inferior parietal cortex might be related with an executive-attentive deficit. As previously suggested (Ehgoetz Martens et al., 2018), cognitive alterations might play a role in the mechanisms underlying FoG together with the emotional interference. Hyperactivation of limbic regions may in part reflect failure to engage executive circuitry (Rolls, 2015), a problem that may be exacerbated in the OFF-medication state. However, further studies with more specific fMRI tasks are needed to deepen the possible altered interaction between cognitive and emotional circuits in these patients.

Finally, observing the FoG phenomenon, PD-FoG patients relative to healthy controls also showed a reduced activity of the SMA. It is well known that the SMA is usually hypoactive during motor tasks in PD-FoG patients (Nutt et al., 2011; Shine et al., 2013; Snijders et al., 2016). We have previously demonstrated that the SMA is less recruited not only during the execution but also during the observation of movements in PD-FoG (Agosta et al., 2017). The SMA organizes the preparation and initiation of movements and an impaired function of this area implies an altered motor output, justifying the inability of starting gait or turning while walking in PD-FoG patients (Nutt et al., 2011; Shine et al., 2013; Snijders et al., 2016). Again we can speculate that watching a person experiencing FoG, PD-FoG patients might show an altered activity of brain areas that usually contribute to the FoG phenomenon. These findings offer an insight into the therapeutic management of FoG in PD supporting the hypothesis that a cognitive and/or cognitive-behavioural therapy in addition to motor rehabilitation could contribute to FoG improvement in daily life situations (Chow et al., 2021; Walton et al., 2017).

This study is not without limitations. First, the sample size is relatively small but the difficulty to recruit a sample of PD-FoG patients able to perform an fMRI should be considered. Second, we did not have a control group of PD patients without FoG, thus results and discussion should be interpreted carefully: indeed, without this group it is difficult to determine whether the PD-FoG group showed self-related emotions in response to the observed FoG, or the mere observation of a severely affected PD patient. Third, we did not obtain a measure of anxiety in our sample to correlate against the fMRI outcome. PD-FoG patients are often anxious and these feelings are worsened by the experience of FoG. It can therefore be hypothesized that these feelings would also be generated during a visual imagery task of FoG. Thus, further studies should implement a more comprehensive neuropsychological evaluation including specific behavioral tests to evaluate anxiety related to FoG experience. Fourth, our results should be carefully considered because we applied an anatomic mask of the emotional brain circuit according to an a priori hypothesis and we did not use whole brain voxel-based or Family Wise Error corrections. Future studies with larger samples are needed to validate our preliminary findings.

5. Conclusions

Our results support the idea that PD-FoG patients watching a patient having FoG might re-evoke their personal FoG experience, reducing the activity of areas that are typically involved in motor programming and executive abilities (reduced activation of the SMA and fronto-parietal MNS) and being emotively involved and self-referred (increased activity of the anterior medial prefrontal cortex). Despite a preserved ability

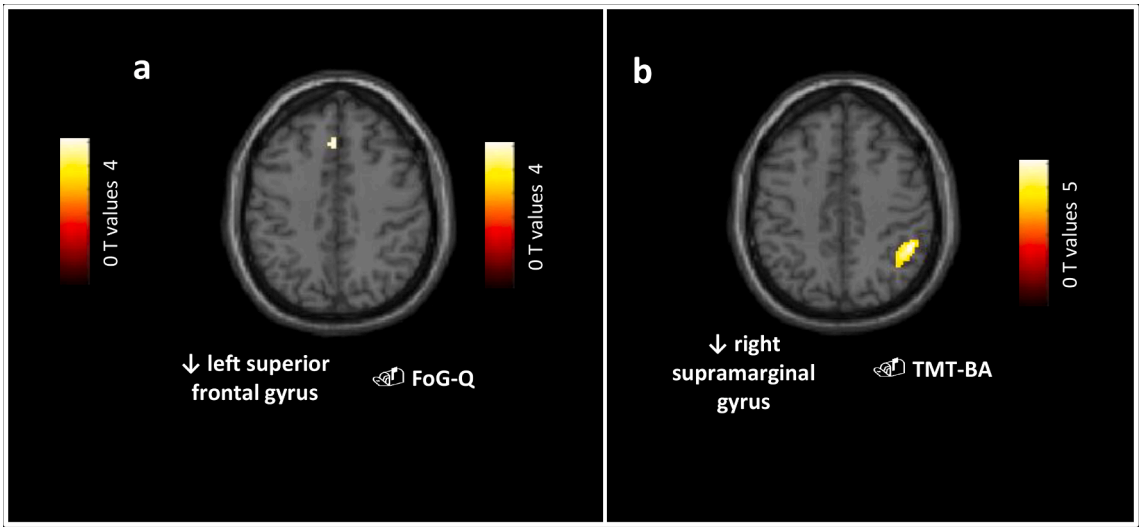

Fig. 3. Clinical-fMRI correlations. A. Negative correlation between the FoG-Questionnaire (FoG-Q) score and the recruitment of the right supramarginal gyrus in PD-FoG patients during the "FoG-observation-task"; B. Negative correlation between the Trail Making Test B-A (TMT-B-A) score and the recruitment of the right medial superior frontal gyrus in PD-FoG patients during the "FoG-observation-task". All findings are shown at $p < 0.001$ uncorrected at the voxel level but only clusters surviving a small volume correction for multiple comparisons, 10 mm radius, cut-off value for significance $p < 0.05$ were presented. Results are shown on axial sections of the Montreal Neurological Institute standard brain. Colour bars denote T values.

to recruit areas involved in cognitive empathy (dorsomedial prefrontal cortex), PD-FoG patients showed a reduced recruitment of these areas relative to healthy controls suggesting a self-focused emotional involvement during FoG observation that might relieve a personal experience usually holding high emotional impact. These findings support an involvement of the limbic circuit and, thus, of the emotional states, in the mechanisms underlying FoG in PD.

Declaration of interest

The authors declare no competing interests related to the current manuscript. Other possible conflicts of interest outside the submitted work are the following:

E. Sarasso, N. Piramide and M.A Volontè report no disclosures.

F. Agosta is Section Editor of *NeuroImage: Clinical*; has received speaker honoraria from Biogen Idec, Novartis, Roche and Philips and receives or has received research supports from the Italian Ministry of Health, AriSLA (Fondazione Italiana di Ricerca per la SLA), and the European Research Council.

E. Canu has received research supports from the Italian Ministry of Health.

M. Filippi is Editor-in-Chief of the *Journal of Neurology* and Associate Editor of *Human Brain Mapping*; received compensation for consulting services and/or speaking activities from Almiral, Alexion, Bayer, Biogen, Celgene, Eli Lilly, Genzyme, Merck-Serono, Novartis, Roche, Sanofi, Takeda, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA).

Funding

This study was partially supported by the Jacques and Gloria Gossweiler Foundation. The funding source had no role in the conduct of the research and/or preparation of the article.

Acknowledgment

The authors thank the patients and their families for the time and effort they dedicated to the research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2021.102649.

References

Abu-Akel, A., Shamay-Tsoory, S., 2011. Neuroanatomical and neurochemical bases of theory of mind. Neuropsychologia 49 (11), 2971–2984.
Adolphs, R., 2002. Neural systems for recognizing emotion. Curr. Opin. Neurobiol. 12 (2), 169–177.
Agosta, F., Gatti, R., Sarasso, E., Volonté, M.A., Canu, E., Meani, A., Sarro, L., Copetti, M., Cattrysse, E., Kerckhofs, E., Comi, G., Falini, A., Filippi, M., 2017. Brain plasticity in Parkinson's disease with freezing of gait induced by action observation training. J. Neurol. 264 (1), 88–101.
Avanzino, L., Lagravinese, G., Abbruzzese, G., Pelosin, E., 2018. Relationships between gait and emotion in Parkinson's disease: A narrative review. Gait Posture 65, 57–64.
Baron-Cohen, S., Wheelwright, S., 2004. The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. J. Autism. Dev. Disord. 34 (2), 163–175.
Caffarra, P., Vezzadini, G., Dieci, F., Zonato, F., Venneri, A., 2002. Rey-Osterrieth complex figure: normative values in an Italian population sample. Neurol. Sci. 22 (6), 443–447.
Canu, E., Agosta, F., Sarasso, E., Volonte, M.A., Basaia, S., Stojkovic, T., Stefanova, E., Comi, G., Falini, A., Kostic, V.S., Gatti, R., Filippi, M., 2015. Brain structural and functional connectivity in Parkinson's disease with freezing of gait. Hum Brain Mapp. 36, 5064–5078.
Chow, R., Tripp, B.P., Rzondzinski, D., Almeida, Q.J., 2021. Investigating Therapies for Freezing of Gait Targeting the Cognitive, Limbic, and Sensorimotor Domains. Neurorehabil Neural Repair 35 (3), 290–299.
Dalgleish, T., 2004. The emotional brain. Nat. Rev. Neurosci. 5 (7), 583–589.
Denny, B.T., Kober, H., Wager, T.D., Ochsner, K.N., 2012. A meta-analysis of functional neuroimaging studies of self- and other judgments reveals a spatial gradient for mentalizing in medial prefrontal cortex. J. Cogn. Neurosci. 24 (8), 1742–1752.
Ehgoetz Martens, K.A., Ellard, C.G., Almeida, Q.J., 2014. Does anxiety cause freezing of gait in Parkinson's disease? PLoS One 9, e106561.
Ehgoetz Martens, K.A., Hall, J.M., Georgiades, M.J., Gilat, M., Walton, C.C., Matar, E., Lewis, S.J.G., Shine, J.M., 2018. The functional network signature of heterogeneity in freezing of gait. Brain 141, 1145-1160.
Fahn, S., 1987. Members of the UPDRS Development Committee. Unified Parkinson's disease rating scale. Rec. Develop. Parkinson's Disease 2, 293–304.
Fan, Y., Duncan, N.W., de Greck, M., Northoff, G., 2011. Is there a core neural network in empathy? An fMRI based quantitative meta-analysis. Neurosci. Biobehav. Rev. 35 (3), 903–911.
Filippi, M., Elisabetta, S., Piramide, N., Agosta, F., 2018. Functional MRI in Idiopathic Parkinson's Disease. Int. Rev. Neurobiol. 141, 439–467.
Filippi, M., Sarasso, E., Agosta, F., 2019. Resting-state Functional MRI in Parkinsonian Syndromes. Mov. Disord. Clin. Pract. 6 (2), 104–117.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12 (3), 189–198.
Giladi, N., Shabtai, H., Simon, E.S., Biran, S., Tal, J., Korczyn, A.D., 2000. Construction of freezing of gait questionnaire for patients with Parkinsonism. Parkinsonism Relat. Disord. 6 (3), 165–170.
Gilat, M., Ehgoetz Martens, K.A., Miranda-Domínguez, O., Arpan, I., Shine, J.M., Mancini, M., Fair, D.A., Lewis, S.J.G., Horak, F.B., 2018. Dysfunctional limbic circuitry underlying freezing of gait in parkinson's disease. Neuroscience 374, 119–132.
Giovagnoli, A.R., Del Pesce, M., Mascheroni, S., Simoncelli, M., Laiacona, M., Capitani, E., 1996. Trail making test: normative values from 287 normal adult controls. Ital. J. Neurol. Sci. 17 (4), 305–309.
Gusnard, D.A., Akbudak, E., Shulman, G.L., Raichle, M.E., 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proc. Natl. Acad. Sci. USA 98 (7), 4259–4264.
Hoehn, M.M., Yahr, M.D., 1998. Parkinsonism: onset, progression, and mortality (Reprinted from Neurology, vol 17, pg 427-442, 1967). Neurology 50, B1-B16.
Iacoboni, M., Lieberman, M.D., Knowlton, B.J., Molnar-Szakacs, I., Moritz, M., Throop, C.J., Fiske, A.P., 2004. Watching social interactions produces dorsomedial prefrontal and medial parietal BOLD fMRI signal increases compared to a resting baseline. Neuroimage 21 (3), 1167–1173.
Johnson, S.C., Baxter, L.C., Wilder, L.S., Pipe, J.G., Heiserman, J.E., Prigatano, G.P., 2002. Neural correlates of self-reflection. Brain 125, 1808–1814.
Lagravinese, G., Pelosin, E., Bonassi, G., Carbone, F., Abbruzzese, G., Avanzino, L., 2018. Gait initiation is influenced by emotion processing in Parkinson's disease patients with freezing. Mov. Disord. 33, 609–617.
Lane, R.D., Fink, G.R., Chau, P.-L., Dolan, R.J., 1997. Neural activation during selective attention to subjective emotional responses. Neuroreport 8 (18), 3969–3972.
Lewis, S.J.G., Barker, R.A., 2009. A pathophysiological model of freezing of gait in Parkinson's disease. Parkinsonism Relat. Disord. 15 (5), 333–338.
Lieberman, A., 2006. Are freezing of gait (FOG) and panic related? J. Neurol. Sci. 248 (1-2), 219–222.
Maldjian, J.A., Laurienti, P.J., Kraft, R.A., Burdette, J.H., 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19 (3), 1233–1239.
Manos, P.J., 1999. Ten-point clock test sensitivity for Alzheimer's Disease in patients with MMSE scores greater than 23. Int. J. Geriat. Psychiatry 14 (6), 454–458.
Monaco, M., Costa, A., Caltagirone, C., Carlesimo, G.A., 2013. Forward and backward span for verbal and visuo-spatial data: standardization and normative data from an Italian adult population. Neurol. Sci. 34 (5), 749–754.
Moore, R.C., Dev, S.I., Jeste, D.V., Dziobek, I., Eyler, L.T., 2015. Distinct neural correlates of emotional and cognitive empathy in older adults. Psychiatry Res. 232 (1), 42–50.
Novelli, G., Papagno, C., Capitani, E., Laiacona, M., 1986. Tre test clinici di memoria verbale a lungo termine: Taratura su soggetti normali. Archivio di psicologia, neurologia e psichiatria.
Nutt, J.G., Bloem, B.R., Giladi, N., Hallett, M., Horak, F.B., Nieuwboer, A., 2011. Freezing of gait: moving forward on a mysterious clinical phenomenon. Lancet. Neurol. 10 (8), 734–744.
Peto, V., Jenkinson, C., Fitzpatrick, R., Greenhall, R., 1995. The development and validation of a short measure of functioning and well being for individuals with Parkinson's disease. Qual. Life Res. 4 (3), 241–248.
Piramide, N., Agosta, F., Sarasso, E., Canu, E., Volontè, M.A., Filippi, M., 2020. Brain activity during lower limb movements in Parkinson's disease patients with and without freezing of gait. J. Neurol. 267 (4), 1116–1126.
Rizzolatti, G., 2005. The mirror neuron system and its function in humans. Anat. Embryol. (Berl) 210 (5-6), 419–421.
Rolls, E.T., 2015. Limbic systems for emotion and for memory, but no single limbic system. Cortex 62, 119–157.
Shine, J.M., Matar, E., Ward, P.B., Bolitho, S.J., Pearson, M., Naismith, S.L., Lewis, S.J.G., Chen, R., 2013. Differential neural activation patterns in patients with Parkinson's disease and freezing of gait in response to concurrent cognitive and motor load. PLoS One 8 (1), e52602. https://doi.org/10.1371/journal.pone.0052602.
Singer, T., Lamm, C., 2009. The social neuroscience of empathy. Ann. NY Acad. Sci. 1156, 81–96.
Snijders, A.H., Takakusaki, K., Debu, B., Lozano, A.M., Krishna, V., Fasano, A., Aziz, T.Z., Papa, S.M., Factor, S.A., Hallett, M., 2016. Physiology of freezing of gait. Ann. Neurol. 80 (5), 644–659.
Spinnler H., G., T., 1987. Standardizzazione e taratura italiana di test neuropsicologici. Masson Italia Periodici, Milano.
Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15 (1), 273–289.
Walton, C.C., Naismith, S.L., Lampit, A., Mowszowski, L., Lewis, S.J.G., 2017. Cognitive Training in Parkinson's Disease. Neurorehabil Neural Repair 31 (3), 207–216.
Worsley, K.J., Friston, K.J., 1995. Analysis of fMRI time-series revisited–again. Neuroimage 2 (3), 173–181.