

Increased inhibitory neurotransmission within the mPFC in PD patients with somatic symptom disorder

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Abstract

The medial prefrontal cortex (mPFC) has been suggested as a neurofunctional substrate for physio-pathological mechanisms underlying somatic symptom disorder (SSD) in Parkinson's Disease (PD). We hypothesize that dysfunction of the mPFC in PD patients with SSD (SSD-PD) may be associated with altered inhibitory/excitatory neurotransmission within the mPFC. With Proton Magnetic Resonance Spectroscopy we investigated the levels of the inhibitory γ -aminobutyric acid (GABA) and excitatory Glx (glutamate + glutamine) within the mPFC. Total creatine (tCr) was used as internal reference. The study cohort included twenty SSD-PD patients, nineteen PD patients without SD (PD) and nineteen age-matched healthy control (HC) subjects. Compared to PD patients and HC subjects, SSD-PD patients showed increased GABA/tCr levels in mPFC. No difference was found by comparing the levels of GABA between PD patients and controls. The Glx/tCr content was not different among groups. We suggest a closed link between inhibitory imbalance within the mPFC and the presence of SSD in PD.

Keywords: medial prefrontal cortex; GABA; Parkinson's Disease; Proton Magnetic Resonance Spectroscopy.

Abbreviations

FAB=the Frontal Assessment Battery

GABA= γ -Aminobutyric acid

Glx=glutamate+glutamine

FLAIR= Fluid attenuation inversion recovery

H&Y=Hoehn and Yahr scale

¹H-MRS=Proton Magnetic Resonance Spectroscopy

MEGA-PRESS=Meshcher-Garwood Point Resolved Spectroscopy sequence

MMSE=Mini Mental State Examination

MRI=Magnetic Resonance Imaging

PD=Parkinson's Disease

mPFC=medial prefrontal cortex

SSD=somatic symptom disorder

TFE= Turbo field echo

UPDRSIII=Unified Parkinson's Disease Rating Scale III

vACC=ventral anterior cingulate cortex

1. Introduction

Parkinson's Disease (PD) is a progressive movement disorder associated with a high prevalence of somatic symptom disorder (SSD)¹ that ranges between 7.0% to 66.7%²⁻⁷ as compared to the general population that, in contrast, is characterized by an occurrence of SSD that ranges between 3.5% and 18.4%.⁸ The peculiar vulnerability of PD patients to SSD, suggested by the prevalence rates, was recently highlighted by a functional Magnetic Resonance Imaging (fMRI) resting state study that demonstrated the presence of specific alterations of Default mode (DMN) and salience (SN) networks connectivity in PD patients with SSD (SSD-PD).⁹

Recent studies suggested a neuro-functional substrate for the aetiology of SSD in the medial prefrontal cortex (mPFC).^{10,11} In particular, fMRI studies on patients with SSD showed hypo-activity of the mPFC and hyper-activation of subcortical structures including striatum, insula, and amygdala.¹²⁻¹⁴ From a physiological standpoint, the mPFC, by through regulating the cortico-subcortical interactions, is critically involved in the integration of body perception with cognitive-affective information.¹⁵ The strength of local intrinsic activity in the mPFC¹⁶ as well as its functional connectivity with the rest of the brain¹⁷⁻¹⁹ is closely dependent on the balance of inhibitory/excitatory (I/E) neurotransmission in the same region.

We thereby hypothesize that altered neurotransmission within the mPFC of the major excitatory and inhibitory neurotransmitters, glutamate and γ -Aminobutyric acid (GABA), characterizes SSD-PD patients.

To explore this hypothesis we investigated the balance of inhibitory/excitatory neurotransmission within the mPFC in a cohort of fifty-eight individuals including twenty SSD-PD patients, nineteen PD patients without SSD (PD), and nineteen healthy age-matched subjects (HC). Proton Magnetic Resonance Spectroscopy (¹H-MRS)^{20,21} with a Meshcher-Garwood Point Resolved Spectroscopy sequence (MEGA-PRESS), was used to assess the levels of GABA and glutamate + glutamine (Glx) within the mPFC in each study participant.

2. Material and Methods

2.1. Study sample

This study was approved by the Local Institutional Ethics Committee and was performed according to the Declaration of Helsinki (1997) and subsequent revisions. All participants gave written informed consent and they were enrolled at Neurology Clinic of the University "G. d'Annunzio" of Chieti-Pescara, Italy. Exclusion criteria were prior history of major medical conditions, head injury, psychiatric or neurological disorders (exception made for those having clinical relevance for the study), history of substance abuse, any MRI contraindication. PD diagnosis was carried-out according to UK Brain Bank Criteria. Mental status and SD were assessed using the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders and by patient interview as detailed in the next section. All patients underwent computerized tomography/MRI and DAT before entering the study. Patients at prodromal or clinical stages of dementia were excluded at enrollment stage. Unified Parkinson's Disease Rating Scale III (UPDRS-III) and Hoehn and Yahr (H&Y) scale investigated the presence of extrapyramidal signs. All subjects were evaluated for global cognition using the Mini Mental State Examination (MMSE). Frontal functioning was assessed using the Frontal Assessment Battery (FAB). Control

subjects were additionally evaluated for: i) attention skills, sustained attention, divided attention, task coordination and set-shifting using the Trail Making Test; ii) selective attention using attentional matrices; iii) verbal short-term and long-term memory using Babcock Story Recall Test; iii) auditory working memory using the forward and backward Digit Span tests. None of the patients or control subjects was treated with anxiolytic or antidepressive drugs. PD patients were under stable doses of dopaminomimetic treatments. Drug treatments for PD were withdrawn the day before MRI acquisition.

2.2 Evaluation of somatic symptom disorders

PD patients were subcategorized in two groups, i.e. PD and SSD-PD. This subdivision was based on direct observation of symptoms in the year (six months before/after) coincident with the diagnosis of neurodegenerative diseases. As a second evaluation, patients underwent semi-structured interviews by a rater blinded to neurological diagnoses. The interviews, which were based on DSM-5, assessed somatic complaints with examples and a checklist presented to patients and caregivers, focusing on somatic symptoms traits (i.e. dependency, mannerism, viscosity, adoption of a sick role, histrionic dramatic descriptions of illness). Past somatic symptoms were also evaluated by including information from prior hospital records and from reports from patient's General Practitioner over the past 4-20 years. The Neuropsychiatry Inventory (NPI) assessed Somatic type delusional disorders.²² Study participants were also tested by the Symptom Questionnaire (SQ).²³ The Diagnostic Criteria for Psychosomatic Research (DCPR) were used to ensure a clinimetrically valid analysis of the symptoms in a neurodegenerative condition.^{9,24-26}

2.3 MR protocol

MR data were collected by means of a Philips Achieva 3 Tesla scanner (Philips Medical Systems, Best, The Netherlands) using a whole-body radiofrequency coil for signal excitation and an 8-channel phased-array head coil for signal reception. Structural images were acquired using a 3D T₁-weighted Turbo Field-Echo sequence (TFE, TR/TE=11/5 ms, slice thickness of 0.8 mm). T₂-weighted fluid attenuation inversion recovery (FLAIR, TR/TE=12000/120 ms, slice thickness of 4 mm, FOV=230 mm x 140 mm x 190 mm) images were also acquired to exclude the presence of concomitant pathologies. MEGA-PRESS sequence (TR/TE=2000/68 ms, 320 averages) was used to assess the GABA, Glx and tCr signals.^{20,21} Spectra were obtained from a ¹H-MRS voxel centered on the mPFC and included the ventral portion of anterior cingulate cortex (ACC), which is highly relevant for emotional processing (**Fig. 1A**).²⁷ To preserve a good signal to noise ratio,²¹ the ¹H-MRS voxel size was of 2.0 (anterior-posterior) x 3.0 (left-right) x 3.0 (skull-caudal) cm³. Thus, inevitably, small portions of CSF and dorsal frontal areas marginally contribute to the ¹H-MRS signal. Methodological details on MEGA-PRESS data acquisition were extensively reported in our recent studies.^{28,29} A representative analyzed patient spectrum is reported in **Fig. 1B**. Because ¹H-MRS could be sensitive to acute pharmacological effects, drug assumption was withdrawn 24 hours before the MRI session.

2.4 Data analysis

GANNET tool (Edden, Puts, Harris, Barker, & Evans, 2014) was used to quantify GABA/tCr and Glx/tCr in each spectrum using default parameters, including frequency and phase correction of time-resolved data using spectral registration (**Fig.**

1C-D). GANNET extension was used to mask the ^1H -MRS voxels and coregister it on structural image (Harris, Puts, & Edden, 2015). The definition of the grey matter (GM) and white matter (WM) within the ^1H -MRS voxel and the measuring of the tissue volumes were obtained by combining the outputs of “recon-all” (FreeSurfer)³⁰ and “fslmaths/fslstats” (FMRIB Software Library-FSL)³¹ command lines. All generated images were visually checked in FSL View to validate the location of the MRS voxel and confidence in tissue segmentation. ^1H -MRS findings were reported as metabolites/tCr because: 1) this quantification shows performance equal to, or better than, water referencing;³² 2) the tCr concentration are independent from anxious-²⁸ or neurodegenerative-related disorders;³³ 3) tCr-referenced metabolite values are expected to be less sensitive to tissue atrophy. Because the edited signal at 3 ppm contains contributions from the both macromolecules and homocarnosine, the signal is labeled as GABA+.³⁴ Due to low signal to noise ratio, Glx/tCr signal were quantifiable for fifty-four patients including nineteen HC subjects, sixteen ns-PD patients, and nineteen s-PD patients.

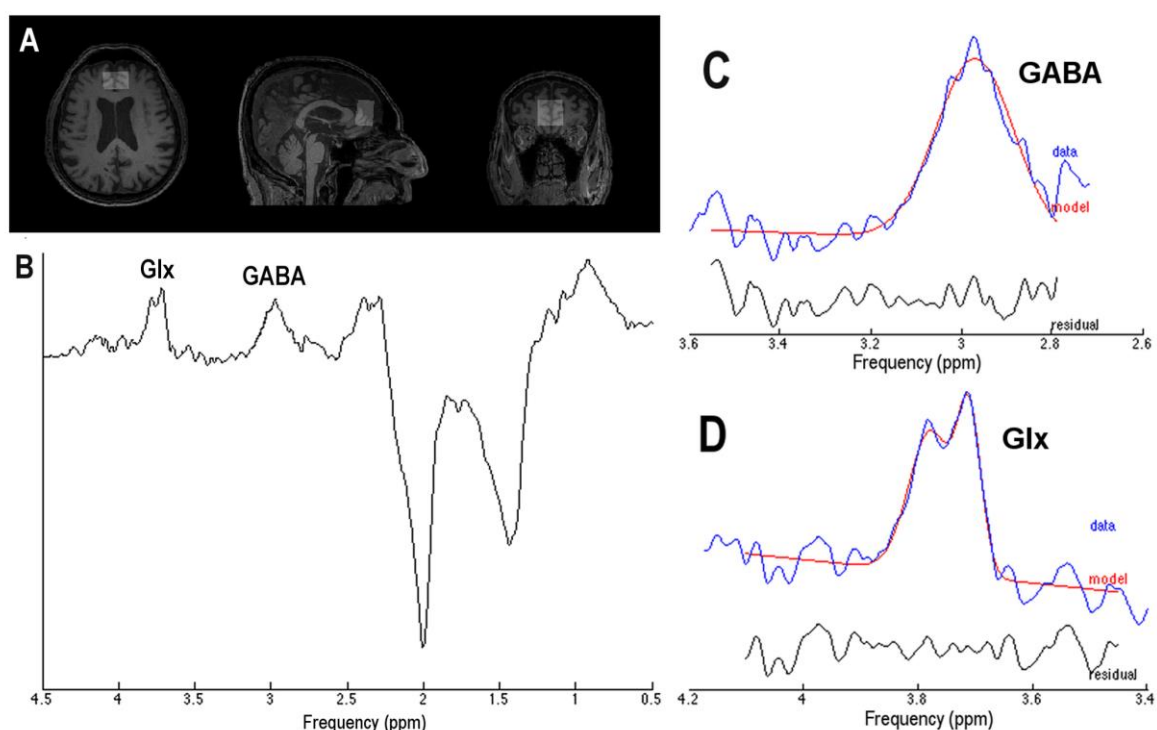


Figure 1. Proton magnetic resonance spectroscopy (^1H -MRS). Panel A: a voxel of 2.0 (anterior-posterior) x 3.0 (left-right) x 3.0 (cranio-caudal) cm^3 is centered on the ventromedial prefrontal GANNET-edited MR spectra. Panel C: representative processing of GABA+ peak at 3.02 ppm. Panel D: representative processing of pseudo-doublet peaks of Glx at 3.65-3.75 ppm. In the panels C-D, the representative GANNET-edited spectrum is reported in blue, the estimated GABA model is indicated in red and noise is shown in black.

2.5 Statistical analysis

One-way analysis of variance and Bonferroni post-hoc test were used to evaluate the group differences regarding demographic/clinical data and levels of metabolites/tCr. Models take in account the effect of age, educational level, gender, and GM within ^1H -MRS voxel. Moreover, t-test for two independent samples was used to assess differences between patient groups on disease duration and scores of the UPDRS-III and H&Y scales. Chi-squared test was carried out to assess the presence of gender group difference.

3. Results

Table 1 summarizes demographic, clinical and structural imaging features. **Suppl. Table 1** summarizes SQ-somatic symptoms subscale items results in each group.

The content of GABA+/tCr within the mPFC was significantly different among groups ($F_{2,57}=23.979$, $p<0.001$). Post-hoc analysis showed that the GABA+/tCr levels were higher in SSD-PD patients as compared to PD patients ($p<0.001$) and HC subjects ($p<0.001$). No difference on GABA+/tCr levels was observed between PD patients and HC subjects ($p=1.000$). Analysis of covariance excluded the effect of age ($F_{1,57}=2.808$, $p=0.100$), educational levels ($F_{1,57}=0.607$, $p=0.440$), gender ($F_{1,57}=0.054$, $p=0.816$), and GM volume ($F_{1,57}=0.023$, $p=0.880$) on GABA+/tCr. Difference on GABA+/tCr between SSD-PD and PD groups was confirmed by performing general linear model on the two groups (group difference: $F_{1,38}=4.791$, $p=0.038$) and including the disease duration as nuisance factor (disease duration effect: $F_{1,38}=44.948$, $p<0.001$). No significant difference in the Glx/tCr amount was found among groups ($F_{1,53}=2.394$, $p=0.102$).

No significant difference was shown among groups for age ($F_{2,57}=0.070$, $p=0.932$), education level ($F_{2,57}=1.946$, $p=0.153$), gender ($\chi^2=2.124$, $p=0.346$), MMSE ($F_{2,57}=0.446$, $p=0.643$), FAB ($F_{2,57}=1.299$, $p=0.281$) and volume of GM within the $^1\text{H-MRS}$ voxel ($F_{2,57}=2.355$, $p=0.104$). The disease duration ($t_{2,57}=3.059$, $p=0.89$) and the scores of UPDRS-III ($t_{37}=0.180$, $p=0.858$) and H&Y ($t_{37}=-0.197$, $p=0.845$) scales were comparable between PD groups.

Table 1. Demographic, clinical and structural imaging features

	HC	PD	SSD-PD	Post-hoc analysis		
				HC vs. PD	HC vs. SSD-PD	PD vs. SSD
Age	66.4±9.2	66.0±8.1	67.0±7.8	NA	NA	NA
Gender	53%	74%	55%	NA	NA	NA
Educational	10.1±4.5	10.5±5.0	7.8±4.3	NA	NA	NA
MMSE	27.8±1.1	27.0±4.1	26.9±4.1	NA	NA	NA
FAB	16.2±1.3	14.8±3.38	15.10±3.06	NA	NA	NA
Disease	0.00±0.00	3.89±1.82	4.90±2.59	NA	NA	NA
UPDRSIII	0.00±0.00	14.11±5.79	14.05±6.47	NA	NA	NA
H&Y	0.00±0.00	1.63±0.50	1.60±0.50	NA	NA	NA
GABA/tCr	0.06±0.01	0.05±0.01	0.07±0.01	1.000	p<0.001	p<0.001
Glx/tCr [#]	0.07±0.02	0.08±0.03	0.08±0.02	NA	NA	NA
GM	10380±715	9811±654	10052±891	NA	NA	NA
NPI-TOT	NA	1.9±0.8	5.5±1.8	PD vs. SSD-PD, $p<0.05$ (t-test)		

Values are expressed as the mean ± standard deviation (SD). Bold values are statistically significant. [#]Data on Glx/tCr was available for fifty-four patients including nineteen HC subjects, sixteen PD patients, and nineteen SSD-PD patients. *Keys:* FAB, Frontal Assessment Battery; HC, healthy control; H&Y, Hoehn and Yahr; MMSE, Mini-Mental State Examination; PD, Parkinson's Disease patients without somatic symptom disorder; SSD-PD, Parkinson's Disease patients with somatic symptom disorder; UPDRS-III, Unified Parkinson's Disease Rating Scale III.

Supplementary Table 1. SQ-subscale items for each group.

	HC	ns-PD	s-PD
Anxiety	4.9±2.2	5.6±3.3	6.2±4.4
Depression	2.7±1.6	2.9±1.6	5.1±3.6
Somatic	4.9±2.2	5.7±2.7	12.9±2.9
Anger, Hostility	3.8±1.2	6.1±3.8	5.2±2.8
Relaxation	0.5±0.8	0.6±0.6	2.0±0.8
Contentment	0.7±1.4	0.8±0.8	2.3±1.7
Physical Wellbeing	1.8±0.8	2.4±0.9	4.2±1.5
Friendliness	0.5±0.6	0.9±1.1	0.9±0.5
Feeling Healthy (%)	100	85	61
Feeling of not enough air (%)	21	9	50
Feeling fit (%)	100	100	50
Heavy (%)	31	28	87
No pain anywhere (%)	31	37	8
Arms (%)	63	76	50
Appetite (%)	8	0	34
Tight (%)	23	26	87
Choking (%)	16	12	26
Feeling of pressure in head or body (%)	8	12	79
Weak arms or legs (%)	8	26	100
No aches anywhere (%)	63	66	21

Values are expressed as the mean ± standard deviation (SD).

4. Discussion

In the present study, we investigated the levels of GABA and Glx within the mPFC in a cohort of SSD-PD patients, PD patients, and HC subjects. The analysis indicated that the content of GABA was increased in SSD-PD patients when compared with PD patients or HC subjects. In contrast, the levels of Glx within the mPFC were not significantly different among groups.

GABA is the leading inhibitory neurotransmitter in the human brain. GABAergic tone induces a persistent ambient level of inhibition, influencing the neuronal excitability as well as the selective activation of cortical neuronal networks.³⁵⁻⁴⁰

The mPFC contains inhibitory GABA-ergic inter-neurons shaping the activity of excitatory glutamatergic projections to a broad set of subcortical structure⁴¹s including the amygdala⁴² and the striatum.⁴³ From a physiological standpoint, the mPFC-amygdala-striatum circuit is implicated in the integration of body perception and cognitive-affective information to sensory stimuli (REF da intro). The mPFC-striatum projections, in addition, are part of the cortico-basal ganglia-thalamo-cortical loop, a key regulator of thalamic filtering on the flow of sensory inputs from midbrain to cortex.⁴⁴ fMRI studies reported that SSD-PD patients are characterized by mPFC hypo-activation¹² as well as hyper-activation in amygdala,^{12-14,45} and striatum.¹² Thus, we speculate on an inhibitory imbalance within the mPFC that results in a persistent GABAergic conductance on glutamatergic projections that triggers an up-regulation of the subcortical activity. This phenomenon could lead to a subsequent over-responsivity of the amygdala and striatum that promotes a dysfunctional integration of body perception and cognitive-affective information with sensory stimuli. Increased striatal activity, in addition, could lead to a neurotransmitter imbalance in the cortico-striato-thalamo-cortical (CSTC) circuit, which, in turn, results in an opening of the thalamic filter and sensory overload of the cortex (**Figure 2**).⁴⁴ The clinical

consequence would be a dysfunctional integration of body perception and cognitive-affective information with emotional state.⁴⁶

In addition, it should be considered that the mPFC is an anterior region of the DMN.⁴⁷ Under physiological conditions, the inverse coupling between the DMN and the salience network (SN) plays a central role in switching attention between external and internal salient stimuli.⁴⁸ In line with the increased GABA levels that we have observed in this study, we have recently found that, in SSD-PD patients, the DMN is hypo-active and hypo-connected to the SN.⁹ Thus, we hypothesize that the reduction of DMN activity as well as of its connectivity with SN could lead to dysfunctional activity in selecting significant peripheral stimuli.

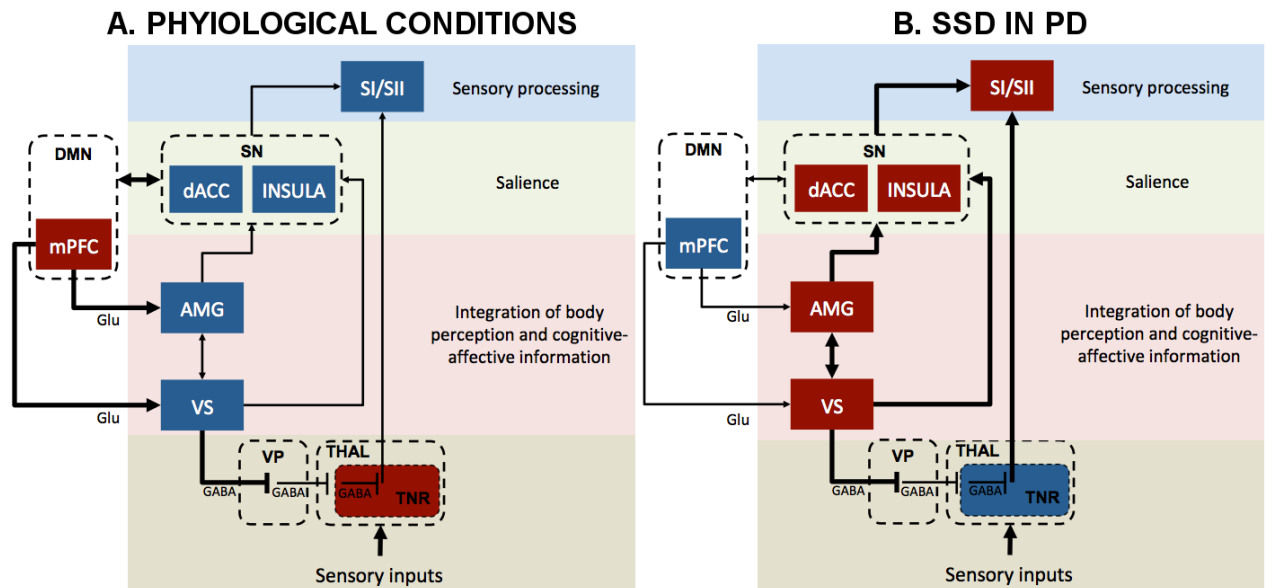


Figure 2. Proposed model for somatic symptom disorder in Parkinson's Disease. Panel A: Under physiological conditions, the mPFC controls the activity of the amygdala and the striatum. Moreover, the CSTC loop regulates the thalamus gates sensory information from midbrain to salience network (SN) and somatosensory cortex. Panel B: In SSD-PD patients, down-regulation of the mPFC results in hyper-activation of amygdala and ventral striatum in response to incoming stimuli, leading to an imbalance in the integration of body perception and cognitive-affective information during sensory processing. The down-regulation of mPFC also promotes opening thalamic filtering and favors an abnormal flux of information that comes from internal and external processes to SN and somatosensory cortices (SI/SII). Moreover, reduced connectivity between the DMN and the SN exacerbates dysfunctional activity in selecting significant peripheral stimuli. **Keys:** AMG, amygdala; dACC, dorsal anterior cingulate cortex; CSTC, cortical-striatal-thalamo-cortical circuit; mPFC, medial prefrontal cortex; PD, Parkinson's Disease; SI/SII, somatosensory cortex; VS, ventral striatum; VP, ventral pallidum; TRN, thalamic reticular nucleus; THAL, thalamus; STR, striatum.

We did not find differences of the levels of Glx within the mPFC among groups. However, methodological limitations could lead to overlook the relevance of glutamate in the patho-physiology of SSD in PD. The Glx signal encompasses contributes of the glutamate and the glutamine and we are unable to provide separate information for the two metabolites. Moreover, much of glutamatergic activity occurs via modulation of the NMDA receptors and these changes cannot be detected by using ¹H-MRS.³⁹

5. Conclusions

We posit a central role of GABA-ergic neurotransmission within mPFC in SSD-PD. Further investigations, combining ¹H-MRS and fMRI could better clarify whether/how the neurotransmission influences the functional interactions within the fronto-subcortical loop and between the DMN and the SN. Even so, we believe that this first evidence could be an important starting point for the definition of new neurochemical substrates, proving more focused neuroprotective strategies in PD.

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