RESEARCH ARTICLE

# High γ-Aminobutyric Acid Content Within the Medial Prefrontal Cortex Is a Functional Signature of Somatic Symptoms Disorder in Patients With Parkinson's Disease

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ABSTRACT: **Background:** The dysfunctional activity of the medial prefrontal cortex has been associated with the appearance of the somatic symptom disorder, a key feature of the Parkinson's disease (PD) psychosis complex.

**Objectives:** The objectives of this study were to investigate whether the basal contents of inhibitory γ-aminobutyric acid and excitatory glutamate plus glutamine neurotransmitter levels are changed in the medial prefrontal cortex of patients with PD with somatic symptom disorder and whether this alteration represents a marker of susceptibility of PD to somatic symptom disorder, thus representing a signature of psychosis complex of PD.

**Methods:** Levels of the γ-aminobutyric acid and glutamate plus glutamine were investigated, at rest, with proton magnetic resonance spectroscopy. Total creatine was used as an internal reference. The study cohort included 23 patients with somatic symptom disorder plus PD, 19 patients with PD without somatic symptom disorder, 19 healthy control subjects, and 14 individuals with somatic symptom disorder who did not show other psychiatric or neurological disorders.

**Results:** We found that, compared with patients with PD without somatic symptom disorder or healthy control individuals, patients with somatic symptom disorder, with or without PD, show increased γ-aminobutyric acid/total creatine levels in the medial prefrontal cortex. The medial prefrontal cortex contents of glutamate plus glutamine/total creatine levels or γ-aminobutyric acid/glutamate plus glutamine were not different among groups.

**Conclusions:** Our findings highlight a crucial pathophysiologic role played by high γ-aminobutyric acid within the medial prefrontal cortex in the production of somatic symptom disorder. This phenomenon represents a signature of psychosis complex in patients with PD. © 2020 International Parkinson and Movement Disorder Society

**Key Words:** somatic symptoms disorder; Parkinson's disease; medial prefrontal cortex; γ-aminobutyric acid; proton magnetic resonance spectroscopy

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Parkinson's disease (PD) is a neurodegenerative condition characterized by movement disorder associated with a high prevalence of neuropsychiatric conditions. The somatic symptom disorder (SSD), along with hallucinations and delusions, is considered as the third component of the PD psychosis complex.[1,2] The notion is supported by epidemiological evidence indicating that, compared with the general population, where the incidence of SSD ranges between 3.5% and 18.4%,[3] patients with PD exhibit an SSD incidence between 7.0% to 66.7%.[4-8]

The pathophysiology of SSD has not been completely unfolded. Imaging-based studies suggest that the hypoactivity of the medial prefrontal cortex (mPFC) and the hyperactivity of subcortical structures connected to frontal regions are neuro-functional substrates for the production of SSD.[9-13] From a physiological standpoint, the mPFC, by controlling the limbic activity, regulates the integration of body perceptions with cognitive-affective information.[14-16] In addition, the mPFC, by shaping the corticostriatal-thalamocortical loop, sets a flux of sensory information to the cortex.[17] The modulatory action of mPFC is locally promoted by inhibitory γ-aminobutyric acid (GABA)-ergic interneurons that in turn tonically trigger a broad range of glutamatergic neurons that project to subcortical structures such as the amygdala,[18] the thalamus,[19] and the striatum.[20] Multimodal imaging studies support this notion by showing that a high content of GABA has been associated with a reduced top-down control of limbic activity and elevated state/trait anxiety.[21-23]

In this study, we have therefore investigated whether a high basal content of GABA in the mPFC is a peculiar and distinct feature of patients with PD with SSD (SSD +PD) and whether this alteration is a functional signature of SSD rather than a marker of PD pathology. The study cohort included 75 individuals, 23 of whom were patients with SSD+PD (SSD+PD), 19 PD patients without SSD (PD); 19 healthy control (HC) subjects; and 14 subjects with SSD who did not show other psychiatric or neurological disorders (SSD). Proton magnetic resonance spectroscopy ($^1$H-MRS) with a Meshcher-Garwood Point Resolved Spectroscopy sequence (MEGA-PRESS)[24,25] was used to enable, noninvasively and in vivo, simultaneous quantification of GABA and excitatory glutamate + glutamine (Glx) levels within the mPFC of the studied subjects.

# Material and Methods

## Study Sample

This study was approved by the local institutional ethics committee and was performed according to the Declaration of Helsinki and subsequent revisions. All participants gave written informed consent, and they were enrolled at the Neurology Clinic of the University "G. d'Annunzio" of Chieti-Pescara, Italy. Exclusion criteria were a prior history of major medical conditions; head injury, including subconcussive trauma; psychiatric or neurological disorders (except PD and SSD); history of substance abuse; or any contraindications to the use of magnetic resonance imaging (MRI). The PD diagnosis was carried out according to the UK Brain Bank Criteria. The mental status and the presence of SSD were assessed in accordance with the *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition*, *Text Revision*[26] and with patient interviews as described in the next section. All patients underwent computerized tomography/MRI and single-photon emission computed tomography before entering the study. Patients at the prodromal or clinical stages of dementia were excluded at the enrollment stage. The Unified Parkinson's Disease Rating Scale Part III and Hoehn and Yahr scale were used to assess the presence and severity of extrapyramidal signs. All subjects were evaluated for global cognition using the Mini-Mental State Examination. Frontal function was assessed using the Frontal Assessment Battery. Control subjects were additionally evaluated for(1) attention skills, sustained attention, divided attention, task coordination, and set-shifting using the Trail Making Test; (2) selective attention using attentional matrices; (3) verbal short-term and long-term memory using the Babcock Story Recall Test; and (4) auditory working memory using the forward and backward Digit Span tests. None of the patients or control subjects were under anxiolytic or antidepressive drugs. Patients with PD were under stable doses of dopaminomimetic compounds. PD drugs were withdrawn the day of MRI acquisition and reinserted after MRI acquisition.

## Evaluation of SSD

Patients were subdivided into 3 groups: PD, SSD, or SSD+PD. This division of patients with PD was based on the direct observation of symptoms at the time of the diagnosis. In a second evaluation, patients underwent semistructured interviews performed by a rater blinded to SSD or PD diagnosis. The interviews, based on the *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition*, *Text Revision*,[26] assessed somatic complaints by using examples and a checklist presented to patients and caregivers. The interviews were focused on the presence of somatic symptoms traits (ie, dependency, mannerism, viscosity, adoption of a sick role, histrionic and dramatic representation of illness). Past somatic symptoms were also evaluated by including information taken from prior hospital records and reports from the patient's general practitioner collected in the previous 4 to 20 years. Supplementary material details methods of SSD categorization. The

Neuropsychiatry Inventory assessed the presence of somatic-type delusional disorders.[27] Study participants were also tested with the symptom questionnaire.[28] The Diagnostic Criteria for Psychosomatic Research were used to ensure a parametric assessment of the symptoms in a neurodegenerative condition.[29-32]

## MRI Protocols

MRI data were collected with 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 3-dimensional $T_1$-weighted turbo field-echo sequence (repetition time/echo time [TR/TE]= 11/5 milliseconds, slice thickness of 0.8 mm). $T_2$-weighted fluid attenuation inversion recovery (TR/TE= 12000/120 milliseconds, slice thickness of 4 mm, field of view = 230 mm × 140 mm × 190 mm) images were also acquired to exclude the presence of concomitant pathologies. Spectra were obtained from a $^1$H-MRS voxel centered on the mPFC and included the ventral portion of anterior cingulate cortex, a region critical for emotional processing (Fig. 1A).[33] To preserve a good signal-to-noise ratio,[25] the $^1$H-MRS voxel size was of 2.0 (anterior–posterior) × 3.0 (left–right) × 3.0 (skull–caudal) cm$^3$. Meshcher-Garwood Point Resolved Spectroscopy sequence (TR/TE = 2000/68 milliseconds, 320 averages; 14-millisecond sinc-Gaussian editing pulses were applied at 1.9 ppm (on) and 7.46 ppm, in a block-interleaved fashion, −40 blocks of 8 TRs, each block (alternately either on or off) was used to acquire 1024 points with a spectral width of 2000 Hz. The sequence generates 2 subspectra, with the editing pulse on in one and off in the other. Specifically, an editing pulse is applied to GABA spins at 1.9 ppm to selectively refocus the evolution of J-coupling to the GABA spins at 3.02 ppm (on spectra). In the other, the inversion pulse is applied elsewhere so that the J-coupling evolves freely throughout the echo time (off spectra). Subtracting off spectra from on spectra, overlying total creatine (tCr) signal was removed from the edited spectrum, revealing the GABA signal in the difference spectrum.[25] The spectrum from a representative patient is reported in Figure 1B. Because acute pharmacological effects can affect $^1$H-MRS spectra, drug assumption was withdrawn the day of MRI session. Point-resolved spectroscopy sequence (PRESS) (TR/TE = 2000/40 milliseconds, 16-step phase cycle, and 128 averages) with and without water suppression was additionally acquired by using chemically shift selective pulses. A total of 1024 points were acquired with a spectral width of 2000 Hz. The PRESS data set was available for 46 of 76 subjects (8/19 HC subjects, 9/14 patients with SSD, 13/19 patients with PD, 16/23 SSD+PD patients).

## Data Analysis

GANNET 3.0 tool[34] 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 $^1$H-MRS voxels and coregister them on the structural image.[35] The definition of the gray matter (GM) and white matter within the $^1$H-MRS voxel and the assessment of the tissue volumes were obtained by combining the outputs of "recon-all" (FreeSurfer) and "fslmaths/fslstats" (FMRIB Software Library)[36] command lines. All the generated images were visually checked with FMRIB Software Library view to validate the location of the MRS voxel and assess the confidence in tissue segmentation. $^1$H-MRS findings were reported as ratios of metabolites/tCr because (1) this quantification exhibits a performance equal to, or better than, water referencing[37]; (2) the tCr concentrations are independent from anxiety-related[21] or neurodegenerative-related disorders[38]; (3) tCr-referenced metabolite values are expected to be less sensitive to changes related to tissue atrophy. Because the edited signal at 3 ppm contains contributions from both the macromolecules and homocarnosine, the signal was labeled as GABA+.[39] PRESS spectra with and without water suppression were analyzed by using JMRUI[40] to calculate the area under the tCr and water peaks, respectively. In detail, spectra with water suppression were filtered to remove residual water by using the Hankel-Lanczos singular values decomposition algorithm. After autophasing, baseline and frequency shifts correction, a priori knowledge database (tCr, 3.03 ppm) was created to put constraints on the advanced magnetic resonance fitting algorithm within the JMRUI package.[40] Peak shifts were restricted to ±0.05ppm of the theoretical location. Spectra with artifact and metabolites fit with Cramer Rao LOWER BOUNDS above 20% were excluded.[38] Thus, water signals were used as an internal reference standard to perform absolute tCr quantification.[38,41-43]

## Statistical Analysis

Analysis of variance and Bonferroni post hoc tests were used to evaluate the group differences regarding demographic and clinical data. Analysis of covariance and Bonferroni post hoc tests were used to evaluate group differences for levels of metabolites/tCr or tCr/water. Brain metabolites were considered as an independent variable and analyzed separately. Each model considered the effect of age, educational levels, sex as well as GM within the $^1$H-MRS voxel. Moreover, a *t* test for 2 independent samples was used to assess differences between patient groups on disease duration and scores of the Unified Parkinson's Disease Rating Scale Part III and Hoehn and Yahr scales. A

chi-squared test was carried out to assess the presence of sex-related group differences.

## Results

The demographic, clinical, and structural imaging features of the study participants are shown in Table 1. Symptom Questionnaire–somatic symptoms subscale items of each group are shown in Supplementary Table 1. Supplementary Table 2 reports the specific symptoms experienced by each patient with SSD+PD and SSD.

The content of GABA+/tCr within the mPFC was found to be significantly different among groups (mean ± standard deviation: HC = 0.0819 ± 0.0201; SSD = 0.1119 ± 0.0263; PD = 0.0848 ± 0.0204; SSD+PD = 0.1095 ± 0.0274; $F_{3,74} = 8.179$, $P < 0.001$). A post hoc analysis of the results showed that, compared with patients with PD and HC subjects, GABA+/tCr levels were higher in the patients with SSD+PD (SSD+PD vs HC, $P = 0.002$; SSD+PD vs PD, $P = 0.008$) or patients with SSD (SSD vs HC, $P = 0.004$; SSD vs PD, $P = 0.011$). No differences in GABA+/tCr levels were observed when comparing patients with PD and HC subjects ($P = 1.000$) and comparing patients with SSD+PD and patients with SSD ($P = 1.000$). Analysis of covariance excluded the effects of age ($F_{2,74} = 1.125$, $P = 0.293$), educational levels ($F_{2,74} = 0.326$, $P = 0.570$), sex ($F_{2,74} = 1.125$, $P = 0.293$), and GM volumes ($F_{2,74} = 0.039$, $P = 0.845$) on GABA+/tCr levels. The difference in GABA+/tCr levels found between patients with SSD+PD and patients with PD was confirmed by performing a general linear model on the 2 groups (group difference: $F_{1,42} = 12.404$, $P = 0.001$) and by including the PD disease duration as nuisance factor (disease duration effect: $F_{1,42} = 1.585$, $P = 0.178$).

The contents of Glx/tCr (mean ± standard deviation: HC, 0.0776 ± 0.0315; SSD = 0.0941 ± 0.0152; PD = 0.0972 ± 0.0437; SSD+PD = 0.0904 ± 0.0186; $F_{3,74} = 1.585$, $P = 0.201$) or tCr/water (HC = $[3.06 \pm 0.42] \times 10^{-4}$; SSD = $[2.87 \pm 1.00] \times 10^{-4}$; PD = $[2.88 \pm 0.58] \times 10^{-4}$; SSD+PD = $[2.70 \pm 0.86] \times 10^{-4}$; $F_{3,45} = 0.391$, $P = 0.760$) did not differ among groups.

No significant differences were found among groups when assessed for age ($F_{3,74} = 0.226$, $P = 0.878$), educational levels ($F_{3,74} = 1.541$, $P = 0.212$), sex ($\mu_3 = 2.173$, $P = 0.527$), Mini-Mental State Examination ($F_{3,74} = 0.555$, $P = 0.646$), Frontal Assessment Battery ($F_{3,74} = 1.748$,

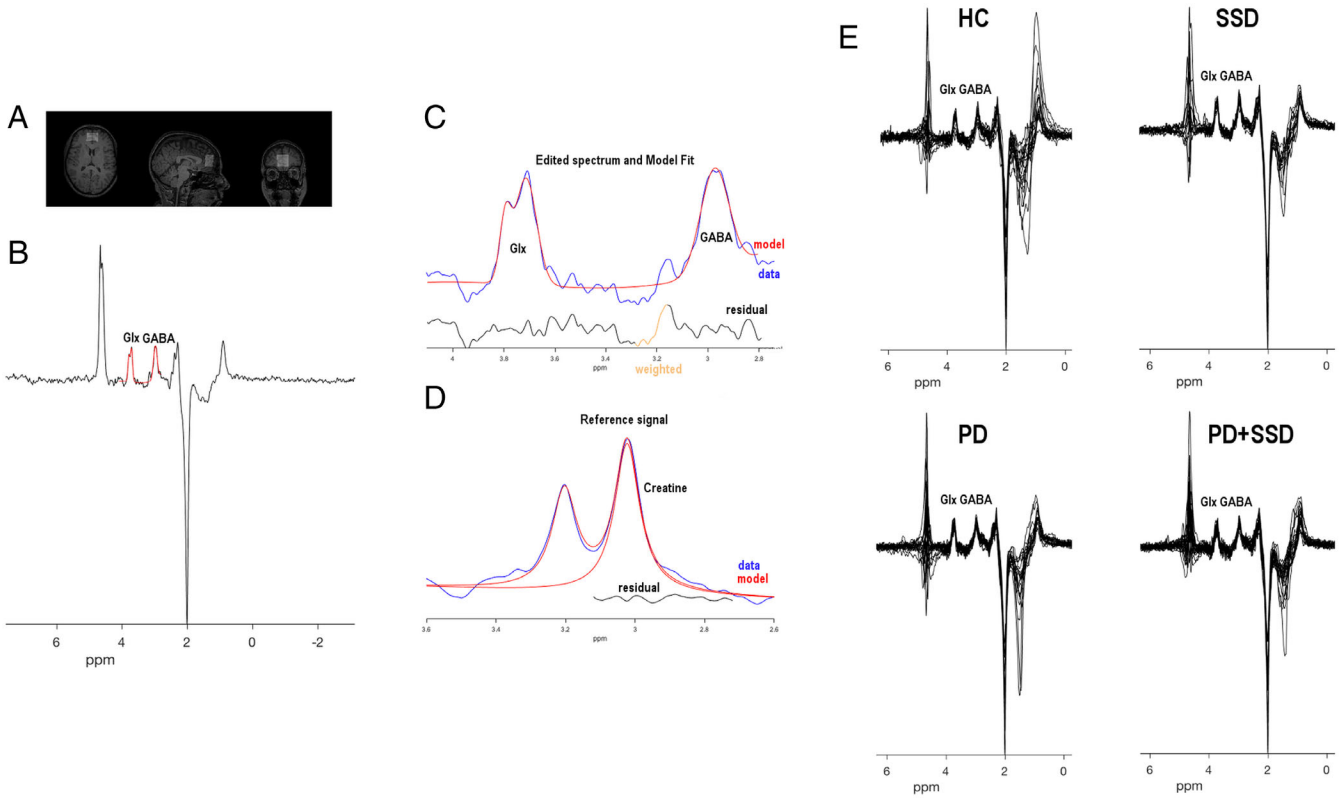


**FIG. 1.** Proton magnetic resonance spectroscopy ($^1$H-MRS). Panel **A** depicts a voxel of 2.0 (anterior–posterior) × 3.0 (left–right) × 3.0 (craniocaudal) cm$^3$ centered on the medial prefrontal cortex. Panel **B** depicts the GABA-edited and Glx-edited MR spectra. Panel **C** shows the GABA+ and Glx peaks from a representative subject. Panel **D** depicts pseudo-doublet peaks of Glx at 3.65 to 3.75 ppm from a representative subject. In the **C,D** panels, the representative GABA-edited and Glx-edited spectrum is depicted in blue, the estimated GABA+ model in red, and noise in black. Panel **E** shows all spectra, collected group by group. GABA, inhibitory γ-aminobutyric acid; Glx, excitatory glutamate + glutamine; HC, healthy control; PD, Parkinson's disease; SSD, somatic symptom disorder. [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 1.** Demographic, clinical, and structural imaging features of the study participants

| Variable | HC | SSD | PD | SSD+PD |
|---|---|---|---|---|
| N | 19 | 14 | 19 | 23 |
| Age | 64.8 ± 10.3 | 64.8 ± 7.3 | 66.7±7.0 | 66.3 ± 7.9 |
| Sex | 58% | 50% | 74% | 61% |
| Educational level, y | 10.4 ± 4.3 | 10.8 ± 3.1 | 9.5 ± 5.2 | 8.0 ± 4.3 |
| GABA+/tCr | 0.0819 ± 0.0201 | 0.1119 ± 0.0263 | 0.0848 ± 0.0204 | 0.1095 ± 0.0274 |
| Glx/tCr | 0.0776 ± 0.0315 | 0.0941 ± 0.0152 | 0.0972 ± 0.0437 | 0.0904 ± 0.0186 |
| tCr/water[a] | 3.06 ± 0.42 | 2.87 ± 1.00 | 2.88 ± 0.58 | 2.70 ± 0.86 |
| GM | 10,045 ± 961 | 10,560 ± 2188 | 9577 ± 2594 | 10,176 ± 656 |
| FAB | 16.5 ± 1.4 | 15.9 ± 1.6 | 14.9 ± 3.6 | 15.1 ± 2.3 |
| MMSE | 27.8 ± 1.2 | 27.3 ± 1.2 | 26.5 ± 3.8 | 27.3 ± 4.0 |
| NPI-TOT | 0.0 ± 0.0 | 3.1 ± 2.0 | 1.4 ± 0.9 | 5.2 ± 1.4 |
| H&Y | NA | NA | 1.7 ± 0.5 | 1.5 ± 0.5 |
| PD duration, y duration | 0.0 ± 0.0 | 0.0 ± 0.0 | 3.5 ± 2.3 | 4.6 ± 2.1 |
| UPDRS-III | 0.0 ± 0.0 | 0.0 ± 0.0 | 13.8 ± 6.0 | 14.3 ± 6.1 |

Values are expressed as mean ± standard deviation. Data on tCr/water refer to 46 of 76 subjects (8/19 HC subjects, 9/14 SSD subjects, 13/19 patients with PD, 16/23 SSD+PD patients).
HC, healthy control; SSD, individuals with SSD who did not show other neurological or psychiatric conditions; PD, Parkinson's Disease patients without somatic symptom disorder; SSD+PD, Parkinson's Disease patients with somatic symptom disorder; GABA, γ-aminobutyric acid; tCr, total creatine; Glx, contributes of glutamate and glutamine; GM, gray matter; FAB, Frontal Assessment Battery; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory Questionnaire; TOT, total; H&Y, Hoehn and Yahr; PD, Parkinson's disease; UPDRS-III, Unified Parkinson's Disease Rating Scale III; NA, not applicable.

$P = 0.165$), and GM within the $^1$H-MRS voxel ($F_{3,74} = 0.991$, $P = 0.402$). The PD disease duration ($t_{40} = -1.679$, $P = 0.101$) and the scores of Unified Parkinson's Disease Rating Scale Part III ($t_{40} = -0.247$, $P = 0.806$) and Hoehn and Yahr ($t_{40} = 1.072$, $P = 0.290$) scales were comparable between PD groups.

No relationship was found, within-group, between the levels of GABA+/tCr and the GM within the $^1$H-MRS voxel (Supplementary Table 3).

## Discussion

In the present study, we investigated the basal content of GABA+ and Glx within the mPFC of a cohort of patients with SSD+PD and patients with PD as well as HC and SSD individuals. The analysis indicates that, when compared with PD or HC individuals, patients with SSD+PD and patients with SSD exhibit increased GABA+ content. In contrast, mPFC levels of Glx were not significantly different among groups.

In the brain, the most significant content of GABA is found in the cytosolic compartment of interneurons, where the neurotransmitter is synthesized from glutamate. The remaining content of intraneuronal GABA is stored in vesicles within presynaptic boutons, while a smaller concentration of GABA is free in the extracellular space.[44-46] Vesicular GABA mediates "phasic" inhibition [47,48] and is less detectable by $^1$H-MRS as the transmitter is bound to macromolecules.[49] Therefore, the $^1$H-MRS-GABA signals primarily represent the metabolic and extracellular pools of GABA. Although the metabolic GABA pool does not affect neural signaling,[47,48] extrasynaptic GABA mediates "tonic" inhibition.[50] Several studies showed that the $^1$H-MRS GABA+ levels negatively correlate with the magnitude of functional MRI signals, the strength of the within-network connectivity, the latency and width of the stimulus-evoked hemodynamic response function curve, and gamma oscillation frequency in the electroencephalographic measures.[22,23,51-59] Nevertheless, the largest study did not find a correlation between gamma activity, as measured with magnetoencephalography, and GABA/glutamate as measured with $^1$H-MRS.[60]

Our data thereby support the notion that, in patients with SSD, increased inhibitory neurotransmission within the mPFC can result in a tonic GABA-mediated inhibition of glutamatergic projections, thereby promoting a persistent overactivation of subcortical structures that participate in the production of SSD. This interpretation is in agreement with functional MRI–based evidence indicating the presence, in patients with SSD, of decreased mPFC activation[10] along with signs of hyperactivation of the amygdala[10,13,61] and striatum.[10] The increased amygdala activity, in particular, can facilitate a failure in the integration of body perceptions and cognitive-affective information with sensory stimuli. On the other hand, an increased striatal activity can produce a neurotransmitter imbalance within the cortical-striatal-thalamocortical circuit that results in defective thalamic filtering and sensory overload of the cortex (Figure 2).[17] In that context, it could be envisioned that the altered cortico-subcortical communication triggers a dysfunctional integration of body perceptions and cognitive-affective information with emotional states, thereby generating the SSD-related symptoms.[2] The mPFC is as a critical anterior hub of the default mode network (DMN)[62] which has been shown, when dysfunctional,[10] to be involved in the genesis of SSD. This functional relationship between mPFC and DMN

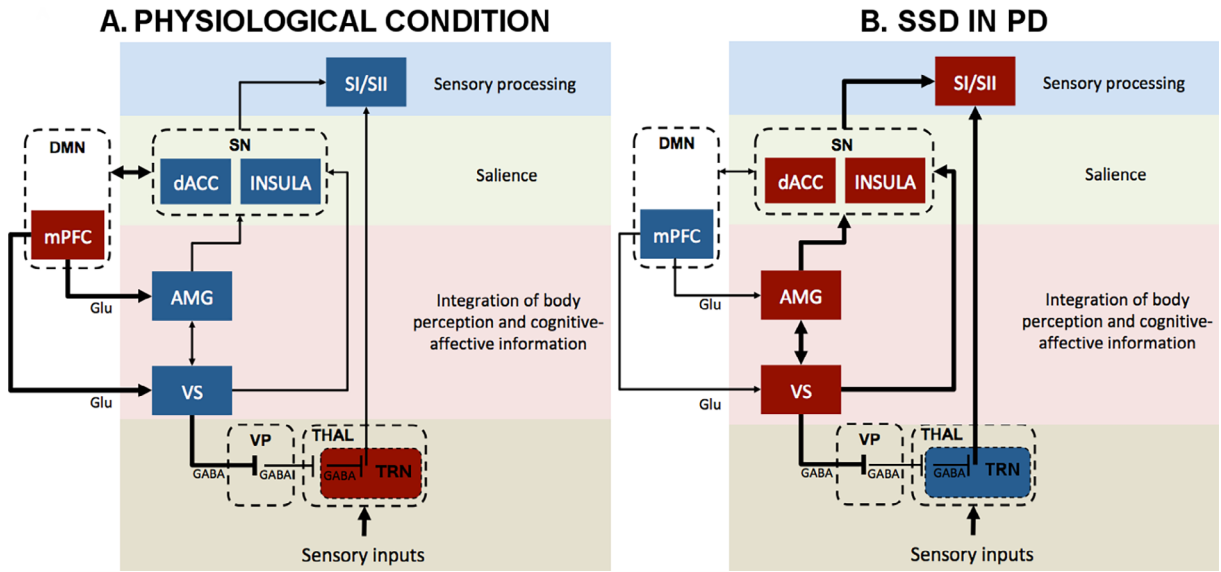


**FIG. 2.** Proposed model for the production of somatic symptom disorder in Parkinson's disease. GABA-ergic interneurons of the mPFC exert top-down inhibitory control over the inputs of excitatory pyramidal neurons, which in turn regulate GABA-ergic interneurons within the amygdala and ventral striatum. (**A**) Under physiological conditions, the mPFC, by acting on glutamatergic projections, downregulates the activity of the amygdala and the striatum. The cortical-striatal-thalamocortical loop regulates the thalamic filtering of sensory information flowing from the midbrain to the SN and the somatosensory cortex. (**B**) In patients with SSD+PD, the downregulation of the mPFC results in the hyperactivation of the amygdala and ventral striatum in response to incoming stimuli, thereby leading to an imbalance in the integration of body perceptions and cognitive-affective information that occurs upon sensory processing. The downregulation of the mPFC also promotes altered thalamic filtering and favors an abnormal flux of information to the SN and somatosensory cortex. Moreover, the reduced connectivity between the DMN and SN exacerbates the dysfunctional activity in the selection and sorting of significant peripheral stimuli. The bold lines indicate the increase in neurotransmission. The red and blue rectangles depict hyperactivity or hypoactivity, respectively. AMG, amygdala; dACC, dorsal anterior cingulate cortex; CSTC, cortical-striatal-thalamocortical circuit; GABA, inhibitory γ-aminobutyric acid; Glu, glutamate; mPFC, medial prefrontal cortex; PD, Parkinson's disease; SI/SII, somatosensory cortex; SN, salience network; SSD, somatic symptom disorder; STR, striatum; VS, ventral striatum; VP, ventral pallidum; TRN, thalamic reticular nucleus; THAL, thalamus. [Color figure can be viewed at wileyonlinelibrary.com]

substantiates our hypothesis because the frontal dysfunction favors the decoupling of the default mode network from task-positive networks. This phenomenon encourages the formation of random connections that link strong autobiographical correlates to trivial sensory stimuli thereby producing SSD.[2] In addition, by destroying the inverse coupling between the DMN and the salience network (SN),[63,64] affects the switching of attention between external and internal salient stimuli, thus favoring a confusion in the judgment and perception of internal stimuli.[2] Moreover, in line with the increased GABA levels that we have observed in this study, we have recently found that, in patients with SSD+PD, the DMN is hypoactive and exhibit decreased connectivity with the SN.[30] Thus, the GABA-mediated reduction of DMN activity that may occur in the patients with SSD can be instrumental in promoting altered functional connectivity with the SN, dysfunctional filtering of significant peripheral stimuli, and, ultimately, the production of SSD symptoms.

The metabolite contents within the mPFC did not differ between the SSD+PD and SSD groups. Therefore, the changes that we observed in inhibitory neurotransmission can be interpreted as functional signatures of SSD that is independent of the presence of PD-related pathology. However, it should be highlighted that our patients with PD were in the early/middle stage of the disease, stages in which the pathological spreading of α-synuclein does not reach significant amounts in the mPFC. On the other hand, it is important to consider that the increased GABA levels in the mPFC,[65,66] the mPFC hypo-connectivity,[67] and the occurrence of SSD[2,68,69] are all prognostic predictors of increased risk of cognitive decline or progression to dementia. Moreover, the mPFC is a preferential and early site of β-amyloid deposition.[70] Therefore, future clinical follow-ups will need to clarify whether the GABAergic dysfunction in the mPFC is predictive for the development of dementia in patients with SSD+PD.

A major limitation of the study concerns the lack of information regarding the role played by glutamate in the production of SSD in our patients with PD. Although a recent study has indicated the presence of increased Glx/Cr in the mPFC of patients affected by functional motor symptoms,[71] we did not find differences in Glx levels among our cohorts. Methodological limitations impact somehow the heuristic value of the finding, as the Glx signal is composed of a mix of glutamate and glutamine, and $^{1}$H-MRS is unable to discriminate the distinct contribution of the 2 metabolites. The separation of Glu and Gln with a 3T is challenging. The use of some sequences has been attempted, but

they are highly dependent on line width and still unsuitable for the investigation of frontal brain regions. The coedited Glx signal is concentration weighted (contains ~4 times more Glu signal that Gln), but also weighted by editing efficiency (how much of the theoretically observable signal can be measured). The editing efficiency of Glu and Gln does not add further weighting. Significant advancements in technology will be required to address this relevant issue. We have analyzed the 40-millisecond PRESS data using different of analysis approaches (full/reduced basis set) within Osprey (https://github.com/schorschinho/osprey; https://www.biorxiv.org/content/10.1101/2020.02.12.944207v2). It has not been possible to make reliable measurements based on these spectra. The coefficient of variations of Glx across the cohort is 81% and the data are not of sufficient quality to separate Glu and Gln. Moreover, much of the glutamatergic activity occurs through the activation of the N-methyl-D-aspartate receptors, activities that, unfortunately, cannot be detected with $^{1}$H-MRS.[57]

$^{1}$H-MRS measures are quantitative, but the choice of reference signals remains a challenge. The tCr concentration has been extensively employed as a standard reference in $^{1}$H-MRS studies[21,38,71,72]; however, its use in PD-related studies is debatable as it is not clear whether the parameter is stable or changes in these patients.[38,73-77] We have attempted to address this issue by performing additional analysis on unsuppressed spectra to calculate the tCr to water ratio for each individual. As no significant difference in tCr/water was found among groups, we reasoned that the use of GABA+/tCr is a reliable method to investigate inhibitory neurotransmission changes that occur within the mPFC of patients with PD.

Finally, we want to point out that, within the $^{1}$H-MRS voxel, we did not find any relationship between metabolite contents and GM. This finding is in line with the current debate on this issue. Although some studies suggest that the tissue volume affects the assessment of GABA+ concentrations,[78] another has shown that variation of GABA levels in the frontal lobe are independent of age and local atrophy.[66]

## Conclusions

Our data converge in supporting a role for GABAergic neurotransmission within the mPFC in the production of SSD in patients with PD. Further investigations combining $^{1}$H-MRS and functional MRI will clarify whether and how GABA influences the functional interactions occurring within the fronto-subcortical loops as well as shapes the interaction between the DMN and SN. Nevertheless, data collected in the present study represent the starting point for the detailed investigation of the neurochemical substrates of SSD+PD as well as the exploration of novel neuroprotective strategies for the patients.

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