The Behavioral Neurology of Parkinson’s Disease

Lead Guest Editor: Pasquale Calabrese
Guest Editors: Per Odin and Elka Stefanova
The Behavioral Neurology of Parkinson’s Disease
The Behavioral Neurology of Parkinson’s Disease

Lead Guest Editor: Pasquale Calabrese
Guest Editors: Per Odin and Elka Stefanova
Editorial Board

Jan Aasly, Norway
Cristine Alves da Costa, France
Ivan Bodis-Wollner, USA
Carlo Colosimo, Italy
Ted Dawson, USA
Francisco Grandas, Spain

Peter Hagell, Sweden
Nobutaka Hattori, Japan
Marjan Jahanshahi, UK
Elan D. Louis, USA
Giovanni Mirabella, Italy
Maral M. Mouradian, USA

Antonio Pisani, Italy
Fabrizio Stocchi, Italy
Eng King Tan, Singapore
Hélio Teive, Brazil
| Title                                                                 | Authors                                                                                                    | Pages   | Volume | Article ID       |
|-----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|---------|---------|------------------|
| Does Dopamine Depletion Trigger a Spreader Lexical-Semantic Activation in Parkinson's Disease? Evidence from a Study Based on Word Fluency Tasks | S. Zabberoni, G. A. Carlesimo, A. Peppe, C. Caltagirone, and A. Costa                                      | 6       | 2017    | 2837685          |
| Correlation of Visuospatial Ability and EEG Slowing in Patients with Parkinson's Disease | Dominique Eichelberger, Pasquale Calabrese, Antonia Meyer, Menorca Chaturvedi, Florian Hatz, Peter Fuhr, and Ute Gschwandtner | 11      | 2017    | 3659784          |
| An Integrated Review of Psychological Stress in Parkinson's Disease: Biological Mechanisms and Symptom and Health Outcomes | Kim Wieczorek Austin, Suzanne Weil Ameringer, and Leslie Jameleh Cloud                                    | 15      | 2016    | 9869712          |
| Mini Review: Anticholinergic Activity as a Behavioral Pathology of Lewy Body Disease and Proposal of the Concept of “Anticholinergic Spectrum Disorders” | Koji Hori, Kimiko Konishi, Misa Hosoi, Hiroi Tomioka, Masayuki Tani, Yuka Kitajima, and Mitsugu Hachisu | 7       | 2016    | 5380202          |
| 5-HT$_{2A}$ Receptor Binding in the Frontal Cortex of Parkinson's Disease Patients and Alpha-Synuclein Overexpressing Mice: A Postmortem Study | Nadja Bredo Rasmussen, Mikkel Vestergaard Olesen, Tomasz Brudek, Per Plenge, Anders Bue Klein, Jenny E. Westin, Karina Fog, Gitta Wörtwein, and Susana Aznar | 8       | 2016    | 3682936          |
| Quantitative EEG and Cognitive Decline in Parkinson's Disease | Vitalii V. Cozac, Ute Gschwandtner, Florian Hatz, Martin Hardmeier, Stephan Rüegg, and Peter Fuhr | 14      | 2016    | 9060649          |
Clinical Study

Does Dopamine Depletion Trigger a Spreader Lexical-Semantic Activation in Parkinson’s Disease? Evidence from a Study Based on Word Fluency Tasks

S. Zabberoni,1,2 G. A. Carlesimo,2,3 A. Peppe,2 C. Caltagirone,2,3 and A. Costa1,2

1Department of Psychology, Niccolò Casu no University, Rome, Italy
2IRCCS Fondazione Santa Lucia, Rome, Italy
3Department of Systems Medicine, Tor Vergata University, Rome, Italy

Correspondence should be addressed to S. Zabberoni; s.zabberoni@hsantalucia.it

Received 11 May 2016; Revised 15 February 2017; Accepted 7 March 2017; Published 11 June 2017

Academic Editor: Elka Stefanova

Copyright © 2017 S. Zabberoni et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

It has been hypothesised that, in Parkinson’s disease (PD), dopamine might modulate spreading activation of lexical-semantic representations. We aimed to investigate this hypothesis in individuals with PD without dementia by assessing word frequency and typicality in verbal fluency tasks. We predicted that the average values of both of these parameters would be lower in PD patients with respect to healthy controls (HC). We administered letter-cued and category-cued fluency tasks to early PD patients in two experimental conditions: the tasks were administered both after 12–18 hours of dopaminergic stimulation withdrawal (“OFF” condition) and after the first daily dose of dopaminergic therapy (“ON” condition). HC were also given the two tasks in two conditions with the same intersession delay as PD patients but without taking drugs. Results showed that in both OFF and ON treatment conditions PD patients did not differ from HC in word frequency or typicality. Moreover, in the PD group, no significant difference was found between the experimental conditions. Our results show that semantic spreading was not altered in the PD sample examined; this suggests that in early PD the functioning of the semantic system is relatively independent from the activity of dopamine brain networks.

1. Introduction

Parkinson’s disease (PD) is frequently accompanied by cognitive deficits. These include dementia or mild cognitive impairment involving attention, executive functions, visual-spatial abilities, and episodic memory [1]. It has been reported that the functional and structural modifications that take place in the frontal-striatal and mesolimbic circuitries in PD are associated with these cognitive changes [2–4].

Increasing attention has been given to the functioning of the lexical-semantic system in PD. Some studies have documented reduced semantic priming in PD patients with respect to healthy controls; this suggests that these patients have delayed lexical/semantic activation [5, 6]. In this vein, there is evidence that PD patients’ performance on priming tasks is affected significantly by dopamine withdrawal [7, 8]. In particular, in addition to confirming reduced priming in PD patients when they were taking levodopa relative to healthy controls, Angwin et al. [8] also showed the lack of any priming effect when PD patients were assessed in the OFF condition. In agreement with previous studies conducted in healthy subjects [9–11], these findings underline the neuromodulatory role of dopamine within the semantic network [12] and also suggest that the speed of activation in PD patients is related to the extent of dopamine depletion [13].

According to the spreading activation theory, in the lexical-semantic network, the activation of individual nodes spreads to neighbouring concepts according to a variety of connections and nodes features [14]. In particular, the strength of association between nodes within the network might be modulated by the frequency of words. Hence, the activation of low frequency words would require greater spreading than the activation of high frequency words.
because the latter would have more and stronger links with other words in the network [15]. For this reason, in a word fluency task (in which subjects are required to generate as many words as possible according to some phonological or semantic constraints), healthy subjects typically produce more frequent words first. In PD patients, dopaminergic depletion could lead to an alteration of the structure of the lexical-semantic system with a reduced activation threshold difference between high and low frequency words as a manifestation of a spreader, less strategic, and seemingly random activation of the lexical units [8]. Accordingly, in a word fluency task, PD patients might generate words with lower frequency of use than healthy subjects. This issue was directly investigated in two studies which, however, reported inconsistent results. Indeed, Foster et al. [16] found that, in a phonological word fluency task, PD patients who were on dopaminergic treatment generated words with a significantly lower frequency of use compared to healthy controls. Conversely, Herrera et al. [17] found no direct effect of the manipulation of dopamine therapy on word use frequency in that PD patients produced words of comparable frequency irrespective of whether or not they were taking dopaminergic medication.

The aim of the present study was to further investigate lexical/semantic spreading activation in PD patients without dementia and its relationship to dopaminergic treatment. For this purpose, in addition to frequency of use computed on words generated in a letter-cued fluency task, we also assessed the typicality of the words produced in a category-cued fluency task. Indeed, the use of word typicality is underpinned by the assumption that each semantic category contains some words that are more representative than others [18]. Similar to what we discussed for high use frequency words, which should have a lower threshold of activation than low use frequency words, in a category-cued fluency task, words representing highly typical exemplars of a certain category should have a greater probability of being recalled than words representing less typical exemplars [18, 19]. To the best of our knowledge, the typicality index has never been used to investigate lexical-semantic spreading in PD patients.

Here we directly investigated the effect of dopaminergic stimulation on lexical-semantic activation in PD by contrasting the frequency of use and the typicality of words generated in fluency tasks in a sample of PD patients after withdrawal from (OFF condition) and after taking (ON condition) dopaminergic medication. Consistent with the assumption that dopamine has a significant neuromodulatory role in the strategic search and generation of words and, conversely, that dopamine depletion results in spreader, less strategic activation of units in the lexical-semantic system [16], we predicted that in the OFF condition PD patients would generate words with reduced frequency of use during the letter-cued fluency task and words less typical in the category-cued fluency task. Taking dopaminergic medication (on condition) should result in normalisation or, at the very least, in a significant increase in the average values of use frequency and typicality of the generated words.

2. Materials and Methods

2.1. Subjects. Twenty PD patients and 18 healthy controls (HC) were enrolled in the study after they gave their written informed consent. All of the patients included in the study were consecutive outpatients who had been referred by their primary care physician to the Parkinson’s disease ambulatory care facilities of the IRCCS Santa Lucia Foundation in Rome. The diagnosis of idiopathic PD was made by a neurologist according to the London Brain Bank criteria [21]. Exclusion criteria for PD patients included (i) disease duration ≥ 5 years, (ii) diagnosis of dementia based on clinical criteria [22] and confirmed by a Mini-Mental State Examination [23] score < 26, and (iii) presence of other neurological and/or psychiatric illnesses in the patient’s clinical history.

The HC participants were volunteers recruited from the patients’ relatives. Exclusion criteria for the HC group included (i) cognitive impairment based on the Mini-Mental State Examination score < 26, (ii) taking medication that affects the central nervous system, and (iii) neurological and/or psychiatric illnesses, traumatic head injury, or substance abuse in the subject’s history.

All PD patients were taking daily doses of dopamine or a dopamine agonist; in particular, seven patients were taking only L-Dopa, six patients were being treated with pramipexole or ropinirole only, and the remaining seven patients were taking both L-Dopa and dopamine agonists (i.e., pramipexole or ropinirole). All the patients presented bilateral akinetic-rigid form of PD and they were good and stable therapy responders. The clinical and demographic characteristics of the two experimental groups are reported in Table 1. L-Dopa equivalent doses are also reported for the patients’ group.

Based on their performance on the tests included in the neuropsychological screening battery [24], 15 PD patients had only executive deficits, three patients had executive and episodic memory disorders, and the two remaining patients had visual-constructive apraxia.

2.2. Experimental Procedure

2.2.1. Tasks. PD patients and HC were given letter-cued (phonemic fluency) and category-cued (semantic fluency) tasks. The experimental procedures were administered by an expert neuropsychologist.

In the letter-cued word fluency task, the subject has to generate as many words as possible that begin with a specified letter in three different trials, each lasting 60 seconds. Two versions of the task were created. In one version, the letters to be used to generate words were “A,” “F,” and “S.” In the other version, the letters to be used were “C,” “E,” and “L.”

Word use frequency was computed for each generated word according to normative values in the COLFIS corpus of Italian words [25].

In the category-cued word fluency task, the subject has to say as many words as possible that belong to a specific taxonomic category in two different trials, each lasting 60 seconds. Also in this case, two versions of the task were created. In one version, the categories to be used in the two
Table 1: Average (SD) of anagraphic data of experimental samples and clinical features of patient’s group.

|                  | PD (n = 20) | HC (n = 18) | F (df) | p     |
|------------------|-------------|-------------|--------|-------|
| Age              | 66.7 (7.6)  | 67.9 (5.6)  | 0.3 (1,37) | 0.57  |
| Years of education| 11.1 (4.2)  | 12.4 (3.4)  | 1.0 (1,37) | 0.31  |
| MMSE (raw score) | 26.5 (0.45) | 29.4 (0.76) | 5.6 (1,37) | 0.23  |
| H&Y (range) [20] | 2.5–3       | —           | —      | —     |
| Disease duration | 2.9 (1.9)   | —           | —      | —     |
| UPDRS “ON”       | 11.8 (4.3)  | —           | 8.2 (1,19) | 0.01  |
| UPDRS “OFF”      | 16.3 (7.6)  | —           | —      | —     |
| L-Dopa equivalents| 352.1 (138.5)| —       | —      | —     |
| Therapy duration (years) | 1.7 (0.6) | —           | —      | —     |

trials of the task were “Trees” and “Furniture” and in the second version “colours” and “animals.”

The typicality value was computed for each word according to the category norms corpus for the Italian language [18].

The administration order of the two tasks was phonemic fluency followed by semantic fluency. At the beginning of each task, a training trial was given to be sure the subjects understood the instructions. Participants were told not to use proper nouns, not to use the same word with a different ending (e.g., arancia, arancione, aranciata), and not to conjugate verbs. In each trial, the number of legal words generated in 60 seconds was recorded. Accuracy in each task was the sum of the number of legal words generated in all trials.

In order to evaluate in more detail the pattern of words generated in the two fluency tasks (in particular, whether the participants in the two groups produced, as expected, more typical/frequent words first and less typical/frequent words later), in each subject, average word use frequency (for the letter-cued fluency task) and average typicality (for the category-cued fluency task) were computed separately for the first half and second half of the words produced in the different trials.

2.2.2. Design. PD patients were submitted to the experimental tasks after they had taken a full dose of stable dopaminergic treatment for one month. They were assessed in two experimental conditions that were performed on different days, with an intersession interval of about one month. In the “OFF” condition PD subjects performed the experimental tasks in the morning after 12/18 hours of drug withdrawal [26]. In the “ON” condition they were examined 90–120 minutes after they had taken their first morning dose of levodopa and/or dopamine agonists. To determine the efficacy of the dopamine compounds in improving extrapyramidal symptoms, in both treatment conditions, PD patients were given the UPDRS-Part III [27].

The tests of the experimental battery were administered to PD patients in both OFF and ON therapy conditions. By contrast, HC were given the tasks in two different sessions, named “blue” and “green,” without any drug administration. The “blue” session was associated with the OFF condition and the “green” session with the ON condition. The order of the experimental conditions (OFF/blue versus ON/green) was counterbalanced across subjects.

2.2.3. Statistical Analysis. Modification of the UPDRS in the PD group as a function of the treatment condition was analysed by means of a repeated measures ANOVA. The average number of words generated on the two fluency tasks was analysed by means of two-way mixed ANOVAs with Group (PD versus HCs) as between subjects variable and Treatment (ON versus OFF condition) as within subjects variable. Finally, data relative to use frequency and typicality of words generated in the letter and category word fluency tasks, respectively, were analysed by means of three-way ANOVAs with Group (PD versus HCs) as between subjects factor and Treatment (OFF/blue versus ON/green condition) and Half (first half versus second half of the generated words) as within subject factors.

3. Results

3.1. UPDRS. Confirming the beneficial effect of dopamine stimulation for extrapyramidal symptoms, the UPDRS scores of patients with PD decreased significantly (Table 1) passing from the OFF (M = 16.3; SD = 7.6) to the ON (M = 11.8; SD = 4.3) treatment condition ($F(1,19) = 8.25; p = 0.01$).

3.2. Letter-Cued Word Fluency Task. The average number of words generated in the phonological word fluency task by PD patients and HC (Table 2) did not differ and it was not influenced by PD patients assuming medication as demonstrated by nonsignificant main effects of Group ($F(1,36) = 1.70; p = 0.20$) and Treatment ($F(1,36) = 0.25; p = 0.61$) and the Group × Treatment interaction ($F(1,36) = 48; p = 0.49$).

The use frequency of words generated during the fluency task (Figure 1) also did not differ between groups and it was not influenced by dopamine stimulation. Indeed, only the main effect of Half was significant ($F(1,36) = 8.83; p = 0.005$), but the main effects of Group ($F(1,36) = 0.69; p = 0.40$) and Treatment ($F(1,36) = 0.01; p = 0.93$) as well as all the interactions (all $p$ consistently >0.40) were not.
In all subjects, word use frequency was higher for the words generated in the first half of the trial \((M = 280.7; SD = 66.9.0)\) than for those generated in the second half \((M = 139.9; SD = 184.1)\). Moreover, planned comparisons documented that PD patients generated words with comparable use frequency while taking dopaminergic medication \((M = 230.97; SD = 467.19)\) and during treatment withdrawal \((M = 229.6; SD = 739.7; F = .01; p = 0.97; Cohen’s d = 0.002)\) and that a comparable decrease in use frequency passing from the first half to the second half of the trial was observed in words generated while patients were in the ON \((M = 209.5; SD = 578.4)\) and the OFF \((M = 136.8; SD = 255.0; F = .51; p = 0.48)\) treatment conditions (Figure 1).

3.3 Category-Cued Word Fluency Task. PD patients and HC did not differ either for the number of words generated in the two trials of the fluency task \((F(1,36) = 0.18; p = 0.67)\). Furthermore, neither the Treatment factor \((F(1,36) = 0.35; p = 0.56)\) nor the Group \(\times\) Treatment interaction \((F(1,36) = 0.35; p = 0.56)\) revealed significant effects, thus demonstrating that the average number of words generated by the PD patients was not affected when patients took L-Dopa medication (Table 1).

The average typicality of words also did not differ between groups and was not affected by the treatment condition (Figure 2). Indeed, also in this case, the Half main effect was significant \((F(1,36) = 124.3; p < 0.001)\), whereas the Group \((F(1.36) = 3.39; p = 0.07)\) and Treatment \((F(1.36) = 3.20; p = 0.08)\) main factors and the second-order and third-order interactions were not \((all p\ consistently >0.30)\). These data indicate that all subjects generated more typical words \((within the semantic category) in the first \((M = 80.7; SD = 52.5)\) than in the second \((M = 53.3; SD = 30.0)\) half of the trials. Moreover, planned comparisons showed that the typicality of the words generated by PD patients when taking dopamine medication \((M = 68.8; SD = 38.7)\) was not different from the typicality of words generated during medication withdrawal \((M = 59.7; SD = 32.0; F = 1.64; p = 0.20; Cohen’s d = 0.002)\) and that the average typicality of the generated word values decreased at the same rate passing from the first half to the second half in the ON \((M = 39.3; SD = 57.2)\) and in the OFF \((M = 22.9; SD = 47.9; F = .54; p = 0.46)\) treatment conditions.

4. Discussion

This study was aimed at investigating whether dopaminergic stimulation has a modulatory effect on the spreading activation of lexical-semantic representations in individuals.
with PD. In particular, we investigated whether reduced dopamine concentration results in increased spreading activation which could potentially influence strategic organization and retrieval of internal representations [16]. For this purpose, we administered a group of PD patients letter-cued and category-cued fluency tasks in two different pharmacological treatment conditions: (a) after a dopaminergic treatment wash-out period (“OFF” treatment condition) and (b) after they took their usual dopaminergic medication dose (“ON” treatment condition). We predicted that in the OFF condition PD patients, unlike HC, would show increased spreading activation documented by the generation of less frequent words in the letter-cued task and of less typical words in the category-cued task. Moreover, we predicted that taking dopamine medication (“ON” condition) would result in significantly less spreading of lexical-semantic activation, thus resulting in the generation of more frequent and more typical words.

Results did not confirm our predictions. Indeed, neither frequency nor typicality of the generated words differed between PD patients (in both OFF and ON treatment conditions) and HC. Moreover, no significant difference in these two parameters was found in the PD group in the two treatment conditions. To the extent that frequency of use and typicality of words generated in fluency tasks are behavioural indices of spreading activation within the lexical-semantic system [16], we can conclude that our PD sample did not present any significant alteration in this lexical-semantic system property and, therefore, that dopamine stimulation has no appreciable effect on the activation level of lexical-semantic representations.

Our findings are consistent with those of Herrera et al. [17]. These authors found no difference between PD patients and matched HC for frequency of use of words generated in a letter-cued fluency task and in two category-cued fluency tasks. The same study failed to reveal any effect of medication administration/withdrawal on the same indices in the PD group. However, these authors [17] found that, in an action-cued fluency task, PD patients in the OFF condition generated action words with greater use frequency than HC. Although the finding of an effect confined to the grammatical class of words is of interest in light of previous evidence of a special role of the frontal lobes in verb generation [28] and of a significant deficit of PD patients on verbs and action words [28], it is difficult to interpret. Indeed, in their PD sample, use frequency of words was not modulated by L-Dopa intake (i.e., there was no significant difference between PD patients in the ON and OFF treatment conditions). Moreover, the average frequency values in the group of PD patients and in HC could have been confounded by the different number of words generated (with higher values in the PD group possibly related to the lower number of words generated).

Therefore, taking together the above observations and the evidence that most PD participants in our study showed dysexecutive deficits, we argue that in the early stage of PD prefrontal lobe dysfunction does not affect processes involved in the maintenance of stable representations, such as those related to semantic knowledge. Coherently, the null effect of the therapy manipulation we found could be interpreted in the view that, in the early phases of PD, dopamine neurotransmission is mainly involved in the modulation of flexibility processes depending on the activity of the D2 dopamine receptors in the caudate nucleus [29–31] and does not affect the on-line processing of consolidated information.

However, our findings are at variance with those of Foster et al. [16]. These authors administered a letter-cued fluency task to groups of PD patients and matched controls and found that the frequency of use of words generated by PD patients was significantly lower than that generated by HC. One way of explaining these contrasting data is that the PD patients enrolled by Foster et al. [16] were in a more advanced stage of the disease compared to the PD patients who participated in our study (Foster et al.’s [16] study: mean disease duration = 6.8 years; mean UPDRS score = 32; our study: mean disease duration = 2.9 years; mean UPDRS score = 16.3). Therefore, we argue that the cortical regions responsible for the integrity of lexical-semantic processing are affected to a lesser extent in our PD sample than in the patients enrolled by Foster et al. [16]. Unfortunately, Foster et al. [16] did not manipulate dopamine treatment; thus we are unable to formulate any hypotheses about the role of dopamine stimulation on the effects they found.

Some limitations of the present study have to be discussed. First, likely because in the early stages of the disease, PD patients in the present study did not generate fewer words in the phonological and category-cued fluency tasks as compared to healthy controls, this could have reduced the possibility of finding significant effects of dopamine stimulation on the use frequency and/or typicality of produced words. Second, the PD patients were assessed while undergoing their usual dopamine therapy, which seemed to be quite heterogeneous as it includes levodopa and/or dopamine agonists. This could be another factor responsible of a lack of an effect of dopamine stimulation on spreading activation. Indeed, it is reported that the different molecules involved in dopaminergic compounds may have different effects on cognitive functions depending on their differential affinity with brain D2 receptors [32].

In conclusion, our results do not show a significant relationship between semantic spreading and dopamine stimulation in early-stage PD patients. However, also taking into account the above limitations, our findings might suggest the relative independence of the functioning of the semantic system and the activity of dopamine brain networks in the early stages of PD.

Finally, studies combining different paradigms (e.g., associative priming and verbal fluency) could be designed to further investigate the effect of dopamine treatment on lexical-semantic processing in PD.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**References**

[1] A. A. Kehagia, R. A. Barker, and T. W. Robbins, "Neuropsychological and clinical heterogeneity of cognitive impairment and
dementia in patients with Parkinson's disease,” The Lancet Neurology, vol. 9, no. 12, pp. 1200–1213, 2010.
[2] G. A. Carlesimo, F. Firas, F. Assogna, F. E. Pontieri, C. Caltagirone, and G. Spalletta, “Hippocampal abnormalities and memory deficits in Parkinson disease: a multimodal imaging study,” Neurology, vol. 78, no. 24, pp. 1939–1945, 2012.
[3] A. Brück, T. Kurki, V. Kaasinen, T. Vahlberg, and J. O. Rinne, “Hippocampal and prefrontal atrophy in patients with early non-demented Parkinson's disease is related to cognitive impairment,” Journal of Neurology, Neurosurgery & Psychiatry, vol. 75, no. 10, pp. 1467–1469, 2004.
[4] I. Ferrer, “Early involvement of the cerebral cortex in Parkinson's disease: Convergence of multiple metabolic defects,” Progress in Neurobiology, vol. 88, no. 2, pp. 89–103, 2009.
[5] A. J. Angwin, H. J. Chenery, D. A. Copland, B. E. Murdoch, and P. A. Silburn, “The speed of lexical activation is altered in Parkinson's disease,” Journal of Clinical and Experimental Neuropsychology, vol. 29, no. 1, pp. 73–85, 2007.
[6] W. L. Arnott, H. J. Chenery, B. E. Murdoch, and P. A. Silburn, “Semantic priming in Parkinson's disease: Evidence for delayed spreading activation,” Journal of Clinical and Experimental Neuropsychology, vol. 23, no. 4, pp. 502–519, 2001.
[7] A. J. Angwin, D. A. Copland, H. J. Chenery, B. E. Murdoch, and P. A. Silburn, “The influence of dopamine on semantic activation in Parkinson's disease: evidence from a multiprimer task,” Neuropsychology, vol. 20, no. 3, pp. 299–306, 2006.
[8] A. J. Angwin, W. L. Arnott, D. A. Copland et al., “Semantic activation in Parkinson's disease patients on and off levodopa,” Cortex, vol. 45, no. 8, pp. 950–959, 2009.
[9] U. Kischka, T. H. Kammer, S. Maier, M. Weisbrod, M. Thimm, and M. Spitzer, “Dopaminergic modulation of semantic network activation,” Neuropsychologia, vol. 34, no. 11, pp. 1107–1113, 1996.
[10] D. Roesch-Ely, S. Weiland, H. Scheffel et al., “Dopaminergic Modulation of Semantic Priming in Healthy Volunteers,” Biological Psychiatry, vol. 60, no. 6, pp. 604–611, 2006.
[11] A. I. Angwin, H. J. Chenery, D. A. Copland, W. L. Arnott, B. E. Murdoch, and P. A. Silburn, “Dopamine and semantic activation: An investigation of masked direct and indirect priming,” Journal of the International Neuropsychological Society, vol. 10, no. 1, pp. 15–25, 2004.
[12] A. S. Pedrizzoli, M. E. Tivarus, P. Agrawal, S. K. Kostyk, K. M. Thomas, and D. Q. Beversdorf, “Dopaminergic modulation of semantic priming in parkinson disease,” Cognitive and Behavioral Neurology, vol. 21, no. 3, pp. 134–137, 2008.
[13] M. Grossman, E. Zurif, C. Lee et al., “Information processing speed and sentence comprehension in Parkinson's disease,” Neuropsychology, vol. 16, no. 2, pp. 174–181, 2002.
[14] A. M. Collins and E. F. Loftus, “A spreading-activation theory of semantic processing,” Psychological Review, vol. 82, no. 6, pp. 407–428, 1975.
[15] P. A. Allen, M. McNeal, and D. Kvak, “Perhaps the lexicon is coded as a function of word frequency,” Journal of Memory and Language, vol. 31, no. 6, pp. 826–844, 1992.
[16] P. S. Foster, V. Drago, D. B. FitzGerald, B. M. Skoblar, G. P Crucian, and K. M. Heilman, “Spreading activation of lexical-semantic networks in Parkinson's disease,” Neuropsychologia, vol. 46, no. 7, pp. 1908–1914, 2008.
[17] E. Herrerra, F. Cuetoa, and R. Ribacoba, “Verbal fluency in Parkinson's disease patients on/off dopamine medication,” Neuropsychologia, vol. 50, no. 14, pp. 3638–3640, 2012.

[18] W. F. Battig and W. E. Montague, “Category norms of verbal items in 56 categories: A replication and extension of the Connecticut category norms,” Journal of Experimental Psychology, vol. 80, no. 3, pp. 1–46, 1969.
[19] M. Boccardi and S. F. Cappa, “Normative values of categorical production for the Italian language,” Giornale Italiano di Psicologia, vol. 24, pp. 425–436, 1997.
[20] M. M. Hoehn and M. D. Yahr, “Parkinsonism: onset, progression, and mortality,” Neurology, vol. 17, no. 5, pp. 427–442, 1967.
[21] G. Brébion, C. Stephan-Otto, E. Huerta-Ramos et al., “Abnormal functioning of the semantic network in schizophrenic patients with thought disorganization. An exemplar production task,” Psychiatrie Research, vol. 205, no. 1-2, pp. 1–6, 2013.
[22] B. Dubois, D. Burn, C. Goetz et al., “Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force,” Movement Disorders, vol. 22, no. 16, pp. 2314–2324, 2007.
[23] M. F. Folstein, S. E. Folstein, and P. R. McHugh, “Mini mental state. A practical method for grading the cognitive state of patients for the clinician,” Journal of Psychiatric Research, vol. 12, no. 3, pp. 189–198, 1975.
[24] A. Laudanna, A. M. T. C. Thornton, B. Brown, C. Burani, and L. Marconi, “Un corpus dell’italiano scritto contemporaneo dalla parte del ricettario,” in II Giornate internazionali di analisi statistica dei dati testuali, S. Bolasco, L. Lebart, and A. Salem, Eds., vol. 1, pp. 103–109, Ciut, Roma, Italy, 1995.
[25] G. A. Carlesimo, C. Caltagirone, G. Gainotti et al., “The mental deterioration battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment,” European Neuropsychologia, vol. 36, no. 6, pp. 378–384, 1996.
[26] J. W. Langston, H. Widner, C. G. Goetz et al., “Core assessment program for intracerebral transplants (CAPIT),” Movement Disorders, vol. 7, no. 1, pp. 2–13, 1992.
[27] S. Fahn, R. L. Elton, and Members of the UPDRS Committee, “Unified Parkinson's disease rating scale,” in Recent development in Parkinson's disease, pp. 153–163, Mac Millan Health Care Information, Florham Park, NJ, USA, 1987.
[28] P. Péran, A. Cherubini, F. Assogna et al., “Magnetic resonance imaging markers of Parkinson's disease nigrostriatal signature,” Brain, vol. 133, no. 11, pp. 3423–3433, 2010.
[29] R. Cools and M. D’Esposito, “Inverted-U-shaped dopamine actions on human working memory and cognitive control,” Biological Psychiatry, vol. 69, no. 12, pp. e113–e125, 2011.
[30] A. Costa, A. Peppe, G. Dell'Agnello, C. Caltagirone, and G. A. Carlesimo, “Dopamine and cognitive functioning in de novo subjects with Parkinson's disease: Effects of pramipexole and pergolide on working memory,” Neuropsychologia, vol. 47, no. 5, pp. 1374–1381, 2009.
[31] A. Costa, A. Peppe, I. Mazzò, M. Longarzo, C. Caltagirone, and G. A. Carlesimo, “Dopamine treatment and cognitive functioning in individuals with Parkinson's disease: The “cognitive flexibility” hypothesis seems to work,” Behavioural Neurology, vol. 2014, Article ID 260896, 2014.
[32] R. Cools, “Dopaminergic modulation of cognitive function-implications for L-DOPA treatment in Parkinson's disease,” Neuroscience and Biobehavioral Reviews, vol. 30, no. 1, pp. 1–23, 2006.
Research Article

Correlation of Visuospatial Ability and EEG Slowing in Patients with Parkinson’s Disease

Dominique Eichelberger,1 Pasquale Calabrese,1 Antonia Meyer,2 Menorca Chaturvedi,2 Florian Hatz,2 Peter Fuhr,2 and Ute Gschwandtner2

1Division of Molecular and Cognitive Neuroscience, Neuropsychology and Behavioural Neurology Unit, University of Basel, Basel, Switzerland
2Department of Neurology, Hospital of the University of Basel, Petersgraben 4, 4031 Basel, Switzerland

Correspondence should be addressed to Pasquale Calabrese; pasquale.calabrese@unibas.ch

Received 10 October 2016; Accepted 5 February 2017; Published 28 February 2017

Copyright © 2017 Dominique Eichelberger et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Visuospatial dysfunction is among the first cognitive symptoms in Parkinson’s disease (PD) and is often predictive for PD-dementia. Furthermore, cognitive status in PD-patients correlates with quantitative EEG. This cross-sectional study aimed to investigate the correlation between EEG slowing and visuospatial ability in nondemented PD-patients.

Methods. Fifty-seven nondemented PD-patients (17 females/40 males) were evaluated with a comprehensive neuropsychological test battery and a high-resolution 256-channel EEG was recorded. A median split was performed for each cognitive test dividing the patients sample into either a normal or lower performance group. The electrodes were split into five areas: frontal, central, temporal, parietal, and occipital. A linear mixed effects model (LME) was used for correlational analyses and to control for confounding factors.

Results. Subsequently, for the lower performance, LME analysis showed a significant positive correlation between ROCF score and parietal alpha/theta ratio (β = .59, p = .012) and occipital alpha/theta ratio (β = 0.50, p = .030). No correlations were found in the group of patients with normal visuospatial abilities.

Conclusion. We conclude that a reduction of the parietal alpha/theta ratio is related to visuospatial impairments in PD-patients. These findings indicate that visuospatial impairment in PD-patients could be influenced by parietal dysfunction.

1. Introduction

Cognitive decline is common in patients with Parkinson’s disease (PD) and may range from mild impairment to overt dementia [1]. The cognitive symptoms are highly relevant as they go hand in hand with quality of life, disease prognosis, and caregiver burden [2]. The cognitive impairment generates far-reaching individual and health economic implications. Cognitive impairment in PD was mainly characterised by executive dysfunction, attentional, memory, and visuospatial deficits [3, 4]. Previous studies showed that visuospatial disturbances are among the first symptoms of cognitive decline to appear in PD [5, 6]. These deficits become more pronounced as the disease progresses [7] and they are independent of the severity of motor dysfunction and of the overall intellectual status. Interestingly, PD-patients with visuospatial deficits or memory impairment show a higher conversion rate to Parkinson’s disease dementia (PDD) than individuals with executive deficits [8, 9].

The cause of the visuospatial deficits remains unclear [10]. Pereira et al. [11] showed that patients with Parkinson’s disease and mild cognitive impairment (PD-MCI) have a greater grey matter atrophy in both occipitotemporal and dorsoparietal cortices compared to healthy controls. Furthermore, previous research found that these patterns correlate with visuoceptual and visuospatial abilities. These results are in line with the dual-stream hypothesis of visual processing which differentiates between two linked visual projection systems [12]. The first system expands from the area 17 (primary visual cortex) over the dorsal visual route towards the areas of the upper temporal lobe and the parietal lobe (occipitoparietal projection system). These areas participate in the analysis of visuospatial information such as movement, depth, position, orientation, and 3D characteristics of objects. The second
projection system, the ventral visual stream, is responsible for pattern recognition (analysis of shapes, colours, objects, and faces). It connects area 17 to the lower temporal lobe.

Biomarker-based detection might lead to a better understanding of the cause of the visuospatial decline in PD-patients. Slowing of oscillatory brain activity (as measured by EEG and MEG) has been proposed as a surrogate marker of cognitive dysfunction [1, 13–15]. Soikkeli et al. [15] and also Olde Dubbelink et al. [16] demonstrated significantly different patterns in EEG frequencies between PD-patients and healthy controls. The authors found a decrease of beta and alpha activity and an analogous increase of theta and delta activity. In PDD-patients, the results are even more marked. A previous study showed that the alphal/theta ratio is a reliable marker for PD-MCI [17]. Furthermore, Schmidt et al. [18] found that alpha/theta ratio discriminates Alzheimer’s disease patients from healthy controls. The study of Kamei et al. [13] verified a positive correlation between deficient executive functions in PD and frontal EEG slowing. This relationship indicates that the deficits in executive tasks in PD could be due to a frontal dysfunction. Based on these findings, it would be interesting to investigate whether visuospatial abilities are related to parietal and occipital EEG activity in PD-patients.

More precisely, it is hypothesized that PD-patients with a visuospatial deficit manifest an EEG slowing which should be particularly pronounced in the parietal and the occipital lobe, compared to frontal, central, and temporal areas. To avoid confounding with overall cognitive performance the EEG slowing is matched with a test of memory span measures (short-term memory). This association in turn, is expected to be stronger in the frontal lobe compared to central, temporal, parietal, and occipital areas.

2. Materials and Methods

2.1. Subjects and Clinical Assessments. Participants were recruited between 2011 and 2015 from the outpatient clinic for movement disorders of the University Hospital Basel or through announcements in the Journal of the Swiss Parkinson’s Disease Association. Altogether 72 patients with PD participated in the study. The data used in this study were baseline data collected from two studies. The first study was a computer-based, multidimensional and disease specific training of cognition in patients with PD that has already been published [20]. The second study is an ongoing group-based stress management training in patients with PD. Clinical assessment was performed with optimally medicated patients by means of the sum score of the motor section of the Unified Parkinson’s Disease Rating Scale (UPDRS) subscale III [21]. Depression was assessed by Beck’s Depression Inventory (BDI) [22]. The levodopa-equivalent (LED) was estimated according to Tomlinson et al. [23]. Inclusion criteria for the study were idiopathic PD according to UK Parkinson’s disease Brain Bank Criteria [24] and signed informed consent was obtained from patients. Patients were excluded if they had other severe brain disorders and insufficient knowledge of the German language or if the EEG and the neuropsychology measurement were set apart more than 60 days.

For this study, the data of 57 patients with PD were included. Fifteen patients were excluded due to a Mini-Mental State Examination (MMSE) score of <24 (n = 3), because of undergoing a deep brain stimulation (n = 6) or due to insufficient EEG quality (n = 6, see below).

2.2. Neuropsychological Assessments. Patients were assessed with a comprehensive neuropsychological test battery. The following tests of this battery were used for this study; Clock Drawing Test, Rey-Osterrieth Complex Figure Test (ROCF) copy task [25], Block Design Test [26], and verbal Digit Span forward [27].

The Clock Drawing Test was scored according to Thalmann et al. [28]. It is a reliable measure of cognitive dysfunction [29, 30]. The Clock Drawing Test correlates with visuospatial tests like the ROCF and the Block Design [31, 32].

The ROCF is a common neuropsychological screening method for visuospatial abilities [33, 34]. Particularly, the copy variant of the task measures visuospatial construction while the delayed variant indicates visuospatial memory performance [34]. In the ROCF, the patients had to copy a complex figure. Afterwards, they had to reproduce it as complete as possible after a delay of 30 minutes. The ROCF was evaluated according to Aebi and Mistridis [35] based on Spreen and Strauss [36]. The sum score ranges from 0 to 36 points. The data were transformed into education and age controlled z-scores according to Aebi and Mistridis [35].

Block Design is a subtest of the revised Hamburg Wechsler Intelligence Scale for Adults [26]. The patients received at the beginning 4 and later 9 blocks, with different colour patterns on each side. With the blocks the patients had to build a predetermined pattern within a restricted period of time. The sum score ranges from 0 to 51 points; lower values are indicating more severe visuospatial disabilities.

Verbal Digit Span was applied to measure short-term memory. This test is a subtest of the Wechsler Memory Scale German adaption [27]. The examiner reads a series of digits aloud which have to be repeated by the subject afterwards. Each correctly repeated series granted a point, adding up to a sum score ranging from 0 to 12 points, where higher values indicate better short-term memory performance.

2.3. EEG Data. During 15 min an eyes-closed, resting-state, 256-channel EEG was recorded (Netstation 300; EGI Inc., Eugene, Oregon, USA). The reference electrode was Cz and referenced to the average. The sampling frequency was 1kHz. Segments of >35 s without artifacts or signs of sleep were visually selected. EEGs were filtered (2,500 order least-square filter; band pass: 0.5–70 Hz, notch: 50 Hz) and bad electrodes were automatically detected (using TAPEEG software) [19] and visually checked for plausibility. Artifacts such as ECG and eye blinks were detected and removed by an application of an independent component analysis. Channels with bad activation were interpolated (spherical spline method). Frequency analysis was performed with the “Welch”-method [37]. Sliding windows of 4 s with 80% Hann windows and the detection of bad windows were analysed with automated routines [19]. Semiautomatic processing
of the data was applied in order to calculate the relative power in alpha (8–18 Hz) and theta (4–8 Hz) frequency bands across the 10 brain regions (see Figure 4). Relative alpha/theta ratios were calculated from the frequency results.

2.4. Statistical Procedure. The R software version 3.2.3 was used for statistical analysis [38]. The level of statistical significance was set at \( p = .05 \).

A linear mixed effects model (LME) with the alpha/theta ratio as the dependent variable was used to test the association between EEG slowing and visuospatial test scores. The test performance was used as fixed factor and the patients as random factor. Consequently, a \( b \)-value below zero indicates that the worse the alpha/theta ratio, the lower the test performance. The LME is a linear model that allows repeated measurement. This model was adopted due to the repeated measurements, resulting from EEG electrode subdivision into the five brain areas. An exhaustive search, according to Stöcklin [39], with age, gender, years of education, motor symptoms (UPDRS III), disease duration, depression scale (BDI), MMSE, and LED showed that gender and age were confounding factors for alpha/theta ratio. The assumptions for LME are homoscedasticity (homogeneous variance), linearity, no influential data points, and independence (collinearity). The plot of the standardized residuals showed a heterogeneous variance relating to the fitted values. A logarithmic transformation was performed in order to achieve a normal distribution as proposed by Crawley [40]. After the logarithmic transformation the residuals in the used LME models were normally distributed around zero and therefore the requested homogeneity of variance was achieved [41]. Plots of the random effects showed an unsystematic arrangement around zero. This confirmed a normal distribution of the errors (linearity) [41]. Influential data points were not found. Furthermore, there was no correlation between the predictor variables.

In a first step, the LME calculations showed no correlation between alpha/theta ratio and the task performance. Because of this finding, a median split was used to separate potentially clinically conspicuous from inconspicuous patients in regard to the visuospatial ability. The median split was calculated separately for each neuropsychological test. Group A included patients from the lowest tasks performance up to the median and group B included patients from the median up to the best tasks performance. Clinical and demographic variables between the median split groups were analysed by means of \( X^2 \)-test or Mann–Whitney \( U \)-test as appropriate. The difference in relative alpha/theta ratio between the left and the right cerebral hemisphere was calculated by a Wilcoxon’s matched-pairs signed rank test. There were no significant differences in the relative alpha/theta ratio between the right- and left-sided electrode in the PD-patients \( (p = .316) \). Therefore, the analyses were based on combined data of the alpha/theta ratio for the right- and left-sided electrode locations. Furthermore, to compare the results, the LME were calculated with \( z \)-scaled Block Design and the Digit Span scores.

### Table 1: Descriptive statistics and tasks performance of total group.

| Parkinson patient | M  | SD  |
|-------------------|----|-----|
| Sex (M/F)         | 40/17 |     |
| Age (years)       | 67.21 (6.96) |     |
| Education (years) | 14.67 (3.01) |     |
| UPDRS III         | 14.77 (11.13) |    |
| MMSE              | 28.70 (1.06) |     |
| Disease duration (years) | 5.25 (0.50) |    |
| Dose of L-dopa (mg/day) | 597.60 (372.06) | |
| BDI               | 7.22 (4.47) |     |
| Clock Drawing Test (incorrectly/correctly drawn) | 16/41 |     |
| ROCF              | 28.83 (4.19) |     |
| Block Design Test | 24.79 (7.56) |     |
| Verbal Digit Span forward | 7.49 (1.72) |     |

Note. Means and standard deviations relate to raw values. UPDRS III = Unified Parkinson’s Disease Rating Scale subscale III (range 0–108); MMSE = Mini-Mental State Examination (range 0–30); BDI = Beck Depression Inventory (range 0–63); ROCF = Rey-Osterrieth Complex Figure Test.

### 3. Results

The visuospatial decrease which would be expected in PD-patients was weak in this population (see Table 1). The descriptive statistics of the clinical performance, split in the two median groups A and B, are shown in Table 2. Significant differences between group A and B had been obtained in the Clock Drawing Test with regard to the MMSE and the BDI and in the Digit Span with regard to the disease duration. Otherwise there were no significant differences between the groups. The exhaustive search had shown that gender and age were confounding factors for all used neuropsychological tests. The EEG alpha/theta ratio was different between males and females in all areas \((\text{parietal } U(57/57) = 211, p = .024, \text{frontal } U(57/57) = 200, p = .014, \text{central } U(57/57) = 205, p = .018, \text{temporal, } U(57/57) = 210, p = .023, \text{and occipital } U(57/57) = 194, p = .010)\].

#### 3.1. Clock Drawing

The LME results for the Clock Drawing Test are shown in Table 3. A significant lower alpha/theta ratio was recognised in PD-patients with an incorrectly drawn clock compared to PD-patients, who had produced a correctly drawn clock. The group difference was more distinct in parietal areas than in central, temporal, and occipital areas.

#### 3.2. ROCF

As shown in Table 4, in group A of the ROCF, the results revealed that the deeper the parietal alpha/theta ratio the worse the ROCF performance. An increase of 1.0 \( z \)-score in the ROCF increased the parietal alpha/theta ratio by \( b = 0.59, t(24) = 2.73, p = .012 \). There was also a significant positive association between occipital alpha/theta ratio and the ROCF performance in the ROCF group A. An increase of 1.0 \( z \)-score in the ROCF increased the occipital alpha/theta ratio \( [b = 0.50, t(24) = 2.31, p = .030] \). No significant association was found in the other cortical areas.
Table 2: Descriptive statistics of median split groups.

| Allocation | Clock Drawing Test | ROCF | Block Design Test | Digit Span |
|------------|--------------------|------|-------------------|------------|
|            | Incorrectly drawn  | Correctly drawn | A   | B   | A   | B   | A   | B   | A   | B   |
| N = 16     | N = 41             |       |                  |             |
| Sex (M/F)  | 11/5               | 29/12 | 1.000            | 19/9       | 20/7 | .833 | 20/9 | 19/8 | 1.000 | 20/9 | 20/8 | 1.000 |
| Age        | 67.5               | 67    | .930             | 66.5       | 69.0 | .295 | 67.0 | 69.0 | .384 | 67.0 | 67.5 | .994 |
| Education  | 15                 | 15    | .964             | 14         | 15   | .572 | 14   | 15   | .922 | 15   | 15   | .413 |
| UPDRS III  | 13.5               | 13.5  | .765             | 170        | 10.0 | .166 | 15.5 | 13.0 | .511 | 14.0 | 13.5 | .818 |
| MMSE       | 28.5               | 29    | .036*            | 29         | 29   | .347 | 29   | 29   | .209 | 29   | 29   | .322 |
| Disease duration | 5.27 | 3.37 | .160             | 4.30       | 3.24 | .508 | 4.12 | 3.37 | .558 | 2.94 | 4.74 | .038* |
| Dose of L-dopa | 666  | 510  | .247             | 650        | 495   | .206 | 590  | 550  | .906 | 510  | 585  | .296 |
| BDI        | 4.5                | 8.0   | .024*            | 6.5        | 7.18 | .901 | 6.5  | 7.35 | .655 | 7    | 7    | .941 |

Note. Values are expressed by median; UPDRS III = Unified Parkinson’s Disease Rating Scale subscale III (range 0–108); MMSE = Mini-Mental State Examination (range 0–30); BDI = Beck Depression Inventory (range 0–63); ROCF = Rey-Osterrieth Complex Figure Test; A = group with lower tasks performance; B = group with higher tasks performance; *p < .050, .p < .1.
The aim of this study was to investigate possible relationships between parietal and occipital EEG slowing and visuospatial deficit in nondemented PD-patients. The EEG slowing was measured by determining the alpha/theta ratio in the frontal, central, temporal, parietal, and occipital lobe. The visuospatial ability was assessed by three different neuropsychological tests: Clock Drawing Test, ROCF, and Block Design Test. A LME was used to explore the association between visuospatial performances and alpha/theta ratio.

In contrast to previous findings, the PD-patients in our study showed only slight deficits in visuospatial ability [3, 4]. This might be explained by the high education level of the patients in our sample. Recent studies indicated that a high education is predictive for a slower cognitive decline [42–44]. In order to separate potentially clinically conspicuous from inconspicuous patients in regard to the visuospatial ability a median split was used.

The results of this study show that PD-patients with a parietal EEG slowing manifest a visuospatial deficit. This result is in line with findings from voxel-based morphometry MRI analysis, indicating correlations between visuospatial ability in PDD-patients and changes in the occipitotemporal and dorsoparietal cortices in comparison to healthy controls [11]. Nombela et al. [45] also reported a correlation between parietal activity and visuospatial performance. In line with our hypothesis, the association between the EEG slowing and the visuospatial task performance is particularly pronounced in parietal areas compared to frontal, central, and temporal areas (see Figure 3). In addition, no differences between the association in parietal and occipital areas were detected in our sample. This finding indicates that the association is not explained by the global EEG slowing, as has been shown in previous studies in patients with PD [46, 47]. Though other previous studies also indicated that the visuospatial ability is not correlated with global EEG slowing measured by median frequency [48], more research is needed to substantiate this point. Our present findings are also in line with the dual-stream hypothesis of the visual processing claiming the occipitoparietal projection system to be responsible for visuospatial performance [12].

In all groups with test scores above the median (i.e., unimpaired visuospatial abilities), no correlations were found between the alpha/theta ratio and the task performances, indicating that a relationship between the visuospatial ability and the EEG is only measurable if the visuospatial ability score decreases below the median.

In contrast to the results of the ROCF and the Block Design Test, the results of the Clock Drawing Test showed that PD-patients drawing an incorrect clock had lower alpha/theta ratio not only in parietal and occipital brain areas but also in all other brain areas. PD-patients with a flawless CDT-performance did not show this association (see Figure 2). The neuroanatomical correlates of Clock Drawing Test performance were investigated in several studies, but
Table 4: Correlation between alpha/theta ratio and Rey-Osterrieth Complex Figure Test.

| brain areas | ROCF A | | | ROCF B | | |
|-------------|--------|-----------------|--------|--------|-----------------|--------|
|             | Comparison b parietal/other areas | p | Comparison b parietal/other areas | p |
| Parietal    | 0.59 (0.21) | .012* | -0.06 (0.17) | .738 |
| Frontal     | 0.39 (0.21) | .079 | .025* | -0.08 (0.17) | .653 | .724 |
| Central     | 0.34 (0.21) | .123 | .005* | -0.00 (0.17) | .984 | .337 |
| Temporal    | 0.34 (0.21) | .123 | .005* | -0.01 (0.17) | .967 | .372 |
| Occipital   | 0.50 (0.21) | .030* | .290 | -0.02 (0.17) | .900 | .530 |

Note. b = beta coefficient (standard errors); using a linear mixed effects model (LME); *P < .05, P < .1.

Table 5: Correlation between alpha/theta ratio and Block Design Test.

| brain areas | Block Design A | | | Block Design B | | |
|-------------|-----------------|-----------------|--------|-----------------|-----------------|--------|
|             | Comparison b parietal/other areas | p | Comparison b parietal/other areas | p |
| Parietal    | 0.49 (0.25) | .062 | 0.02 (0.15) | .886 |
| Frontal     | 0.32 (0.25) | .202 | .090 | -0.01 (0.15) | .938 | .588 |
| Central     | 0.33 (0.25) | .198 | .096 | 0.05 (0.15) | .711 | .578 |
| Temporal    | 0.25 (0.25) | .328 | .013* | 0.05 (0.15) | .735 | .631 |
| Occipital   | 0.40 (0.25) | .121 | .353 | -0.01 (0.15) | .962 | .640 |

Note. b = beta coefficient (standard errors); using a linear mixed effects model (LME); *P < .05, P < .1.

Table 6: Correlation between alpha/theta ratio and verbal Digit Span forward.

| Brain areas | Digit Span A | | | Digit Span B | | |
|-------------|-----------------|-----------------|--------|-----------------|-----------------|--------|
|             | Comparison b parietal/other areas | p | Comparison b parietal/other areas | p |
| Parietal    | -0.34 (0.17) | .054 | 0.25 (0.22) | .272 |
| Frontal     | -0.35 (0.17) | .051 | .944 | 0.13 (0.22) | .548 | .156 |
| Central     | -0.29 (0.17) | .096 | .469 | 0.08 (0.22) | .707 | .041* |
| Temporal    | -0.28 (0.17) | .107 | .381 | 0.15 (0.22) | .505 | .218 |
| Occipital   | -0.19 (0.17) | .265 | .030* | 0.13 (0.22) | .510 | .210 |

Note. b = beta coefficient (standard errors); using a linear mixed effects model (LME); *P < .05, P < .1.

The findings are inconsistent [49–52]. This discrepancy might probably stem from the fact that the Clock Drawing Test measures also executive function, numerical and verbal memory, and visuospatial ability [34,53]. Furthermore, Matsuo et al. [52] explored the relationship between regional cerebral blood flow and different scoring criteria of the Clock Drawing Test in patients with Alzheimer’s disease, revealing that different criteria correlate with different brain regions. Consequently, it can be concluded that for a more realistic analysis between different brain areas and CDT-performance an overall classification into errorless and incorrect CDT-performance might be too simple. Hence, future studies should adopt differential scoring CDT-scoring criteria to unravel this relationship.

In our study, the results of the ROCF and the Block Design Test are consistent. In line with our findings, for both tests, neuroanatomical correlations in parietal and occipital areas were also found in previous studies [54–57]. However, the results are more specific in ROCF than in the Block Design Test. This result could be partly explained by the somewhat different cognitive processes required by the different tasks. Hence, while the ROCF is mainly a visuo-constructional task, with a preponderance on visuoperceptive, visuospatial as well as graphomotor abilities without time limitation, the Block Design Test on the other hand requires mental rotation as well as geometric fragmentation analysis under time-restriction [26].

The Digit Span measures working memory. Studies on healthy subjects, using either transcranial magnetic stimulation [58] or functional neuroimaging [59], were able to show an involvement of the right dorsolateral prefrontal cortex in Digit Span processing. Furthermore, Gerton et al.
reported that parietal and occipital areas are activated during the Digit Span forward task. In the present study no association was found between EEG slowing and the Digit Span performance. Nevertheless, a slight tendency towards a negative correlation between the alpha/theta ratio and the Digit Span performance was observed in frontal, central, and parietal areas (see Figure 3). The involvement of parietal areas could be explained by the use of the visual imagination strategies the subjects used during the Digit Span test [34]. An explanation for the negative tendency could be that the Digit Span performance is not relating to EEG slowing caused by a shifting in alpha/theta ratio but by a shifting in others frequency range (e.g., theta/delta ratio or beta/alpha).

The results from our present study do not reveal significant differences regarding confounding factors between the median split groups of the ROCF and Block Design Test. However, there were significant differences between the subgroups according to their Clock Drawing Test performance. Patients with an incorrectly drawn clock had a lower MMSE score than patients with a flawless CDT-performance. This finding is not surprising since both CDT and MMSE are also measures of global cognitive dysfunction [28–30, 60]. In addition, many studies found a correlation between these two tests [53, 61–63]. Furthermore, PD-patients with an incorrectly drawn clock had a lower BDI score than PD-patients with a correctly drawn clock. These findings were unexpected as it is well-known that there is an association between depression and cognitive performance [64]. The results exploring the association between severity of depression and the performance in the Clock Drawing Test are inconsistent. Some authors have reported a significant negative relation [65, 66], whereas others have found minimal or no effect between the severity of depression and the Clock Drawing performance [61, 67–70]. In the present study, the BDI score has no significant influence on the used LME model. Nevertheless, the results cannot predict whether severity of depression has an influence on the cognitive performance. Another group difference is found between Digit Span performance and disease duration. Patients in the Digit Span group A have a shorter disease duration than patients in the Digit Span group B. This result contrasts with some recent findings which have shown a reduction of working memory capacity in PD-patients as the disease progresses [71, 72]. Hence, our result might be caused by the sampling process based on a right-skewed sample.

While in the present study gender (see Figure 1) and age are identified as confounding factors and were consequently controlled in the LME, other authors have found only a small influence for these variables on the EEG activity [46, 73]. Hence, further studies are needed to determine the influence of gender and age on EEG slowing in PD-patients.

One limitation of our study is that the calculation of z-scores for the ROCF was based on a norm population whereas the calculations of z-scores for the Block Design Test and the Digit Span were based on our study population, limiting a comparison of the tests. Moreover, since only 17 of 57 patients in our sample were female, the gender influence on EEG limits a generalization. Although the unequal distribution of gender is well-known in PD [74, 75] an equal gender distribution should be considered in future studies. Another limitation of this study is that there were no healthy controls included. Therefore, the conclusion that the findings are specific for patients with PD cannot be drawn. Moreover, since we adopted a priori hypothesis based models, we relinquished to account for multiple comparisons. Therefore the interpretation of the results should be treated with caution, bearing in mind that the probability of correlative findings increases with the number of tests performed. In conclusion, in PD-patients with only slight deficits in visuospatial abilities the visuospatial performance is related to parietal and occipital EEG slowing. The association between the EEG slowing and the visuospatial task performance is particularly pronounced in parietal areas compared to frontal, central, and temporal areas.
Figure 3: Correlation between alpha/theta ratio and tasks performance in different brain areas; ROCF is Rey-Osterrieth Complex Figure Test; A is group with lower tasks performance; B is group with higher tasks performance.

Figure 4: Electrodes allocation for frequency analysis [19].
Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The study was supported in part by grants from the Jacques and Gloria Gossweiler Foundation, Parkinson Schweiz, the Hedwig Widmer Foundation, and the Swiss National Science Foundation (SPUM 33CM30). Peter Fuhr’s research is supported by Swiss National Science Foundation, Swiss Multiple Sclerosis Society, Synopsis Foundation, Parkinson Schweiz, Novartis Research Foundation, Gossweiler Foundation, Freiwillige Akademische Gesellschaft Basel, Mach-Gaensslen-Stiftung, Botnar Foundation, Bangerter Foundation, and unconditional research grants from industry (Roche, AbbVie, Biogen, and General Electric).

References

[1] J. N. Caviness, J. G. Hentz, V. G. Evidente et al., "Both early and late cognitive dysfunction affects the electroencephalogram in Parkinson’s disease," Parkinsonism and Related Disorders, vol. 13, no. 6, pp. 348–354, 2007.
[2] E. Kalbe and A. Petrelli, "Leichte kognitive Störungen und Demenz bei Patienten mit Morbus Parkinson," Zeitschrift für Neuropsychologie, vol. 25, no. 1, pp. 17–30, 2014.
[3] M. Emre, "Dementia associated with Parkinson’s disease," Lancet Neurology, vol. 2, no. 4, pp. 229–237, 2003.
[4] J. Gratwicke, M. Jahanshahi, and T. Foltynie, "Parkinson’s disease dementia: a neural networks perspective," Brain, vol. 138, no. 6, pp. 1454–1476, 2015.
[5] A. Antal, F. Bandini, S. Kéri, and I. Bodis-Wollner, "Visuo-cognitive dysfunctions in Parkinson’s disease," Clinical Neuroscience, vol. 5, no. 2, pp. 147–152, 1998.
[6] F. Girotti, P. Soliveri, F. Carella et al., "Dementia and cognitive impairment in Parkinson’s disease," Journal of Neurology, Neurosurgery & Psychiatry, vol. 51, no. 12, pp. 1498–1502, 1988.
[7] B. E. Levin, M. M. Llabre, S. Reisman et al., "Visuospatial impairment in Parkinson’s disease," Neurology, vol. 41, no. 3, pp. 365–369, 1991.
[8] D. Muslimović, B. Post, J. D. Speelman, and B. Schmand, "Cognitive profile of patients with newly diagnosed Parkinson disease," Neurology, vol. 65, no. 8, pp. 1239–1245, 2005.
[9] C. H. Williams-Gray, T. Foltynie, C. E. G. Brayne, T. W. Robbins, and R. A. Barker, "Evolution of cognitive dysfunction in an incident Parkinson’s disease cohort," Brain, vol. 130, no. 7, pp. 1787–1798, 2007.
[10] S. Laatu, A. Revonsuo, L. Phikho, R. Portin, and J. O. Rinne, "Visual object recognition deficits in early Parkinson’s disease," Parkinsonism & Related Disorders, vol. 10, no. 4, pp. 227–233, 2004.
[11] J. B. Pereira, C. Junqué, M.-J. Marti, B. Ramirez-Ruiz, N. Bargalló, and E. Tolosa, "Neuroanatomical substrate of visuospatial and visuo-perceptual impairment in Parkinson’s disease," Movement Disorders, vol. 24, no. 8, pp. 1193–1199, 2009.
[12] L. G. Ungerleider and J. V. Haxby, "‘What’ and ‘where’ in the human brain," Current Opinion in Neurobiology, vol. 4, no. 2, pp. 157–165, 1994.
[13] S. Kamei, A. Morita, K. Serizawa, T. Mizutani, and K. Hirayagni, "Quantitative EEG analysis of executive dysfunction in Parkinson disease," Journal of Clinical Neurophysiology, vol. 27, no. 3, pp. 193–197, 2010.
[14] A. Morita, S. Kamei, and T. Mizutani, "Relationship between slowing of the EEG and cognitive impairment in Parkinson disease," Journal of Clinical Neurophysiology, vol. 28, no. 4, pp. 384–387, 2011.
[15] R. Soikkeli, J. Partanen, H. Soininen, A. Pääkkönen, and P. Riekkinen Sr., "Slowing of EEG in Parkinson’s disease," Electroencephalography and Clinical Neurophysiology, vol. 79, no. 3, pp. 159–165, 1991.
[16] K. T. E. Olde Dubbelink, D. Stoffers, J. B. Deijen, J. W. R. Twisk, C. J. Stam, and H. W. Berendse, "Cognitive decline in Parkinson’s disease is associated with slowing of resting-state brain activity: a longitudinal study," Neurobiology of Aging, vol. 34, no. 2, pp. 408–418, 2013.
[17] H. Bousleiman, M. Chaturvedi, U. Gschwandtner et al., "Pi22: Alpha1/theta ratio from quantitative EEG (qEEG) as a reliable marker for mild cognitive impairment (MCI) in patients with Parkinson’s disease (PD)," Clinical Neurophysiology, vol. 126, no. 8, pp. e150–e151, 2015.
[18] M. T. Schmidt, P. A. M. Kanda, L. F. H. Basile et al., "Index of alpha/theta ratio of the electroencephalogram: A new marker for Alzheimer’s disease," Frontiers in Aging Neuroscience, vol. 5, 2013.
[19] F. Hatz, M. Hardmeier, H. Bousleiman, S. Rüegg, C. Schindler, and P. Fehr, "Reliability of fully automated versus visually controlled pre- and post-processing of resting-state EEG," Clinical Neurophysiology, vol. 126, no. 2, pp. 268–274, 2015.
[20] R. Zimmermann, U. Gschwandtner, N. Benz et al., "Cognitive training in Parkinson disease: cognition-specific vs nonspecific computer training," Neurology, vol. 82, no. 14, pp. 1219–1224, 2014.
[21] S. Fahn and R. I. Elton, "Unified Parkinson’s disease rating scale," in Recent Developments in Parkinson’s Disease, S. Fahn, C. D. Marsden, D. B. Calne, and M. Goldstein, Eds., vol. 2, Macmillan Health Care Information, Florham Park, NJ, USA, 1987.
[22] A. Schrag, P. Barone, R. G. Brown et al., "Depression rating scales in Parkinson’s disease: critique and recommendations," Movement Disorders, vol. 22, no. 8, pp. 1077–1092, 2007.
[23] C. L. Tomlinson, R. Stowe, S. Patel, C. Rick, R. Gray, and C. E. Clarke, "Systematic review of levodopa dose equivalency reporting in Parkinson’s disease," Movement Disorders, vol. 25, no. 15, pp. 2649–2653, 2010.
[24] W. R. G. Gibb and A. J. Lees, "The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson’s disease," Journal of Neurology, Neurosurgery and Psychiatry, vol. 51, no. 6, pp. 745–752, 1988.
[25] J. F. Duley, J. W. Wilkins, S. L. Hamby, D. G. Hopkins, R. D. Burwell, and N. S. Barry, "Explicit scoring criteria for the Rey-Osterrieth and Taylor complex figures," Clinical Neuropsychologist, vol. 7, no. 1, pp. 29–38, 1993.
[26] D. Wechsler, Hamburger Wéchsler Intelligenztest für Erwachsene Revision, Huber, Bern, Switzerland, 1991.
[27] C. Härtling, H. J. Markowitsch, H. Neufeld, P. Calabrese, K. Deisinger, and J. Kessler, Wechsler Gedächtnis-Test-Revidierte Fassung (WMS-R), Testmanual, Deutsche Adaptation der Revidierten Fassung der Wechsler Memory Scale von David Wechsler, Hans Huber, Bern, Switzerland, 2000.
[28] B. Thalmann, R. Spiegel, H. B. Stahelin et al., "Dementia screening in general practice: optimised scoring for the clock drawing test," Brain Aging, vol. 2, no. 2, pp. 36–43, 2002.

[29] D. A. Cahn-Weiner, E. V. Sullivan, P. K. Shear et al., "Brain structural and cognitive correlates of clock drawing performance in Alzheimer’s disease," Journal of the International Neuropsychological Society, vol. 5, no. 6, pp. 502–509, 1999.

[30] O. Riedel, J. Klotsche, H. Förstl, and H.-U. Wittchen, "Clock drawing test: is it useful for dementia screening in patients having parkinson disease with and without depression?" Journal of Geriatric Psychiatry and Neurology, vol. 26, no. 3, pp. 151–157, 2013.

[31] E. Pinto and R. Peters, "Literature review of the Clock Drawing Test as a tool for cognitive screening," Dementia and Geriatric Cognitive Disorders, vol. 27, no. 3, pp. 201–213, 2009.

[32] A. Hochrein, L. Jonitz, E. Plaum, and R. R. Engel, Kompetenzbeurteilung und Kompetenzzmessung bei Dementen—ein Vergleich zwischen Verfahren zur Quantifizierung demenzbedingter Beeinträchtigungen des Alltagsverhaltens, Springer, Berlin, Germany, 1996.

[33] K. Karádi, T. Lucza, Z. Aschermann et al., "Visuospatial impairment in Parkinson’s disease: the role of laterality," Laterality, vol. 20, no. 1, pp. 112–127, 2015.

[34] M. D. Lezak, D. B. Howieson, E. D. Bigler, and D. Tranel, Neuropsychological Assessment, Oxford University Press, New York, NY, USA, 2012.

[35] S. Aebi and P. Mistridis, "Die komplexe Figur von Rey-Osterieth. Eine Normierungsstudie zur Bewertung der Reproduktionsgenauigkeit nach deutschen Kriterien," in Unveröffentlichte Masterarbeit, Universität Basel, Basel, Switzerland, 2009.

[36] O. Spreen and E. Strauss, A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary, University Press, New York, NY, USA, 1991.

[37] P. D. Welch, "The use of fast fourier transform for the estimation of power spectra: a method based on time averging over short, modified periodograms," IEEE Transactions on Audio and Electroacoustics, vol. 15, no. 2, pp. 70–73, 1967.

[38] R-Core-Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2015, https://www.r-project.org/.

[39] M. Stocklin, Statistik 3 Eine Einführung mit R, Fakultät für Psychologie-Universität Basel, Basel, Switzerland, 2014.

[40] M. J. Crawley, Statistik mit R, John Wiley & Sons, New York, NY, USA, 2012.

[41] R. Leonhart and S. Lichtenberg, Lehrbuch Statistik, H. Huber, 2009.

[42] H. H. Kornhuber, "Prävention von Demenz (einschließlich Alzheimer-Krankheit)," Das Gesundheitswesen, vol. 66, no. 5, pp. 346–351, 2004.

[43] Y. Stern, "Cognitive reserve," Neuropsychologia, vol. 47, no. 10, pp. 2015–2028, 2009.

[44] X. Meng and C. D’Arcy, "Education and dementia in the context of the cognitive reserve hypothesis: a systematic review with meta-analyses and qualitative analyses," PLoS ONE, vol. 7, no. 6, Article ID e38268, 2012.

[45] C. Nombela, J. B. Rowe, S. E. Winder-Rhodes et al., "Genetic impact on cognition and brain function in newly diagnosed Parkinson’s disease: ICICLE-PD study," Brain, vol. 137, no. 10, pp. 2743–2758, 2014.

[46] B. T. Klassen, J. G. Hentz, H. A. Shill et al., "Quantitative EEG as a predictive biomarker for Parkinson disease dementia," Neurology, vol. 77, no. 2, pp. 118–124, 2011.

[47] K. T. E. O. Dubbelink, A. Hillebrand, J. W. R. Twisk et al., "Predicting dementia in Parkinson disease by combining neurophysiologic and cognitive markers," Neurology, vol. 82, no. 3, pp. 263–270, 2014.

[48] R. Zimmermann, U. Gschwandtner, F. Hatz et al., "Correlation of EEG slowing with cognitive domains in nondemented patients with Parkinson’s disease," Dementia and Geriatric Cognitive Disorders, vol. 39, no. 3–4, pp. 207–214, 2014.

[49] T. Matsuoka, J. Narumoto, K. Shibata et al., "Neural correlates of performance on the different scoring systems of the clock drawing test," Neuroscience Letters, vol. 487, no. 3, pp. 421–425, 2011.

[50] D. Y. Lee, E. H. Lee, I. H. Choo et al., "Neural correlates of the clock drawing test performance in Alzheimer’s disease: a FDG-PET study," Dementia and Geriatric Cognitive Disorders, vol. 26, no. 4, pp. 306–313, 2008.

[51] J. M. Shon, D. Y. Lee, E. H. Seo et al., "Functional neuroanatomical correlates of the executive clock drawing task (CLOX) performance in Alzheimer’s disease: a FDG-PET study," Neuroimage, vol. 246, pp. 271–280, 2013.

[52] T. Matsuoka, J. Narumoto, A. Okamura et al., "Neural correlates of the components of the clock drawing test," International Psychogeriatrics, vol. 25, no. 8, pp. 1317–1323, 2013.

[53] K. I. Shulman, "Clock-drawing: is it the ideal cognitive screening test?" International Journal of Geriatric Psychiatry, vol. 15, no. 6, pp. 548–561, 2000.

[54] R. J. Melrose, D. Harwood, T. Khoo, M. Mandelkern, and D. L. Sultzter, "Association between cerebral metabolism and Rey-Osterrieth Complex Figure Test performance in Alzheimer’s disease," Journal of Clinical and Experimental Neuropsychology, vol. 35, no. 3, pp. 246–258, 2013.

[55] T. N. Chase, P. Fedio, N. L. Foster, R. Brooks, G. Chirow, and L. Mansi, "Wechsler adult intelligence scale performance: cortical localization by fluorodeoxyglucose F18-positron emission tomography," Archives of Neurology, vol. 41, no. 12, pp. 1244–1247, 1984.

[56] E. K. Warrington, M. James, and C. Maciejewski, "The WAIS as a lateralizing and localizing diagnostic instrument: a study of 656 patients with unilateral cerebral lesions," Neuropsychologia, vol. 24, no. 2, pp. 223–239, 1986.

[57] M. C. Wilde, C. Boake, and M. Sherer, "Wechsler adult intelligence scale-revised block design broken configuration errors in nonpenetrating traumatic brain injury," Applied Neuropsychology, vol. 7, no. 4, pp. 208–214, 2000.

[58] A. Aleman and M. van’t Wout, "Repetitive transcranial magnetic stimulation over the right dorsolateral prefrontal cortex disrupts digit span task performance," Neuropsychobiology, vol. 57, no. 1-2, pp. 44–48, 2008.

[59] B. K. Gerton, T. T. Brown, A. Meyer-Lindenberg et al., "Shared and distinct neurophysiological components of the digits forward and backward tasks as revealed by functional neuroimagining," Neuropsychologia, vol. 42, no. 13, pp. 1781–1787, 2004.

[60] C. Ploenes, S. Sharp, and M. Martin, "The Clock Test: drawing a clock for detection of cognitive disorders in geriatric patients," Zeitschrift für Gerontologie und Geriatrie, vol. 27, no. 4, pp. 246–252, 1994.

[61] L. K. Klein, Vergleichende neuropsychologische Untersuchungen bei älteren Patienten mit früh und spät beginnenden depressiven Störungen unter besonderer Berücksichtigung von Uhrentests, Universität Tübingen, Tübingen, Germany, 2015.

[62] H. Brodaty and C. M. Moore, "The clock drawing test for dementia of the Alzheimer’s type: a comparison of three scoring
methods in a memory disorders clinic,” *International Journal of Geriatric Psychiatry*, vol. 12, no. 6, pp. 619–627, 1997.

[63] J. Heinik, I. Solomesh, and P. Berkman, “Correlation between the CAMCOG, the MMSE, and three clock drawing tests in a specialized outpatient psychogeriatric service,” *Archives of Gerontology and Geriatrics*, vol. 38, no. 1, pp. 77–84, 2004.

[64] D. C. Steffens and G. G. Potter, “Geriatric depression and cognitive impairment,” *Psychological Medicine*, vol. 38, no. 2, pp. 163–175, 2008.

[65] P. O. Harvey, G. Le Bastard, J. B. Pochon et al., “Executive functions and updating of the contents of working memory in unipolar depression,” *Journal of Psychiatric Research*, vol. 38, no. 6, pp. 567–576, 2004.

[66] C. Sarapas, S. A. Shankman, M. Harrow, and J. F. Goldberg, “Parsing trait and state effects of depression severity on neurocognition: evidence from a 26-year longitudinal study,” *Journal of Abnormal Psychology*, vol. 121, no. 4, pp. 830–837, 2012.

[67] V. Elderkin-Thompson, K. B. Boone, S. Hwang, and A. Kumar, “Neurocognitive profiles in elderly patients with frontotemporal degeneration or major depressive disorder,” *Journal of the International Neuropsychological Society*, vol. 10, no. 5, pp. 753–771, 2004.

[68] C. R. Quinn, A. Harris, K. Felmingham, P. Boyce, and A. Kemp, “The impact of depression heterogeneity on cognitive control in major depressive disorder,” *Australasian and New Zealand Journal of Psychiatry*, vol. 46, no. 11, pp. 1079–1088, 2012.

[69] M. Kirby, A. Denihan, I. Bruce, D. Coakley, and B. A. Lawlor, “The clock drawing test in primary care: sensitivity in dementia detection and specificity against normal and depressed elderly,” *International Journal of Geriatric Psychiatry*, vol. 16, no. 10, pp. 935–940, 2001.

[70] N. Herrmann, D. Kidron, K. I. Shulman et al., ”Clock tests in depression, Alzheimer’s disease, and elderly controls,” *International Journal of Psychiatry in Medicine*, vol. 28, no. 4, pp. 437–447, 1998.

[71] C. Warden, J. Hwang, A. Marshall, M. Feney, and K. L. Poston, “The effects of dopamine on digit span in Parkinson’s disease,” *Journal of Clinical Movement Disorders*, vol. 3, no. 1, article 5, 2016.

[72] D. K. Johnson, Z. Langford, M. Garnier-Villarreal, J. C. Morris, and J. E. Galvin, “Onset of mild cognitive impairment in parkinson disease,” *Alzheimer Disease and Associated Disorders*, vol. 30, no. 2, pp. 127–133, 2016.

[73] M. L. Morgan, E. A. Witte, I. A. Cook, A. F. Leuchter, M. Abrams, and B. Siegman, “Influence of age, gender, health status, and depression on quantitative EEG,” *Neuropsychobiology*, vol. 52, no. 2, pp. 71–76, 2005.

[74] M. Baldereschi, A. Di Carlo, W. A. Rocca et al., “Parkinson’s disease and parkinsonism in a longitudinal study: two-fold higher incidence in men,” *Neurology*, vol. 55, no. 9, pp. 1358–1363, 2000.

[75] S. K. Van Den Eeden, C. M. Tanner, A. L. Bernstein et al., “Incidence of Parkinson’s disease: variation by age, gender, and race/ethnicity,” *American Journal of Epidemiology*, vol. 157, no. 11, pp. 1015–1022, 2003.
Review Article

An Integrated Review of Psychological Stress in Parkinson’s Disease: Biological Mechanisms and Symptom and Health Outcomes

Kim Wieczorek Austin,1 Suzanne Weil Ameringer,1 and Leslie Jameleh Cloud2

1Virginia Commonwealth University School of Nursing, 1100 East Leigh Street, Richmond, VA 23219, USA
2Virginia Commonwealth University Parkinson’s and Movement Disorders Center and VCU Health Neuroscience, Orthopaedic, and Wellness Center, 11958 West Broad Street, Richmond, VA 23233, USA

Correspondence should be addressed to Kim Wieczorek Austin; kim@kwaustin.net

Received 8 May 2016; Revised 28 September 2016; Accepted 1 November 2016

Academic Editor: Elka Stefanova

Copyright © 2016 Kim Wieczorek Austin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Parkinson’s disease (PD) is characterized by complex symptoms and medication-induced motor complications that fluctuate in onset, severity, responsiveness to treatment, and disability. The unpredictable and debilitating nature of PD and the inability to halt or slow disease progression may result in psychological stress. Psychological stress may exacerbate biological mechanisms believed to contribute to neuronal loss in PD and lead to poorer symptom and health outcomes. The purpose of this integrated review is to summarize and appraise animal and human research studies focused on biological mechanisms, symptom, and health outcomes of psychological stress in PD. A search of the electronic databases PubMed/Medline and CINAHL from 1980 to the present using the key words Parkinson’s disease and stress, psychological stress, mental stress, and chronic stress resulted in 11 articles that met inclusion criteria. The results revealed significant associations between psychological stress and increased motor symptom severity and loss of dopamine-producing neurons in animal models of PD and between psychological stress and increased symptom severity and poorer health outcomes in human subjects with PD. Further research is needed to fully elucidate the underlying biological mechanisms responsible for these relationships, for the ultimate purpose of designing targeted interventions that may modify the disease trajectory.

1. Introduction

Parkinson’s disease (PD) is characterized by complex symptom patterns that fluctuate in onset, severity, responsiveness to treatment, and associated level of disability. The classic motor symptoms of tremor, rigidity, bradykinesia, and postural instability are compounded by nonmotor symptoms such as depression, cognitive impairments, sleep disturbances, fatigue, pain, and autonomic dysfunction. Many of these nonmotor symptoms respond poorly to available treatment options and significantly contribute to poorer quality of life and increased functional disability [1]. Early in the course of the disease, medications for PD typically improve motor symptoms. However, as the disease progresses, higher medication doses become necessary which can then cause debilitating dystonia and dyskinesia [2]. Reductions in medication dosages are often required to lessen the severity of these complications, resulting in breakthrough primary motor symptomology. Further complicating the illness experience, prolonged and/or high dose treatment with PD medications has been associated with on-off phenomena, which leads to unpredictable motor symptom exacerbations and periods of immobility. The ability to reduce these medication-induced complications while still achieving motor symptom benefits becomes more difficult as the disease progresses, leading to greater functional disability and poorer quality of life.

The unpredictable and debilitating nature of the symptoms associated with PD combined with the inability to halt or slow disease progression has the potential to result
in psychological stress. Psychological stress is a complex phenomenon that involves cognitive, emotional, behavioral, and biological responses to events or experiences that are perceived as threatening [3]. An individual's ability to cope with and adapt to psychological stress can be influenced by the number and significance of stressful events experienced within a given period of time, the degree to which stressors are perceived as threatening or harmless, and biological responses designed to promote adaptation [4, 5]. The inability to cope with or adapt to psychological stress has been associated with poorer symptom and health outcomes that may be relevant in PD. For example, in non-PD populations, significant relationships have been demonstrated between psychological stress and increased motor symptomology [6], pain [7, 8], fatigue, [6, 8], cognitive decline [9], and functional disability [9, 10].

Biological responses of the neuroendocrine and immune systems represent plausible mechanisms that may explain relationships between psychological stress and poorer symptom and health outcomes. A complex network of bidirectional links between the neuroendocrine and immune systems serve as major regulatory mechanisms for mounting effective biological responses to psychological stress [11]. The hypothalamic-pituitary-adrenal axis (HPAA) coordinates these responses by stimulating the release of cortisol in humans and corticosterone in humans and rodents. Glucocorticoids play an important role in mediating immunological responses to psychological stress by regulating microglial activation and proinflammatory cytokine and transcription factor expression and release [12]. Stress-induced dysregulation of relationships between the neuroendocrine and immune systems has been associated with neuroinflammation, oxidative stress, and loss of dopamine (DA) producing neurons within the central nervous system [13–18]. Prolonged exposure to psychological stress has also been shown to sensitize the neuroendocrine and immune systems to the detrimental effects of future insults, thereby exaggerating inflammatory responses, oxidative stress, and neuronal loss [14, 19, 20].

Based upon work in non-PD animal models, a number of underlying biological mechanisms have been implicated in stress-induced neuroinflammation, oxidative stress, and neuronal loss to include microglial activation, upregulation of proinflammatory cytokines, transcription factors, and isoenzymes, increased production of reactive oxygen species (ROS), and imbalances in the production of ROS and antioxidant reduction. Table 1 summarizes the literature on these biological mechanisms. Specifically, studies have demonstrated that both stress and the administration of exogenous corticosterone increase microglial activation, reactivity, and proliferation [14, 16, 20]. Under normal conditions, microglia cells, which are found in particularly high concentrations in the substantia nigra, exist in a resting state. Once activated, microglia provide the first line of defense by releasing immune mediators that coordinate innate and adaptive immune responses within the central nervous system to include the expression and release of proinflammatory cytokines and transcription factors [21, 22]. Whereas acute activation of microglia facilitates tissue repair, prolonged or exaggerated responses result in increased neuroinflammation and oxidative stress, both of which have been associated with dopaminergic neurotoxicity [14, 21–23]. Dopaminergic neurons are particularly vulnerable to neuroinflammatory and oxidative processes due to the high rate of oxygen consumption and limited antioxidant defenses within the central nervous system [24].

The upregulation of proinflammatory cytokines and transcription factors may further perpetuate stress-induced neuroinflammation and oxidative stress within the central nervous system. Cytokines play an important role in stimulating and coordinating the cellular interactions necessary for mounting effective immune responses to infection, injury, and disease [25]. Psychological stress has been associated with significant elevations in tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), proinflammatory cytokines that have been implicated in oxidative stress and apoptosis within the central nervous system [26–29]. Proinflammatory cytokines also stimulate activation of nuclear transcription factor-kappa B (NF-kB) pathways [30, 31], which have been implicated in apoptosis of nigral dopaminergic neurons in animal models of PD [17, 32]. Activation of NF-kB pathways also results in the production of cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin mediated inflammatory responses [33]. While COX-2 is normally expressed in relatively constant amounts within the central nervous system, stress-induced upregulation of COX-2 has been associated with increased generation of ROS, oxidative stress, neurotoxicity, and apoptosis within the central nervous system [30, 34, 35].

Stress-induced oxidative mechanisms have also been associated with neuronal loss within the central nervous system. Oxidation, and the subsequent production of ROS, occurs normally throughout the body as a result of aerobic metabolism. The greatest concentrations of ROS are found within mitochondria, the main site of adenosine triphosphate (ATP) production [36]. Under normal conditions, ROS are maintained at relatively constant levels as a result of balances between the rate of ROS production and removal by antioxidant substrates [37]. Oxidative stress occurs as a result of imbalances in the production of ROS and antioxidant reduction, resulting in excessive lipid peroxidation and tissue injury within the central nervous system. Psychological stress has been associated with increased mitochondrial production of ROS [38] and increased production of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a membrane-bound enzyme involved in neutrophil respiratory bursts [39]. Normally, NADPH remains latent in neutrophils and is tightly regulated by hormones, cytokines, and a variety of other mechanisms. Stress-induced activation of NADPH has been associated with increased production of ROS, neuroinflammation, and degeneration of dopaminergic and nondopaminergic neurons [39]. Psychological stress has also been associated with decreased expression and release of antioxidants [38, 40]. Dysregulation of the antioxidant system, coupled with increased ROS production, has been shown to perpetuate oxidative stress, lipid peroxidation, and tissue damage within the central nervous system [26, 38, 41].
| Study author/date       | Major study findings                                                                                                                                 |
|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lucca et al., 2009 [38]| Chronic mild psychological stress resulted in significant elevations in superoxide, a reactive oxygen species, in the submitochondrial particles of the prefrontal cortex, cortex, and hippocampus in subjects when compared to controls. The results also demonstrated significant elevations in TBARS, a measure of lipid peroxidation, in the cortex of stressed subjects. |
| De Pablo et al., 2006 [14]| Induction of chronic variate psychological stress enhanced LPS-induced neuroinflammation in the PFC of stressed subjects when compared to nonstressed LPS-induced subjects and controls. Significant findings included increased microglial activation, levels of DA and its metabolite DOPAC, expression of proinflammatory cytokine mRNA (TNF-α, IL-1β, and IL-6), activation of MAP kinases, and loss of NeuN-positive neurons in the PFC. |
| Munhoz et al., 2006 [18]| Chronic, unpredictable psychological stress potentiated NF-κB binding activity in the frontal cortex and hippocampus and proinflammatory gene expression of IL-β, TNF-α, and NOS-2 as mediated by elevated GC levels in LPS-induced subjects when compared to controls. |
| Kim et al., 2005 [41]  | Acute psychological stress resulted in elevated BH4 and DA levels in striatal tissues and led to greater lipid peroxidation, protein-bound quinone, neuromelanin, and antioxidant enzyme activities, markers of oxidative stress, in the substantia nigra and striatum of subjects when compared to controls. Furthermore, in subjects exposed to stress, TH-immunoreactive DA neurons demonstrated strong Fluoro-Jade staining, indicating selective degeneration of dopaminergic neurons. In contrast, no Fluoro-Jade staining was identified in controls. |
| Munhoz et al., 2004 [26]| Repeated psychological stress was associated with time-dependent markers of oxidative stress in brain tissue to include increase in Ca²⁺-independent NOS-2 activity, lipid peroxidation, TNF-α, and TACE activity in subjects when compared to controls. |
| Madrigal et al., 2003 [33]| Acute psychological stress was associated with higher levels of PGE₂, a marker of COX-2 neuronal activity, MDA and oxidized glutathione, markers of lipid peroxidation, and NOS-2 in the cortex of subjects when compared to controls. |
| Madrigal et al., 2002 [30]| Acute psychological stress induced the expression of iNOS in the brain cortex, which was preceded by increased expression of TACE and the subsequent release of TNF-α in subjects as compared to controls. Furthermore, the results demonstrated that increased production of TNF-α was involved in stress-induced expression of iNOS as mediated by activation of NF-κB. |

TBARS, thiobarbituric acid reactive species; LPS, lipopolysaccharide; PFC, prefrontal cortex; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic; mRNA, messenger ribonucleic acid; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; MAP, mitogen-activated protein; NeuN-positive, neuronal nuclei positive; GC, glucocorticoids; BH4, tetrahydrobiopterin; TH-immunoreactive, tyrosine hydroxylase; Ca²⁺, calcium²⁺; NOS-2, inducible nitric oxide synthase; TACE, TNF-α converting enzyme; iNOS, inducible nitric oxide synthase; PGE₂, prostaglandin E2; COX-2, cyclooxygenase-2; MDA, malondialdehyde.
Stress-induced dysregulation of the neuroendocrine and immune systems may play an important role in symptom and health outcomes in PD. While the exact cause remains unknown, progressive loss of dopaminergic neurons within the substantia nigra pars compacta (SNc) and nondopaminergic neurons within the central, peripheral, and autonomic nervous system are believed to contribute to symptom and disease progression [42]. As briefly summarized in Table 2, a considerable body of research exists to suggest that biological mechanisms associated with neuroinflammation and oxidative stress contribute to the loss of dopaminergic neurons in PD. These biological mechanisms include but are not limited to increased microglial activation [43–45], atypical production of select proinflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) [41, 46, 47], enhanced activation of NF-κB pathways [32, 48], increased upregulation of COX-2 [49] and NADPH oxidases [50], and increased production of select biomarkers of oxidative stress [51–53]. As such, stress-induced neuroendocrine and immune system dysregulation may exacerbate pathogenic mechanisms in PD, resulting in poorer symptom and health outcomes. The purpose of this integrated review is to summarize and critically appraise the current state of the science regarding biological mechanisms and symptom and health outcomes of psychological stress in individuals with and animal models of PD. Limitations of the existing literature as well as directions for future research will also be discussed.

2. Methods

An integrated review was conducted to examine human and animal research studies that focused on biological mechanisms and symptom and health outcomes of psychological stress in PD. A title search of the electronic databases

| Study author/date | Major study findings |
|-------------------|----------------------|
| **Microglia activation** |
| Gerhard et al., 2006 [43] | In vivo PET imaging revealed widespread and longitudinal microglial activation in subjects with PD when compared to controls |
| Ouchi et al., 2005 [44] | Microglial activation was associated with damage in nigrostriatal pathway in drug-naïve subjects with PD when compared to controls |
| Depino et al., 2003 [45] | Induction of PD in animals (6-OHDA model) resulted in increased microglial activation and atypical production of proinflammatory cytokine mRNA when compared to controls |
| **Proinflammatory cytokine production** |
| Lindqvist et al., 2012 [54] | Serum levels of IL-6 significantly higher in subjects with PD than controls |
| Scalzo et al., 2010 [46] | Serum levels of IL-6 significantly higher in subjects with PD than controls |
| Reale et al., 2009 [47] | Basal and bacterial LPS-induced production of IL-1β, TNF-α, and IFN-γ significantly higher in subjects with PD than controls |
| **Proinflammatory transcription pathway activation** |
| Tobón-Velasco et al., 2013 [48] | Induction of PD in animals (6-OHDA model) resulted in enhanced NF-κB activation which was associated with increased TNF-α and COX-2 levels when compared to controls |
| Liang et al., 2007 [32] | Induction of PD in animals (6-OHDA model) resulted in activation of NF-κB pathways which contributed to oxidative stress-induced degeneration of dopaminergic neurons when compared to controls |
| **Proinflammatory isoenzyme production** |
| Hernandez et al., 2013 [50] | Induction of PD in animals (6-OHDA) demonstrated that NADPH oxidases contribute to dopaminergic neurodegeneration in the nigrostriatal pathway |
| Teismann et al., 2003 [49] | Brain tissue samples of subjects with and animal models of PD (6-OHDA) demonstrated increased COX-2 upregulation in dopaminergic neurons when compared to controls |
| **Oxidative stress** |
| Lin et al., 2012 [51] | Induction of PD in animals (rotenone model) associated with significantly higher levels of oxidative proteins in the striatum leading to greater levels of apoptotic cell death of dopaminergic neurons within the nigrostriatal system when compared to controls |
| Seet et al., 2010 [52] | Biomarkers of oxidative stress (F₂-isoprostanes, hydroxyeicosatetraenoic acid products, 7B- and 27-hydroxycholesterol, 7-ketocholesterol, neuroprostanes, and urinary 8-hydroxy-2’-deoxyguanosine) significantly higher in subjects with PD when compared to controls |
| Keeney et al., 2006 [53] | Misassembled mitochondrial complex I as reflected by significant loss of its 8 kDa subunits associated with oxidative damage in brain tissue of subjects with PD when compared to controls |

PET, position emission tomography; PD, Parkinson’s disease; 6-OHDA, 6-hydroxydopamine; mRNA, messenger ribonucleic acid; IL-6, interleukin-6; LPS, lipopolysaccharide; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; NF-κB, nuclear factor-kappa-light-chain-enhancer of activated B cells; COX-2, cyclooxygenase-2; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase.
PubMed/Medline and CINAHL was conducted from 1980 to the present using the key words Parkinson’s disease and stress, psychological stress, mental stress, and chronic stress. A total of 221 articles were identified (Figure 1). Articles were reviewed based on the following inclusion criteria: (1) research studies involved human subjects with PD or animal models of PD with a primary aim of examining the effects of psychological stress on biological mechanisms and symptom and health outcomes; (2) animal studies reported using a recognized model of PD to include 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), or rotenone induction; (3) animal studies involved the induction of psychological stress; (4) human studies involved the induction of psychological stress and/or quantified psychological stress levels; and (5) the study was written in English. Based on these inclusion criteria, a title/abstract review resulted in the exclusion of 200 articles, the majority of which focused on oxidative, nitrosative, and/or endoreticulum stress (n = 171) or caregiver stress (n = 9) in PD. Full-text review and assessment for inclusion criteria was conducted on the remaining 21 articles. An additional 15 articles were excluded, the majority of which were literature reviews and involved biological mechanisms of psychological stress in non-PD animal models or case-studies that did not involve the induction of psychological stress and/or quantify psychological stress levels. The remaining six articles met the above identified inclusion criteria and were included in this review. An additional five articles were identified after a manual review of the references cited in the included articles and excluded articles that underwent a full-text review. A total of eleven articles met the inclusion criteria and were included in this review.

3. Results and Discussion

Of the 11 studies included in this review, seven examined biological mechanisms of psychological stress that contribute to pathophysiological processes and symptom outcomes in animal models of PD [55–61]. In contrast, the remaining four studies focused on biological mechanisms, symptom, and/or health outcomes of psychological stress that may modify the illness trajectory in human subjects with PD [62–65]. Each of these studies is further discussed below.

3.1. Biological Mechanisms of Psychological Stress That Contribute to Pathophysiological Processes and Symptom Outcomes in Animal Models of PD

Seven of the studies included in this review examined biological mechanisms and symptom outcomes of psychological stress in animal models of PD [55–61]. Health outcomes of psychological stress were not examined in these studies. Biological mechanisms in the reviewed studies included biomarkers of dopamine production and metabolism [55, 56, 58–61], serotonin (5-HT) [55], norepinephrine [61], and/or dopaminergic neurodegeneration [57, 58]. In addition, two studies examined biological mechanisms associated with stress responses of the neuroendocrine system, specifically the effects of corticosterone administration [57] and norepinephrine levels [61] on motor symptom outcomes. Of particular importance, one study [55] examined the effects of psychological stress on the expression of α-synuclein, a misfolded protein complex that is recognized as a pathological hallmark of PD. All of the animal studies included in this review examined symptom outcomes specific to the motor manifestations of PD [55–61]. Only one study examined the effects of psychological stress on behaviors associated with depression, a common nonmotor symptom in PD [55]. In addition, one study focused on the effects of psychological stress on the neuroprotective effects of voluntary exercise on symptom and biological outcomes [58]. These studies are further discussed below and summarized in Table 3.

Using a MPTP/probenecid (MPTP/p) animal model of PD, researchers examined the effects of chronic mild psychological stress on biological mechanisms involving DA and 5-HT levels and the expression of dopaminergic markers of the nigrostriatal (substantia nigra and striatum) and non-nigrostriatal (hippocampus, cortex, and cerebellum) systems and symptom outcomes specific to depression [55]. Symptom
| Study author & date | Study purpose & sample | Method(s) of psychological stress induction | Measures of biological mechanisms & symptom outcomes | Major study findings |
|---------------------|------------------------|--------------------------------------------|----------------------------------------------------|----------------------|
| Janakiraman et al., 2016 [55] | **Purpose:** to examine the effects of psychological stress on symptom outcomes of depression and biological mechanisms of DA, 5-HT, and α-synuclein  
**Sample:** male C57BL/6 mice (n = 72); MPTP/probenecid induction | "Cage tilting, damp sawdust, placement in empty cage, group housing, placement of a foreign object in cage, inversion of light/dark cycle, food or water deprivation, lights on for a short period of time during the dark phase, and switching cages" (p. 3) | **Biological mechanisms:** DA, 5-HT, TH, DAT, VMAT-2, α-synuclein levels in nigrostriatal (substantia nigra and striatum) and nonnigrostriatal tissues (hippocampus, cortex, and cerebellum)  
**Symptom outcomes:** depression as measured by behavioral deficits and anhedonia using the (a) open field test; (b) narrow beam walking test; and (c) sucrose intake test | Biological mechanisms: increased depletion of DA, 5-HT, TH, DAT, and VMAT-2 was identified in stress-treated lesioned subjects. Stress exaggerated the expression of nigrostriatal and nonnigrostriatal α-synuclein.  
Symptom outcomes: stress increased behavioral deficits and anhedonia in stress-treated lesioned subjects. |
| Hemmerle et al., 2014 [56] | **Purpose:** to examine the effects of psychological stress on motor symptom outcomes and biological mechanisms associated with loss of DA neurons  
**Sample:** rats (sample size/gender not provided); 6-OHDA induction | Chronic variable stress (protocol not provided) | **Biological mechanisms:** TH cell counts in the SNc  
**Symptom outcomes:** forelimb asymmetry tests (not defined) | Biological mechanisms: stress was associated with significantly lower TH cell counts in the SNc in lesioned subjects.  
Symptom outcomes: stress was associated with significant forelimb asymmetry in lesioned subjects. |
| Smith et al., 2008 [57] | **Purpose:** to examine the effects of psychological stress and corticosterone administration on motor symptom outcomes and biological mechanisms of DA neurodegeneration  
**Sample:** female rats (n = 71); 6-OHDA induction | Restraint in Plexiglas tubes | **Biological mechanisms:** plasma concentrations of corticosterone and TH positive cells, Fluoro-Jade B cells, and GFAP immunoreactivity in the MTA, VTA, and SNc  
**Symptom outcomes:** skilled forelimb reaching, skilled walking, open field behavior, and apomorphine-induced rotations | Biological mechanisms: in stress- and corticosterone-treated lesioned subjects, the loss of TH positive cells was associated with significant increases in Fluor-Jade B cells in the SNc. Significant reductions in Nissl-positive cells in the VTA and SNc and enhanced GFAP immunoreactivity in the SNc were also demonstrated in stress- and corticosterone-treated lesioned subjects.  
Symptom outcomes: stress and elevated corticosterone levels impaired skilled limb reaching and limb coordination, impeded spontaneous recovery, and altered exploratory behavior in lesioned subjects. |
| Howells et al., 2005 [58] | **Purpose:** to examine the effects of psychological stress on the neuroprotective effects of voluntary exercise on motor symptom outcomes and biological mechanisms of dopaminergic neurodegeneration  
**Sample:** male rats (n = 31); 6-OHDA induction | Running wheel immobilization and shifting light/dark cycles | **Biological mechanisms:** TH positive cells in the SNc  
**Symptom outcomes:** number of running wheel revolutions and apomorphine-induced rotations | Biological mechanisms: stressed runners demonstrated lower TH positive cells in the SNc.  
Symptom outcomes: stressed runners demonstrated a significant increase in rotational behavior. |
| Study author & date | Study purpose & sample | Method(s) of psychological stress induction | Measures of biological mechanisms & symptom outcomes | Major study findings |
|---------------------|------------------------|--------------------------------------------|------------------------------------------------|-------------------|
| Keefe et al., 1990 [59] | Purpose: to examine the extent to which psychological stress affects motor symptom outcomes specific to DA concentrations. Sample: male rats (sample size not provided); 6-OHDA. | Tail-shock stress | Biological mechanisms: extracellular striatal DA, DOPAC, and HVA levels in vivo and in brain tissue specimens. Symptom outcomes: akinesia defined as latency to move all four paws when placed on a flat surface within 120 seconds and catalepsy defined as latency to return all four paws to the table surface within 120 seconds. | Measures of biological mechanisms: extracellular striatal DA, DOPAC, and HVA levels in vivo and in brain tissue specimens. Symptom outcomes: akinesia defined as latency to move all four paws when placed on a flat surface within 120 seconds and catalepsy defined as latency to return all four paws to the table surface within 120 seconds. | Major study findings: Subjects exposed to tail-shock stress demonstrated significantly lower striatal DA, DOPAC, and HVA levels in all but one subject, these levels did not reach levels comparable to those demonstrated in control animals. A significant negative correlation was shown between post-stress striatal DA concentrations and motor behaviors. A significant difference was shown between stress-treated lesioned and control groups. No significant difference was shown between DOPAC or HVA levels in stress-treated lesioned and control groups. |
outcomes of depression included measures of locomotion, activity, and anhedonia. Subjects were randomly assigned to a saline-treated control group, MPTP/p group, stress group, MPTP/p followed by stress group, stress followed by MPTP/p group, and stress before and after MPTP/p group. Both the before and after MPTP/p stress groups demonstrated greater DA depletion in all studied brain regions when compared to the stress or MPTP/p alone groups, with greater levels of DA depletion identified in the MPTP/p followed by stress group. Serotonin levels were also decreased in both the stress and MPTP/p groups, with the greatest reduction identified in all studied brain regions in the MPTP/p followed by stress group. In the MPTP/p followed by stress group, greater reductions in tyrosine hydroxylase (TH), dopamine transporter (DAT), and vesicular monoamine transporters (VMAT-2) expression were identified in all studied brain regions, suggesting that stress affects the biosynthesis and transport of DA. In stress-treated MPTP/p subjects, stress exaggerated the expression of nigrostriatal and nonnigrostriatal α-synuclein, which plays a major role in the development and progression of PD. While no significant changes were identified in locomotion, activity, and anhedonia in the stress or MPTP/p groups, greater behavioral changes in all parameters were identified in the MPTP/p followed by stress group. Cumulatively, these findings provide important evidence to suggest that psychological stress may contribute to biological mechanisms, symptom outcomes, and disease progression in PD.

In a 6-OHDA animal model of PD, researchers examined the effects of chronic variable psychological stress on biological mechanisms involving DA as measured by TH cell counts and motor symptom outcomes [56]. Subjects were assigned to 6-OHDA lesioned stressed and nonstressed groups, a sham-lesioned group, and a control group. Both 6-OHDA groups demonstrated impaired contralateral forelimb use when compared to the sham-lesioned group. However, following four weeks of chronic, variable stress, the 6-OHDA lesioned group demonstrated significantly more forelimb asymmetry when compared to the 6-OHDA lesioned nonstressed group. Significantly lower TH cell counts were identified in the SNc of stressed 6-OHDA subjects when compared to nonstressed 6-OHDA subjects. These findings suggest chronic variable stress may exacerbate motor symptom outcomes as a result of biological mechanisms associated with dopamine deficiencies in PD.

In a 6-OHDA animal model of PD, researchers examined the effects of restraint stress and corticosterone administration on biological mechanisms associated with dopaminergic neurodegeneration and motor symptom outcomes [57]. The results revealed psychological stress and elevated corticosterone levels in lesioned subjects impaired skilled limb reaching and limb coordination, impeded spontaneous recovery and compensation, and altered exploratory behavior. Fluoro-Jade positive cells, a biomarker of neuronal degeneration, were detected earlier in stress-treated lesioned subjects than controls. In stress- and corticosterone-treated lesioned subjects, the loss of TH positive cells, a biomarker of dopamine-producing cells, was associated with a significant increase in Fluoro-Jade positive cells in the SNc. Stress- and corticosterone-treated lesioned subjects demonstrated significant reductions in Nissl-positive cells in the ventral tegmental area (VTA) and SNc, suggesting greater neurodegeneration in these areas when compared to controls. Stress- and corticosterone-treated lesioned subjects also demonstrated enhanced glial fibrillary acidic protein (GFAP) immunoreactivity in the SNc when compared to controls, indicating greater reactive gliosis in the central nervous system. In stress-treated lesioned subjects, motor impairments were associated with higher numbers of Fluoro-Jade positive cells in the SNc and VTA. Cumulatively, these findings provide important evidence to suggest that psychological stress and stress-response hormones may contribute to pathogenic mechanisms involved in motor symptom outcomes in PD as a result of biological mechanisms associated with dopaminergic neurodegeneration.

Using a 6-OHDA animal model of PD, researchers examined the effects of psychological stress on the neuroprotective effects of voluntary exercise to include motor symptom outcomes as measured by rotational behavior and biological mechanisms associated with dopaminergic neurodegeneration [58]. Subjects were randomly assigned to one of three groups: runners or stressed runners, both of which had free access to running wheels, or nonrunners who had immobilized running wheels. Nonrunners demonstrated significantly higher numbers of apomorphine-induced contralateral rotations, a measure indicative of DA depletions exceeding 80%, when compared to stressed and nonstressed runners. This finding suggests voluntary exercise exerted neuroprotective effects on dopaminergic neurons in both stressed and nonstressed runners. The administration of apomorphine resulted in a significant increase in rotational behavior in stressed runners when compared to nonstressed runners, suggesting that stress may ameliorate the neuroprotective effects of voluntary exercise. Nonstressed runners demonstrated a nonsignificant decrease in the percentage of dopaminergic neurons lost when compared to both the stressed runners (4%) and nonrunners (14%). Significant differences were also identified in the number of baseline wheel rotations and the amount of DA destruction in the stressed runners and between the stressed and nonstressed runners. Significant group differences were found in the number of apomorphine-induced rotations and loss of DA between the stressed and nonstressed runners, the stressed runners and nonrunners, and all three groups. These findings suggest psychological stress may cancel the neuroprotective effects of voluntary exercise and contribute to greater DA deficiencies and poorer motor symptom outcomes.

In a 6-OHDA animal model of PD, researchers examined the extent to which motor symptom outcomes as measured by akinetic and cataleptic behaviors and biological mechanisms specific to DA concentrations were affected by psychological stress [59]. Biological mechanisms investigated in this study included the extent to which nigrostriatal DA neurons were capable of responding to the additional demand for DA during tail-shock stress and relationships between extracellular striatal DA concentrations and motor symptom outcomes before and after psychological stress exposures. When compared to baseline, subjects exposed to tail-shock stress demonstrated significantly increased striatal
extracellular DA, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) levels. These findings suggest residual nigrostriatal DA neurons are capable of producing DA in response to psychological stress. However, in all but one subject, these levels did not reach levels comparable to those demonstrated in nonlesioned animals following tail-shock stress. While no consistent pattern was demonstrated between stress and akinetiic and cataleptic motor behaviors, a significant negative correlation was shown between poststress latencies for catalepsy and extracellular DA concentrations, with a similar trend identified for akinesia. These findings suggest psychological stress may lead to poorer motor symptom outcomes as a consequence of lower than normal DA responsiveness in the striatum.

In a MPTP animal model of PD, researchers examined the effects of immersion immobilization stress on motor symptom outcomes associated with locomotor activity and biological mechanisms involving DA content, metabolites (DOPAC and HVA), and indices (DOPAC + HVA/DA) [60]. Subjects were allocated to one of four subgroups: MPTP-treated group, saline-treated group, MPTP and stress-treated group, or saline and stress-treated group. The results revealed the induction of psychological stress in the MPTP-treated group was associated with a more pronounced but transient decrease in locomotor activity when compared to the stress-treated saline group. Striatal DA content was significantly lower in the stress-treated MPTP group when compared to the MPTP-treated group. Striatal DA indices were significantly elevated in both the MPTP- and saline-treated stress groups, indicating increased DA turnover. There was no significant difference in the striatal DA metabolites DOPAC or HVA between the two MPTP-treated groups or the two saline-treated groups. These findings provide evidence to suggest that psychological stress may contribute to motor symptom outcomes and biological mechanisms associated with dopamine deficiencies in PD.

Finally, in a 6-OHDA animal model of PD, researchers examined the effects of psychological stress on biological mechanisms involving DA and noradrenaline that may contribute to motor symptom outcomes of akinesia [61]. The results revealed the induction of acute psychological stress precipitated the development of transient akinesia in all lesioned subjects but had no effect on controls. Dopamine deficiencies in the striatum were more predictive of stress-induced akinesias than in other areas of the brain. While some subjects demonstrated moderate depletion in hippocampal noradrenaline levels, there was no consistent relationship with stress-induced akinesia. These findings suggest DA deficiencies may affect the ability to maintain normal motor function under conditions of psychological stress.

Collectively, these studies suggest important relationships exist between psychological stress and biological mechanisms that contribute to pathophysiological processes and symptom outcomes in animal models of PD. Specifically, significant relationships have been demonstrated between psychological stress and increased motor symptom severity [55–61] and depression [55] as well as dopamine deficiencies [55, 56, 58–61], dopaminergic neurodegeneration [55, 57], and the expression of α-synuclein [55]. One study demonstrated significant relationships between corticosterone administration, a key mediator of neuroendocrine stress responses, and increased motor symptoms and loss of dopaminergic neurons [57]. However, another study failed to demonstrate a significant relationship between hippocampal noradrenaline levels, another biomarker of neuroendocrine-mediated stress responses, and stress-induced akinesia [61], suggesting additional research is needed in order to fully elucidate the role the neuroendocrine system plays in biological mechanisms and symptom outcomes of psychological stress in PD. None of the reviewed studies examined underlying biological mechanisms of neuroinflammation and oxidative stress that may contribute to symptom and health outcomes in PD.

3.2. Symptom and Health Outcomes of Psychological Stress That May Modify the Illness Trajectory in Human Subjects with PD

The remaining four studies in this review examined symptom and health outcomes of psychological stress that may modify the illness trajectory in human subjects with PD [62–65]. Three of these studies focused on symptom outcomes of psychological stress to include freezing of gait (FoG) [63], the ability to experience pleasure, reach-to-grasp movements [64], and nonmotor symptom frequencies [65]. One study examined relationships between psychological stress and health outcomes [65]. In contrast to research in animal models of PD, only one study examined biological outcomes of psychological stress, specifically sympathetic skin responses (SSR), a biomarker of sympathetic cholinergic sudomotor function associated with autonomic dysfunction [62]. Each of these studies is further discussed below and summarized in Table 4.

In subjects with PD (n = 29) and controls (n = 27), researchers examined the effects of psychological stress on biological mechanisms of autonomic dysfunction, specifically SSR [62]. SSR is a noninvasive biomarker of sympathetic cholinergic sudomotor function associated with autonomic dysfunction. Autonomic dysfunction is common in PD and can result in nonmotor symptoms such as diaphoresis, hypotension, and urinary and gastrointestinal dysfunction. In this study, SSR onset latencies, peak-to-peak, and amplitude recordings were obtained before and after a series of mental stressors designed to induce psychological stress. The results revealed no significant difference in SSR parameters before or after mental stress between subjects with PD and controls. However, the exclusion of subjects presenting with clinical autonomic dysfunction may explain the lack of significant findings in this study.

In a cross-sectional study, researchers examined factors that influence symptom outcomes of FoG in subjects with PD (n = 130) [63]. FoG, a common motor symptom of PD, results in a sudden and transient inability to walk typically in response to obstacles or situations that affect visual or proprioceptive input. Early in the disease, FoG is often triggered when initiating walking or by turning, confined spaces, rushed situations, or approaching a destination. As the disease progresses, FoG begins to occur in the absence of these triggers, perpetuating the unpredictability of this
| Study author & date   | Study purpose & sample                                                                 | Method(s) of psychological stress induction                        | Measures of biological mechanisms, symptom, and health outcomes                                                                 | Major study findings                                                                 |
|----------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Giza et al., 2012    | *Purpose:* to examine the effects of psychological stress on biological mechanisms of autonomic dysfunction, specifically SSR parameters. *Sample:* male and female subjects with PD and controls (*n* = 56). | Arithmetic calculations using the WAIS-R arithmetic subscale    | Biological mechanisms: 4-channel Nihon Kohen Neuropack S/MFrB 5504K used to record SSR in accordance with International Federation of Clinical Neurophysiology Guidelines | Biological mechanisms: no significant differences in SSR parameters were demonstrated between subjects or controls before or after the induction of psychological stress. |
| Rahman et al., 2008  | *Purpose:* to examine factors that influence symptom outcomes of FoG in subjects with PD. *Sample:* male and female subjects with PD (*n* = 130). | Not applicable                                               | Symptom outcomes: factors influencing walking/freezing questionnaire (tool not specified)                                    | Symptom outcomes: stress was identified as a trigger of FoG by 53.1% of subjects.   |
| Macht et al., 2007   | *Purpose:* to examine the effects of psychological stress on symptom outcomes, specifically goal directed movements and hedonic responsiveness. *Sample:* male and female subjects with PD and controls (*n* = 38). | Arithmetic calculations while listening to loud music (protocol not specified) | Symptom outcomes: Eshkol and Wachman coding system for reach-to-grasp movements, duration of forward and backward movements, and emotional state questionnaire (tool not specified) | Symptom outcomes: stress was associated with significant deteriorations in mood and reduced hedonic responsiveness in subjects with PD. Stress did not result in significant differences in each-to-grasp movements between subjects and controls. |
| Macht et al., 2005   | *Purpose:* to examine patterns of psychological problems in subjects with PD to include symptom frequencies and health outcomes. *Sample:* male and female subjects with PD (*n* = 3075). | Not applicable                                               | Symptom outcomes: Inventory of Psychosocial Stress in PD Health outcomes: Inventory of Psychosocial Stress in PD and benefits of social support questionnaire (tool not specified) | Symptom outcomes: Symptom increases with even small amounts of stress were reported by approximately two-thirds of subjects. Higher stress levels were associated with greater frequencies in depressive moods, sleep disturbances, anxiety, sexual problems, and communication difficulties. Health outcomes: higher stress was associated with greater difficulties coping with PD, worsening social relationships, less enjoyment of life, and the need for more psychological support. |

PD, Parkinson’s disease; FoG, freezing of gait; WAIS-R, Wechsler Adult Intelligence Scale-Revised; SSR, sympathetic skin responses.
symptom. While turning around and fatigue were the most prevalent factors cited as contributing to FoG, 53.1% of subjects (n = 105) cited being in a stressful situation as a significant trigger of FoG.

In subjects with PD (n = 19) and matched controls (n = 19), researchers examined the effects of psychological stress on symptom outcomes, specifically goal directed movements and hedonic responsiveness [64]. Impaired goal directed movements and reduced hedonic responsiveness, defined in this study as impaired reach-to-grasp movements and decreased ability to experience physical or social pleasure, respectively, are two common symptoms in PD. Subjective ratings of mood state and pleasure associated with eating as well as reach-to-grasp movement measurements were obtained at baseline and after the induction of emotional stress. Deterioration in mood and reduction in hedonic responsiveness following the induction of psychological stress were significantly more pronounced in subjects with PD than controls. Psychological stress did not result in significant differences in reach-to-grasp movements between subjects and controls. However, the majority of subjects in this study were receiving treatment with a combination of dopamine replacement therapy and dopamine agonists, which may have ameliorated the effects of psychological stress on motor symptoms.

In a cross-sectional study, researchers examined patterns of psychological problems in subjects with PD (n = 3075) to include symptom and health outcomes of psychological stress [65]. Cluster analysis revealed four patterns of psychological problems: general low stress, general high stress, sexual and social problems, and nonsocial problems. Approximately two-thirds of the total subjects (69% of women; 67% of men) reported symptom increases with even small amounts of stress. Subjects with high stress reported greater frequencies of depressive moods, sleep disturbances, anxiety, sexual problems, and communications difficulties. Subjects with high stress also reported a greater frequency of symptom increases with even small amounts of stress as well as poorer health outcomes to include greater difficulty coping with PD, poorer social relationships, less enjoyment of life, and needing more psychological support.

These studies provide preliminary evidence to support relationships between psychological stress and poorer symptom and health outcomes in individuals with PD. Significant relationships were demonstrated between psychological stress and symptom outcomes to include increased FoG [63], decreased ability to experience pleasure [64], and increased symptom frequencies for select nonmotor symptoms such as depressive mood, sleep disturbances, anxiety, sexual problems, and communication difficulties [65]. Psychological stress was not associated with significant differences in reach-to-grasp movements between subjects and controls [64]. Greater psychological stress was also associated with poorer health outcomes such as difficulty coping with PD, poorer social relationships, less enjoyment of life, and the need for more psychological support [65]. Evidence is lacking regarding biological mechanisms of psychological stress that may contribute to symptom and health outcomes in human subjects with PD.

4. Conclusion

This integrated review supports the notion that psychological stress affects biological mechanisms and symptom and health outcomes in PD. Evidence in animal models has demonstrated that significant relationships exist between psychological stress and poorer symptom outcomes to include increased motor symptom severity and behaviors associated with depression. Significant relationships have also been demonstrated between psychological stress and biological mechanisms involving biomarkers of dopamine production and metabolism, serotonin, and dopaminergic neurodegeneration. Specifically, the evidence suggests psychological stress in animal models of PD results in greater DA depletions in the nigrostriatal and nonnigrostriatal systems, exaggerated expression of nigrostriatal and nonnigrostriatal α-synuclein, increased neurodegeneration of dopaminergic neurons as indicated by increased Fluoro-Jade positive cells and GFAP in the SNc, and decreased 5-HT levels. These findings suggest the induction of psychological stress in animal models of PD contributes to poorer motor symptom outcomes by exacerbating underlying pathogenic features associated with PD.

Preliminary evidence also exists to suggest psychological stress may play a role in symptom and health outcomes in individuals with PD. In human subjects with PD, evidence supports relationships between psychological stress and increased symptom severity and poorer health outcomes. Significant relationships have been demonstrated between psychological stress and poorer symptom outcomes to include increased FoG, depressive moods, sleep disturbances, anxiety, sexual problems, and communication difficulties and decreased hedonic responsiveness. Greater levels of psychological stress have also been associated with poorer health outcomes such as difficulty coping with PD, poorer social relationships, less enjoyment of life, and the need for more social support. Evidence is lacking regarding underlying biological mechanisms of psychological stress that contribute to these findings in human subjects with PD.

Cumulatively, the studies included in this review provide evidence of the potential importance of psychological stress to biological mechanisms and symptom and health outcomes in PD. It should be noted however that many of the reviewed studies demonstrated limitations that may affect the validity and generalizability of these findings. These limitations include issues associated with translating experimental outcomes in animal models to human populations, key differences in the primary outcomes examined, methods for inducing psychological stress, and underlying stress paradigms and study design and methodologies issues.

A number of limitations exist when attempting to translate outcomes of animal experimentation to human populations. Routine laboratory procedures and conditions, such as artificial and restricted housing environments, noise, human handling, and contagious anxiety, have been associated with elevations in stress-related biomarkers, factors that may confound experimental results [66, 67]. The complexity of human diseases is often difficult to replicate, resulting in discrepancies between animal models of select diseases and Parkinson's Disease. 11
actual human conditions [66–68]. Animal models involve the induction of diseases in healthy, homogenous subjects that lack the many predisposing factors and comorbidities that contribute to disease development and progression in human populations [67, 68]. Differences in physiology, behavior, pharmacokinetics, and genetics may limit the ability to generalize experimental data from animal models to human populations [66, 67]. Experimental animal studies often lack fundamental aspects of study design that are required in human clinical trials, specifically randomization, blinding of research personnel, and sample size calculations, which may result in overestimated outcome effects [67, 68]. Furthermore, the small sample sizes typically used in animal experimentation often lead to underpowered studies, which may increase the risk of erroneously detecting treatment effects [67, 68]. Each of these issues confounds the ability to translate findings in experimental models to human populations with PD.

Key differences were identified in the primary outcomes examined in the reviewed studies. The majority of the animal studies included in this review focused on the effects of psychological stress on biological mechanisms that may contribute to pathophysiological processes in PD whereas the studies involving human subjects focused on the effects of psychological stress on symptom and health outcomes that may modify the illness trajectory. Extreme caution should be exercised when attempting to extrapolate biological outcomes in animal models as a means of explaining factors that modify symptom and health outcomes in those living with PD. As such, this highlights the need for additional research that focuses on biological mechanisms of psychological stress specific to underlying pathophysiological processes that may modify symptom and health outcomes in human populations with PD.

Important differences were demonstrated in the manner in which psychological stress was induced in animal models of and human subjects with PD. In the reviewed studies, the induction of psychological stress in individuals with PD involved performing strenuous mental tasks [62, 64]. In contrast, the induction of psychological stress in animal models of PD involved physiological insults such as glucodeprivation, osmotic diuresis, hypothermia, and painful stimuli [59–61]. Stress induction techniques used in animal models may result in the activation of biological pathways unrelated to psychological stress responses. For example, the dopaminergic system has been implicated in biological responses associated with pain perception and processing variability as well as central control of thermoregulation [69, 70]. Activation of these pathways may have confounded the ability to interpret outcomes of psychological stress in animal models of PD. Furthermore, the induction of stress in animal models of PD does not adequately reflect the multidimensional and dynamic nature of stress experiences in individuals living with PD. Each of these factors necessitates the use of caution when attempting to translate these findings to human populations with PD.

Key differences in acute versus chronic stress paradigms are important to consider when evaluating the studies included in this review. The underlying stress paradigms in the majority of the reviewed studies focused on the implementation of acute psychological stressors that varied in timing and type so as to limit predictability [55, 57–62, 64]. Only one study specifically focused on chronic variable stress [56]. Important differences exist in biological mechanisms responsible for mediating physiological responses to acute versus chronic psychological stressors. For example, activation of the HPAA and the release of glucocorticoids in response to acute psychological stress have been associated with transient immunosuppressive effects [22, 71, 72]. In contrast, sustained activation of the HPAA and release of glucocorticoids, as seen with chronic psychological stress, have been associated with exaggerated inflammatory responses, cell damage, and death [18, 22, 73]. Chronic psychological stress has also been associated with dysregulation of glucocorticoid feedback mechanisms. Under normal circumstances, transient increases in circulating glucocorticoids exert inhibitory effects on further HPAA stimulation. Dysregulation of these feedback mechanisms, as seen with chronic psychological stress, has been associated with increased expression and release of glucocorticoids [18]. Furthermore, the increased energy demands needed to respond to chronic psychological stress have been associated with increased production of ROS, oxidative stress, lipid peroxidation, and tissue damage within the central nervous system [14, 26, 38, 74]. Given that individuals with PD often face a variety of stressors across the illness trajectory, additional research is needed that considers differences in biological mechanisms, symptoms, and health outcomes associated with acute versus chronic stress paradigms.

A number of design and methodological issues were identified in the reviewed studies. For instance, between-subject procedural variability [61], the introduction of potential confounders [58, 61], and inadequately operationalized key variables/procedures [56, 59, 60] were identified in several of the reviewed animal studies. With regard to the human studies, the limited number of studies and relatively small sample sizes in all but one study represent limitations to the generalizability of these findings. In several studies, inadequate operationalization of key variables and/or unknown psychometric properties for the measurement tools that were utilized may have affected the validity and reliability of the results [62–65]. The operationalization of hedonic responsiveness as pleasurable food experiences in one study may also present a potential limitation given individuals with PD often experience loss of taste and swallowing difficulties, factors that were not controlled for and may have confounded differences in hedonic responsiveness between subjects and controls [64]. Finally, in another study, advertising the study as a mobility study may have led to the overrepresentation of subjects with mobility problems [63].

As this review has demonstrated, significant gaps exist in our understanding of biological mechanisms and symptom and health outcomes of psychological stress in individuals living with PD. Much of what is currently known about biological mechanisms and symptom outcomes of psychological stress in PD has been conducted in animal models of PD and/or predicated on research in non-PD animal models. While important, this knowledge may not adequately
reflect these constructs in individuals living with the disease, particularly given the multifaceted nature of stress in human populations and similarities in key pathogenic features of PD that may be exacerbated by underlying biological mechanisms of psychological stress. As a result, additional research is needed in order to further elucidate underlying biological mechanisms and symptom and health outcomes of psychological stress in PD. Psychoneuroimmunological frameworks provide an important opportunity for gaining insight into plausible biological mechanisms of the neuroendocrine and immune systems that may contribute to stress-induced neuroinflammation, oxidative stress, and loss of dopaminergic neurons in PD, which may lead to poorer symptom and health outcomes [11]. Rigorously designed studies that provide a deeper understanding of these relationships would provide the foundation for designing interventions specifically targeted to biological mechanisms and symptom and health outcomes of psychological stress in PD.

Healthcare providers need to be aware of multifaceted factors that may contribute to symptom and health outcomes in PD. Given the progressive and unpredictable nature of PD, individuals living with the disease must cope with and adapt to a variety of stressors over a protracted period of time. The cumulative costs of psychological stressors may further exacerbate pathogenic mechanisms in PD thereby perpetuating neuronal loss within the central nervous system. Consideration of these factors in the design of future research studies as well as the clinical care of individuals with PD represents an important opportunity to improve symptom and health outcomes in those living with the disease.

Disclosure

The development of this manuscript was not associated with any specific grant funding from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests

The authors declare that there is no conflict of interests regarding publication of this paper.

References

[1] A. Antonini, P. Barone, R. Marconi et al., “The progression of non-motor symptoms in Parkinson’s disease and their contribution to motor disability and quality of life,” Journal of Neurology, vol. 259, pp. 2621–2631, 2012.
[2] C. C. Aquino and S. H. Fox, “Clinical spectrum of levodopa-induced complications,” Movement Disorders, vol. 30, no. 1, pp. 80–89, 2015.
[3] M. S. Clark, M. J. Bond, and J. R. Hecker, “Environmental stress, psychological stress and allostatic load,” Psychology, Health and Medicine, vol. 12, no. 1, pp. 18–30, 2007.
[4] T. H. Holmes and R. H. Rahe, “The social readjustment rating scale,” Journal of Psychosomatic Research, vol. 11, no. 2, pp. 213–218, 1967.
[5] J. K. Kiecolt-Glaser, L. McGuire, T. F. Robles, and R. Glaser, “Emotions, morbidity, and mortality: new perspectives from psychoneuroimmunology,” Annual Review of Psychology, vol. 53, pp. 83–107, 2002.
[6] M. Sorenson, L. Janusek, and H. Mathews, “Psychological stress and cytokine production in multiple sclerosis: correlation with disease symptomatology,” Biological Research for Nursing, vol. 15, no. 2, pp. 226–233, 2013.
[7] M. C. Davis, A. J. Zautra, and J. W. Reich, “Vulnerability to stress among women in chronic pain from fibromyalgia and osteoarthritis,” Annals of Behavioral Medicine, vol. 23, no. 3, pp. 215–226, 2001.
[8] A. W. M. Evers, E. W. M. Verhoeven, H. Van Middendorp et al., “Does stress affect the joints? Daily stressors, stress vulnerability, immune and HPA axis activity, and short-term disease and symptom fluctuations in rheumatoid arthritis,” Annals of the Rheumatic Diseases, vol. 73, no. 9, pp. 1683–1688, 2014.
[9] T. E. Seeman, B. H. Singer, J. W. Rowe, R. I. Horwitz, and B. S. McEwen, “Price of adaptation—allostatic load and its health consequences,” Archives of Internal Medicine, vol. 157, no. 19, pp. 2259–2268, 1997.
[10] J. Kulmala, M. B. von Bonsdorff, S. Stenhelm et al., “Perceived stress symptoms in midlife predict disability in old age: a 28-year prospective cohort study,” The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, vol. 68, no. 8, pp. 984–991, 2013.
[11] N. L. McCain, D. P. Gray, J. M. Walter, and J. Robins, “Implementing a comprehensive approach to the study of health dynamics using the psychoneuroimmunology paradigm,” Advances in Nursing Science, vol. 28, no. 4, pp. 320–332, 2005.
[12] F. S. Dhabhar, “Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology,” NeuroImmunoModulation, vol. 16, no. 5, pp. 300–317, 2009.
[13] C. J. Bannum, T. W. W. Pace, F. Hu, G. N. Neigh, and M. G. Ten- seny, “Psychological stress in adolescent and adult mice increases neuroinflammation and attenuates the response to LPS challenge,” Journal of Neuroinflammation, vol. 9, article 9, 2012.
[14] R. M. De Pablos, R. F. Villarán, S. Argüelles et al., “Stress increases vulnerability to inflammation in the rat prefrontal cortex,” Journal of Neuroscience, vol. 26, no. 21, pp. 5709–5719, 2006.
[15] M. G. Frank, M. V. Baratta, D. B. Spruner, L. R. Watkins, and S. F. Maier, “Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses,” Brain, Behavior, and Immunity, vol. 21, no. 1, pp. 47–59, 2007.
[16] M. G. Frank, B. M. Thompson, L. R. Watkins, and S. F. Maier, “Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses,” Brain, Behavior, and Immunity, vol. 26, no. 2, pp. 337–345, 2012.
[17] J. L. M. Madrigal, B. García-Bueno, J. R. Caso, B. G. Pérez-Nievas, and J. C. Leza, “Stress-induced oxidative changes in brain,” CNS and Neurological Disorders—Drug Targets, vol. 5, no. 5, pp. 561–568, 2006.
[18] C. D. Munhoz, L. B. Lepsch, E. M. Kawamoto et al., “Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-κB in the frontal cortex and hippocampus via glucocorticoid secretion,” The Journal of Neuroscience, vol. 26, no. 14, pp. 3813–3820, 2006.
[19] J. B. Buchanan, N. L. Sparkman, J. Chen, and R. W. John- son, “Cognitive and neuroinflammatory consequences of mild
repeated stress are exacerbated in aged mice,” *Psychoneuroendocrinology*, vol. 33, no. 6, pp. 755–765, 2008.

[20] M. G. Frank, S. A. Hershman, M. D. Weber, L. R. Watkins, and S. F. Maier, “Chronic exposure to exogenous glucocorticoids primes microglia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus,” *Psychoneuroendocrinology*, vol. 40, no. 1, pp. 191–200, 2014.

[21] M. L. Block, L. Zecca, and J.-S. Hong, “Microglia-mediated neurotoxicity: uncovering the molecular mechanisms,” *Nature Reviews Neuroscience*, vol. 8, no. 1, pp. 57–69, 2007.

[22] H. A. Jurgens and R. W. Johnson, “Dysregulated neuronal–microglial cross-talk during aging, stress and inflammation,” *Experimental Neurology*, vol. 233, no. 1, pp. 40–48, 2012.

[23] H.-M. Gao, J. Jiang, B. Wilson, W. Zhang, J.-S. Hong, and B. Liu, “Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to parkinson’s disease,” *Journal of Neurochemistry*, vol. 81, no. 6, pp. 1285–1297, 2002.

[24] G. Lucca, C. M. Comim, S. S. Valvassori et al., “Increased oxidative stress in submitchondrial particles into the brain of rats submitted to the chronic mild stress paradigm,” *Journal of Psychiatric Research*, vol. 43, no. 9, pp. 864–869, 2009.

[25] J. S. Myers, “Proinflammatory cytokines and sickness behavior: implications for depression and cancer-related symptoms,” *Oncology Nursing Forum*, vol. 35, no. 5, pp. 802–807, 2008.

[26] C. Munhoz, J. L. M. Madrigal, B. García-Bueno et al., “TNF-α accounts for short-term persistence of oxidative status in rat brain after two weeks of repeated stress,” *European Journal of Neuroscience*, vol. 20, no. 4, pp. 1125–1130, 2004.

[27] J.-P. Gouin, R. Glaser, W. B. Malarkey, D. Beversdorf, and J. Kiecolt-Glaser, “Chronic stress, daily stressors, and circulating inflammatory markers,” *Health Psychology*, vol. 31, no. 2, pp. 264–268, 2012.

[28] S. Holmín and T. Mathiesen, “Intracerebral administration of interleukin-1β and induction of inflammation, apoptosis, and vasogenic edema,” *Journal of Neurosurgery*, vol. 92, no. 1, pp. 108–120, 2000.

[29] D. Zhou, A. W. Kusnecov, M. R. Shurin, M. DePaoli, and B. S. Rabin, ”Exposure to physical and psychological stressors elevates plasma interleukin 6: Relationship to the activation of hypothalamic-pituitary-adrenal axis,” *Endocrinology*, vol. 133, no. 6, pp. 2523–2530, 1993.

[30] J. L. M. Madrigal, O. Hurtado, M. A. Moro et al., ”The increase in TNF-α levels is implicated in NF-xB activation and inducible nitric oxide synthase expression in brain cortex after immobilization stress,” *Neuropsychopharmacology*, vol. 26, no. 2, pp. 155–163, 2002.

[31] A. Bierhaus, J. Wolf, M. Andrassy et al., ”A mechanism converting psychosocial stress into mononuclear cell activation,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 4, pp. 1920–1925, 2003.

[32] Z.-Q. Liang, Y.-L. Li, X.-L. Zhao et al., ”NF-xB contributes to 6-hydroxydopamine-induced apoptosis of nigral dopaminergic neurons through p53,” *Brain Research*, vol. 1145, no. 1, pp. 190–203, 2007.

[33] J. L. M. Madrigal, M. A. Moro, I. Lizasoain et al., ”Induction of cyclooxygenase-2 accounts for restraint stress-induced oxidative status in rat brain,” *Neuropsychopharmacology*, vol. 28, no. 9, pp. 1579–1588, 2003.

[34] S. Vesce, D. Rossi, L. Brambilla, and A. Volterra, ”Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation,” *International Review of Neurobiology*, vol. 82, pp. 57–71, 2007.

[35] K. Yamagata, K. I. Andresson, W. E. Kaufmann, C. A. Barnes, and P. F. Worley, ”Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids,” *Neuron*, vol. 11, no. 2, pp. 371–386, 1993.

[36] S. R. Subramaniam and M.-F. Chesnelet, ”Mitochondrial dysfunction and oxidative stress in parkinson’s disease,” *Progress in Neurobiology*, vol. 106–107, pp. 17–32, 2013.

[37] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, ”Free radicals and antioxidants in normal physiological functions and human disease,” *The International Journal of Biochemistry & Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.

[38] G. Lucca, C. M. Comim, S. S. Valvassori et al., ”Effects of chronic mild stress on the oxidative parameters in the rat brain,” *Neurochemistry International*, vol. 54, no. 5–6, pp. 358–362, 2009.

[39] H.-M. Gao, H. Zhou, and J.-S. Hong, ”NADPH oxidases: novel therapeutic targets for neurodegenerative diseases,” *Trends in Pharmacological Sciences*, vol. 33, no. 6, pp. 295–303, 2012.

[40] I. Paduraru, O. Paduraru, G. Manolidis, W. Bild, and I. Haulica, ”Antioxidant activity in rat models of nociceptive stress,” *Revista medico-chirurgicala a Societatis de Medicis si Naturalisti din Iasi*, vol. 114, no. 1, pp. 175–179, 2010.

[41] S. T. Kim, J. H. Choi, J. W. Chang, S. W. Kim, and O. Hwang, ”Immobilization stress causes increases in tetrahydrobiopterin, dopamine, and neuromelanin and oxidative damage in the nigrostriatal system,” *Journal of Neurochemistry*, vol. 95, no. 1, pp. 89–98, 2005.

[42] D. T. Dextera and P. Jenner, ”Parkinson disease: from pathology to molecular disease mechanisms,” *Free Radical Biology and Medicine*, vol. 62, pp. 132–144, 2013.

[43] A. Gerhard, N. Pavese, G. Hotton et al., ”In vivo imaging of microglial activation with [11C][R]-PK11195 PET in idiopathic Parkinson’s disease,” *Neurobiology of Disease*, vol. 21, no. 2, pp. 404–412, 2006.

[44] Y. Ouchi, E. Yoshikawa, Y. Sekine et al., ”Microglial activation and dopamine terminal loss in early Parkinson’s disease,” *Annals of Neurology*, vol. 57, no. 2, pp. 168–175, 2005.

[45] A. M. Depino, C. Earl, E. Kaczmarchyk et al., ”Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson’s disease,” *European Journal of Neuroscience*, vol. 18, no. 10, pp. 2731–2742, 2003.

[46] P. Scalzo, A. Kümmcr, F. Cardoso, and A. L. Teixeira, ”Serum levels of interleukin-6 are elevated in patients with Parkinson’s disease and correlate with physical performance,” *Neuroscience Letters*, vol. 468, no. 1, pp. 56–58, 2010.

[47] M. Reale, C. Iarloli, A. Thomas et al., ”Peripheral cytokines profile in Parkinson’s disease,” *Brain, Behavior, and Immunity*, vol. 23, no. 1, pp. 55–63, 2009.

[48] J. C. Tobón-Velasco, J. H. Limón-Pacheco, M. Orozco-Ibarra et al., ”6-OHDA-induced apoptosis and mitochondrial dysfunction are mediated by early modulation of intracellular signals and interaction of Nrf2 and NF-xB factors,” *Toxicology*, vol. 304, pp. 109–119, 2013.

[49] P. Teismann, K. Tieu, D.-K. Choi et al., ”Cyclooxygenase-2 is instrumental in Parkinson’s disease neurodegeneration,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 9, pp. 5473–5478, 2003.
M. Macht, S. Brandstetter, and H. Ellgring, “Stress affects Parkinson’s disease,” Behavioural Brain Research, vol. 177, no. 1, pp. 171–174, 2007.

M. Macht, R. Schwarz, and H. Ellgring, “Patterns of psychological problems in Parkinson’s disease,” Acta Neurologica Scandinavica, vol. 111, no. 2, pp. 95–101, 2005.

A. Akhtar, “The flaws and human harms of animal experimentation,” Cambridge Quarterly of Healthcare Ethics, vol. 24, no. 4, pp. 407–419, 2015.

D. G. Hachman, “Translating animal research into clinical benefit,” British Medical Journal, vol. 334, article ID 163, 2007.

M. I. Martić-Kehl, R. Schibili, and P. A. Schubiger, “Can animal data predict human outcome? Problems and pitfalls of translational animal research,” European Journal of Nuclear Medicine and Molecular Imaging, vol. 39, no. 9, pp. 1492–1496, 2012.

S. K. Jääskeläinen, P. Lindholm, T. Valmunen et al., “Variation in the dopamine D2 receptor gene plays a key role in human pain and its modulation by transcranial magnetic stimulation,” Pain, vol. 155, no. 10, pp. 2180–2187, 2014.

P. J. Schwartz and S. D. Erk, “Regulation of central dopamine-2 receptor sensitivity by a proportional control thermostat in humans,” Psychiatry Research, vol. 127, no. 1-2, pp. 19–26, 2004.

N. K. Leidy, “A physiologic analysis of stress and chronic illness,” Journal of Advanced Nursing, vol. 14, no. 10, pp. 868–876, 1989.

J. K. Kiecolt-Glaser, L. McGuire, T. F. Robles, and R. Glaser, “Psychoneuroimmunology: psychological influences on immune function and health,” Journal of Consulting and Clinical Psychology, vol. 70, no. 3, pp. 537–547, 2002.

A. M. Espinosa-Oliva, R. M. de Pablos, R. E. Villarán et al., “Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus,” Neurobiology of Aging, vol. 32, no. 1, pp. 85–102, 2011.

E. Izzo, P. P. Sanna, and G. F. Koob, “Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor,” Pharmacology, Biochemistry, and Behavior, vol. 81, no. 4, pp. 701–708, 2005.
Mini Review: Anticholinergic Activity as a Behavioral Pathology of Lewy Body Disease and Proposal of the Concept of “Anticholinergic Spectrum Disorders”

Koji Hori,1,2 Kimiko Konishi,3 Misa Hosoi,2 Hiroi Tomioka,2 Masayuki Tani,4 Yuka Kitajima,5 and Mitsugu Hachisu6

1Department of Neuropsychiatry, St. Marianna University, School of Medicine, Kanagawa, Japan
2Department of Psychiatry, Showa University Northern Yokohama Hospital, Kanagawa, Japan
3Tokyo Metropolitan Tobu Medical Center for Persons with Developmental/Multiple Disabilities, Tokyo, Japan
4Department of Psychiatry, Showa University East Hospital, Tokyo, Japan
5Department of Anesthesiology, School of Medicine, Juntendo University, Tokyo, Japan
6Department of Pharmaceutical Therapeutics, Division of Clinical Pharmacy, School of Pharmacy, Showa University, Tokyo, Japan

Correspondence should be addressed to Koji Hori; kojihori@med.showa-u.ac.jp

Received 7 May 2016; Accepted 26 July 2016

Academic Editor: Per Odin

Copyright © 2016 Koji Hori et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Given the relationship between anticholinergic activity (AA) and Alzheimer’s disease (AD), we rereview our hypothesis of the endogenous appearance of AA in AD. Briefly, because acetylcholine (ACh) regulates not only cognitive function but also the inflammatory system, when ACh downregulation reaches a critical level, inflammation increases, triggering the appearance of cytokines with AA. Moreover, based on a case report of a patient with mild AD and slightly deteriorated ACh, we also speculate that AA can appear endogenously in Lewy body disease due to the dual action of the downregulation of ACh and hyperactivity of the hypothalamic-pituitary-adrenal axis. Based on these hypotheses, we consider AA to be a behavioral pathology of Lewy body disease. We also propose the concept of “anticholinergic spectrum disorders,” which encompass a variety of conditions, including AD, Lewy body disease, and delirium. Finally, we suggest the prescription of cholinesterase inhibitors to patients in this spectrum of disorders to abolish AA by upregulating ACh.

1. Introduction

The neurotransmitter acetylcholine (ACh) and anticholinergic activity (AA) can bind to the muscarinic acetylcholine receptor [1]. AA includes every substance that binds the muscarinic acetylcholine receptor; however, generally speaking AA antagonizes ACh to the muscarinic acetylcholine receptor. Therefore, AA disturbed the function of ACh. AA predominantly disturbs memory function [2, 3], with psychotic symptoms more prominent than cognitive dysfunctions [4, 5]. The main cause of AA is prescribed medication [6], but physical illness [7] and mental stress which cause elevated cortisol [8] also cause AA. We previously reported that AA was also caused by ACh downregulation and proposed our hypothesis of the endogenous appearance of AA in Alzheimer’s disease (AD) [9, 10]. That is, because ACh regulates not only cognitive function but also the inflammatory system, when ACh downregulation reaches a critical level, inflammation is increased, triggering the appearance of cytokines with AA. These processes might also accelerate AD pathologies [9, 10]. Moreover, we speculated that, in addition to AD, other neurocognitive disorders such as Lewy body disease (LBD) and delirium are related to AA and that the appearance of AA in LBD is also related to an endogenous factor [11]. In this article, we rereview our hypotheses surrounding the endogenous appearance of AA in AD [9, 10] and LBD [11]. We also theorize that the onset of clinical symptoms in LBD depends on the endogenous appearance of AA in LBD and propose a new concept of “anticholinergic spectrum disorders.”
2. Hypothesis of Endogenous Anticholinergic Activity in Alzheimer’s Disease

We previously evaluated the relationship between clinical symptoms and AA in 76 AD patients [12]. Serum anticholinergic activity (SAA), a peripheral marker of anticholinergic burden, was positive in 26 of these patients; the other 50 were negative for SAA. Delusions, hallucinations, diurnal rhythm disturbances, and global cognitive dysfunctions were significantly more severe in the SAA-positive group than in the SAA-negative group, and the patients in the SAA-positive group took more psychotropic medicines. Moreover, SAA positivity was more related to psychotic symptoms than cognitive dysfunctions. Our results indicated that clinical symptoms and prescription of psychotropic medicines are factors related to SAA, particularly psychotic symptoms. Because these results were from a cross-sectional study, we could not elucidate the causal relationships among these three factors (psychotic symptoms, the prescription of psychotropic medicines, and SAA). However, we speculated that there might be a cyclic relationship among the factors. Accordingly, we named this association the “vicious cycle of anticholinergic activity in AD (VCAA)” [12].

Positive SAA can be caused by various medicines and AA worsens clinical psychiatric symptoms. However, psychotropic medicines are generally prescribed for the clinical psychiatric symptoms of agitation and psychosis in AD [13, 14]. Therefore, the relationship among prescribed psychotropic medicines, SAA, and clinical symptoms—especially hallucinations and a disturbed diurnal rhythm—might be cyclic. Moreover, because psychotropic medicines are typically prescribed for the clinical psychiatric symptoms of agitation and psychosis in AD [13, 14], psychotropic medicines might not be the first step in this cycle. There might be a causal relationship between the other two factors comprising the VCAA, namely, psychotic symptoms and the use of psychotropic medicines, with AA worsening psychiatric symptoms. Therefore, we consider the appearance of AA to be the first step in the VCAA: that is, AA appears endogenously in AD [12]. In fact, there is a high probability that the pathogenesis of AD involves neuronal degeneration due to oxidative stress, and it has been shown that amyloid might be able to generate free radicals [15]. On the other hand, an endogenous ligand of the muscarinic receptor is found to a greater extent in the AD brain than in the nondemented control brain, and the endogenous ligand of the muscarinic receptor seems to be a low-molecular weight substance of 100–1,000 Da that is catalyzed by oxidation [16]. Thus, SAA might not always derive purely from prescribed psychotropic medicines; it may also derive from endogenous oxidative products [12].

In short, we hypothesized that the relationship among psychotic symptoms, positive SAA, and the prescription of psychotropic medicines is a cyclic one and that AA appears endogenously in AD [12], because psychotropic medicines are prescribed for psychotic symptoms [13, 14]. We also speculated about the mechanism behind the endogenous appearance of AA in AD [9, 10]. ACh regulates not only cognitive function but also the inflammatory system [17, 18]. Therefore, we surmised that because AD is characterized by the downregulation of ACh, the inflammatory system is upregulated in AD when the level of ACh reaches a specific threshold (i.e., moderately severe disease) [9, 10]. Thus, downregulation of ACh would cause downregulation of the anti-inflammatory pathway (the cholinergic anti-inflammatory pathway), permitting upregulation of the inflammatory pathway [17, 18]. The hyperactive inflammation generates cytokines with AA, such as C-reactive protein [19].

Moreover, stimulation of muscarine 1 receptor is favorable for reducing amyloid pathology [20, 21]. Therefore, we considered that AA accelerates amyloid pathology. In fact, anticholinergic loads are reported to increase amyloid pathology [22, 23]. We thus proposed the hypothesis of the endogenous appearance of AA in AD.

3. Dual Actions of Anticholinergic Inserts Cause Anticholinergic Activity and the Endogenous Appearance of Anticholinergic Activity in Lewy Body Disease

We then encountered a 74-year-old woman with positive SAA, although her cognitive decline was not at a sufficiently critical level to elicit endogenous AA [24]. In this instance, we hypothesized that the SAA positivity was induced by the addition of mental stress to a preexisting ACh downregulation [24]. We consider mental stress and other factors besides ACh downregulation to be capable of inducing SAA, to be so-called “AA inserts.” In this context, because there are other AA inserts besides ACh downregulation [9, 10], such as the prescribed medication [6], physical illness [7], and mental stress [8], dual actions of AA inserts can also cause AA when the ACh level does not reach a critical level (i.e., at the stage of mild cognitive impairment or at a mild stage of the disease) [24]. Based on this case, we speculated that the appearance of AA might cause psychiatric symptoms such as delusions, hallucinations, and diurnal rhythm disturbances in delirium and LBD based on the dual actions of ACh downregulation [11] (if ACh is not deteriorated or overloaded, the intact ACh system can be upregulated and compensate for another AA insert [10]) and the effects of another AA insert.

We also previously evaluated the relationship between postoperative delirium and SAA [11]. Although delirium is considered an important issue among elderly patients in various settings, the mechanism underlying delirium is poorly understood [25]. We concluded that delirious patients fail to compensate for the increase in AA, making it important to pay close attention to the perioperative transition of the SAA level in relation to delirium, rather than focusing on a single SAA level [11].

The factor suggesting the endogenous appearance of AA in LBD is the dual action of a deteriorated autonomic parasympathetic nervous system and a relatively minor decrease in ACh [11]. This is attributable to the induction of AA in LBD, with the deteriorated autonomic parasympathetic nervous system increasing the activity of the hypothalamic-pituitary-adrenal (HPA) axis [26] and hypercortisolism [27, 28]. If inflammatory processes are caused
by AA, it is plausible to conclude that corticosteroids would inhibit the action of AA because of their anti-inflammatory properties.

Nonetheless, corticosteroids have been reported to induce or increase AA in the brain [8] and cause delirium [25]. Typically, the corticosteroid level in plasma is high early in the morning and rapidly declines thereafter. We theorized that this rapid reduction might cause immune system disinhibition, with immune system activation in the afternoon, evening, and night [10], because cortisol levels after awakenings, such as in the morning, might be affected in neurocognitive and neuropsychiatric disorders [10]. If the blood level of corticosteroids rises above normal, the subsequent decline in the level can be expected to be greater, where it is expected that the inflammatory state activated the AA. Therefore, it seems logical that even if AA does not appear early in the morning, it may appear by noon or later. This mechanism might explain why patients with delirium can appear calm in the morning but delirious in the late afternoon and at night (i.e., sundowning) [10]. We also believe that deteriorated parasympathetic autonomic nervous function causes AA in patients with LBD based on the small degree of downregulation of ACh and that AA appears endogenously in LBD [11].

Briefly, AA appears earlier in LBD than in AD because of the combination of HPA axis hyperactivity and the small degree of ACh downregulation. In delirium, AA appears, as in LBD, due to the combination of these two factors. In LBD, HPA axis hyperactivity occurs endogenously by way of dysfunction of the parasympathetic nervous system [27, 28]. In contrast, HPA axis hyperactivity occurs exogenously in delirium due to mental stress and/or physical illnesses. AA is thus related to the pathogenesis of AD, LBD, and delirium.

We can explain the onset of clinical symptoms using our hypothesis of the endogenous appearance of AA in LBD. This hypothesis is shown in Figure 1. Briefly, based on a small degree of ACh downregulation, HPA axis hyperactivity caused by a deteriorated autonomic parasympathetic nervous system gradually worsens and finally induces hyperactive inflammation, which also causes AA. Continuous and recurrent appearance of AA exacerbates the amyloid pathology and further downregulates ACh. We divided this entire process into the following three stages, in this order: (1) deterioration of the parasympathetic nervous system (and hyperactivity of the HPA axis); (2) appearance of AA; and (3) downregulation of ACh caused by continuous and recurrent appearance of AA.

At the stage of parasympathetic nervous system deterioration, which corresponds to the prodromal stage of LBD, HPA axis hyperactivity gradually worsens but does not reach the level at which AA is induced. Therefore, symptoms related to dysfunction of the parasympathetic nervous system occur in LBD, such as REM behavioral symptoms, syncope, and constipation [29]. Moreover, depression, which is related to HPA axis hyperactivity, also develops (Figure 2). We have already reported that anxiety and affective disturbances in AD patients are connected to delusion, hallucination, and aggressiveness by aging and the disease progress [30]. Therefore, if the degree of ACh dysfunction is relatively large, depression appears as behavioral and psychological symptoms of dementia in AD.

At the stage of AA appearance, which corresponds to the early stage of LBD, HPA axis hyperactivity reaches a critical level and induces AA. In the early stage of LBD, symptoms related to AA develop, including psychotic symptoms such...
4 Parkinson's Disease

Figure 3: At the stage of AA appearance, which corresponds to the early stage of LBD, HPA axis hyperactivity reaches a critical level and induces AA. In the early stage of LBD, symptoms related to AA develop, including psychotic symptoms such as visual hallucinations, delusions, and diurnal rhythm disturbances. These symptoms are similar to those of delirium and are included as the core symptoms of the diagnostic criteria.

Figure 4: At the stage of ACh downregulation, corresponding to the late stage of LBD, the patient develops symptoms related to ACh downregulation, which is induced by the continuous and recurrent appearance of AA. These symptoms include memory disturbances and disorientation to time and place. AA: anticholinergic activity, ACh: acetylcholine, AD: Alzheimer’s disease, HPA axis: hypothalamic-pituitary-adrenal axis, and LBD: Lewy body disease.

AA appears when the ACh deterioration reaches a critical level, that is, at a moderate stage in AD. Therefore, AA is related not only to behavioral symptoms, such as delusions, hallucinations, and diurnal rhythm disturbances [12], but also to cognitive dysfunctions, such as memory disturbances and executive dysfunction. However, the ACh deterioration in LBD and delirium is not as severe as that of AD when AA appears. Therefore, only behavioral symptoms are prominent in LBD and delirium, such as delusions, hallucinations, and diurnal rhythm disturbances. This is why the symptoms of LBD and delirium are similar. We consider the pathophysiology of the clinical symptoms of LBD to be related to AA.

4. Proposal of “Anticholinergic Spectrum Disorders” in Neurocognitive Disorders

Based on these hypotheses, we propose that certain neurocognitive disorders, such as AD, LBD, and delirium, be considered “anticholinergic spectrum disorders.” In AD, AA appears endogenously when the downregulation of ACh reaches a critical level. In contrast, the AA in LBD and delirium involves a combination of HPA axis hyperactivity and a slight ACh downregulation. When the ACh deterioration is small, a larger AA insert of HPA axis dysfunction is necessary for the appearance of AA. However, if ACh is not deteriorated or overloaded, the intact ACh system can be upregulated and compensate for any other AA inserts. We refer to the conditions encompassed by this concept as “anticholinergic spectrum disorders,” which include AD, LBD, and delirium. AD and LBD are primary NCDs, whereas delirium is a secondary NCD. AA: anticholinergic activity, ACh: acetylcholine, AD: Alzheimer’s disease, HPA axis: hypothalamic-pituitary-adrenal axis, LBD: Lewy body disease, and NCD: neurocognitive disorder.

Possible pharmacotherapies for anticholinergic spectrum disorders are of course also important. Yilmaz et al. [32] described the case of a 19-year-old man with anticholinergic...
Figure 6: ChEI enhances ACh and enhanced ACh compensates for other AA inserts besides ACh downregulation. We prescribe three main ChEIs—donepezil, galantamine, and rivastigmine—to patients with mild or moderate stage AD. For donepezil, 5 mg doses are allowed in Japan (a). Of these ChEIs (including generic medicines), only Aricept is permitted for LBD patients in Japan (since September 2014). In general, the dose of Aricept is 10 mg for LBD (10 mg of donepezil is permitted only for severe AD patients in Japan) (b). We believe that the difference in required dosage between AD and LBD is because there is no other AA insert in AD besides ACh downregulation. However, another AA insert (e.g., HPA axis hyperactivity) is present in LBD, so it is important to upregulate ACh to compensate for this AA insert. AA: anticholinergic activity, ACh: acetylcholine, AD: Alzheimer’s disease, ChEI: cholinesterase inhibitor, HPA axis: hypothalamic-pituitary-adrenal axis, and LBD: Lewy body disease. 5 mg (in (a)): 5 mg doses of donepezil; 10 mg (in (b)): 10 mg doses of Aricept (donepezil). This figure is reproduced from Konishi et al. [24] with the permission of Karger Publishers, Basel, Switzerland.

Abbreviations

AA: Anticholinergic activity
ACh: Acetylcholine
AD: Alzheimer’s disease
ChEI: Cholinesterase inhibitor
HPA axis: Hypothalamic-pituitary-adrenal axis
LBD: Lewy body disease
SAA: Serum anticholinergic activity.

Competing Interests

Koji Hori has received lecture fees from Eisai Co. Ltd., Pfizer Japan Inc., Novartis Pharma KK, Daiichi Sankyo Inc., Ono Pharmaceutical Co. Ltd., Janssen Pharmaceutical KK, Yoshitomi Yakuhin Co., Meiji Seika Pharma Co. Ltd., and Mitsubishi Tanabe Pharma Co. Koji Hori has received funding from Eisai Co. Ltd., Daiichi Sankyo Inc., Ono Pharmaceutical Co. Ltd., Dainippon Sumitomo Pharm Co. Ltd., Eli Lilly Japan KK, and Shionogi & Co. Ltd. Mitsugu Hachisu has received funding from Astellas Pharma Inc., Meiji Seika Pharma Co. Ltd., Dainippon Sumitomo Pharm Co. Ltd., Eli Lilly Japan KK, and Shionogi & Co. Ltd. and has received lecture fees from Meiji Seika Pharma Co. Ltd. and Mitsubishi Tanabe Pharma Co.

References

[1] L. Tune and J. T. Coyle, “Serum levels of anticholinergic drugs in treatment of acute extrapyramidal side effects,” Archives of General Psychiatry, vol. 37, no. 3, pp. 293–297, 1980.

[2] T. Sunderland, P. N. Tariot, R. M. Cohen, H. Weingartner, E. A. Mueller III, and D. L. Murphy, “Anticholinergic sensitivity in patients with dementia of the Alzheimer type and age-matched controls: a dose-response study,” Archives of General Psychiatry, vol. 44, no. 5, pp. 418–426, 1987.

[3] O. J. Thienhaus, A. Allen, J. A. Bennett, Y. M. Chopra, and F. P. Zezman, “Anticholinergic serum levels and cognitive
performance,” *European Archives of Psychiatry and Clinical Neurosciences*, vol. 240, no. 1, pp. 28–33, 1990.

[4] J. L. Cummings, “Cholinesterase inhibitors: a new class of psychotropic compounds,” *The American Journal of Psychiatry*, vol. 157, no. 1, pp. 4–15, 2000.

[5] A. W. Lemstra, P. Eikelenboom, and W. A. van Gool, “The cholinergic deficiency syndrome and its therapeutic implications,” *Gerontology*, vol. 49, no. 1, pp. 55–60, 2003.

[6] L. Tune, S. Carr, E. Hoag, and T. Cooper, “Anticholinergic effects of drugs commonly prescribed for the elderly: potential means for assessing risk of delirium,” *The American Journal of Psychiatry*, vol. 149, no. 10, pp. 1393–1394, 1992.

[7] J. M. Flacker and L. A. Lipsitz, “Serum anticholinergic activity changes with acute illness in elderly medical patients,” *The Journal of Gerontology Series A: Biological Sciences and Medical Sciences*, vol. 54, no. 1, pp. M12–M16, 1999.

[8] K. Plaschke, J. Kopitz, J. Mattern, E. Martin, and P. Teschendorf, “Increased cortisol levels and anticholinergic activity in cognitively unimpaired patients,” *Journal of Neuropsychiatry and Clinical Neurosciences*, vol. 22, no. 4, pp. 433–441, 2010.

[9] K. Hori, K. Konishi, R. Akita et al., “Proposal of endogenous anticholinergic hypothesis in Alzheimer disease,” *Japanese Journal of Neuropsychopharmacology*, vol. 33, no. 3, pp. 117–126, 2013 (Japanese).

[10] K. Hori, K. Konishi, M. Tani et al., “Serum anticholinergic activity: a possible peripheral marker of the anticholinergic burden in the central nervous system in Alzheimer’s disease,” *Disease Markers*, vol. 2014, Article ID 459013, 7 pages, 2014.

[11] Y. Kitajima, K. Hori, K. Konishi et al., “A review of the role of anticholinergic activity in Lewy body disease and delirium,” *Neurodegenerative Diseases*, vol. 15, no. 3, pp. 162–167, 2015.

[12] K. Hori, K. Konishi, K. Watanabe et al., “Influence of anticholinergic activity in serum on clinical symptoms of Alzheimer’s disease,” *Neuropsychobiology*, vol. 63, no. 3, pp. 147–153, 2011.

[13] I.-C. Chien, J.-H. Hsu, S.-H. Bih et al., “Prevalence, correlates, and disease patterns of antipsychotic use in Taiwan,” *Psychiatry and Clinical Neurosciences*, vol. 62, no. 6, pp. 677–684, 2008.

[14] A. Wood-Mitchell, I. A. James, A. Waterworth, A. Swann, and C. Ballard, “Factors influencing the prescribing of medications by old age psychiatrists for behavioural and psychological symptoms of dementia: a qualitative study,” *Age and Ageing*, vol. 37, no. 5, pp. 547–552, 2008.

[15] P. L. McGeer and E. G. McGeer, “The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases,” *Brain Research Reviews*, vol. 21, no. 2, pp. 195–218, 1995.

[16] W. H. Frey II, C. R. Emory, M. E. Wiebenga et al., “Inhibitor of antagonist binding to the muscarinic receptor is elevated in Alzheimer’s brain,” *Brain Research*, vol. 655, no. 1-2, pp. 153–160, 1994.

[17] T. R. Bernik, S. G. Friedman, M. Ochani et al., “Pharmacological stimulation of the cholinergic antiinflammatory pathway,” *The Journal of Experimental Medicine*, vol. 195, no. 6, pp. 781–788, 2002.

[18] J. G. Mabrey, P. Packer, and C. Szabo, “Activation of the cholinergic antiinflammatory pathway reduces ricin-induced mortality and organ failure in mice,” *Molecular Medicine*, vol. 15, no. 5-6, pp. 166–172, 2009.

[19] P. G. Nazarov, I. B. Krylova, N. R. Evdokimova, G. I. Nezhinskaya, and A. A. Butyugov, “C-reactive protein: a pentraxin with anti-acetylcholine activity,” *Life Sciences*, vol. 80, no. 24-25, pp. 2337–2341, 2007.

[20] A. Fisher, “Therapeutic strategies in Alzheimer’s disease: M1 muscarinic agonists,” *Japanese Journal of Pharmacology*, vol. 84, no. 2, pp. 101–112, 2000.

[21] C. K. Jones, A. E. Brady, A. A. Davis et al., “Novel selective allosteric activator of the M1 muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats,” *Journal of Neuroscience*, vol. 28, no. 41, pp. 10422–10433, 2008.

[22] E. K. Perry, L. Kilford, A. J. Lees, D. J. Burn, and R. H. Perry, “Increased Alzheimer pathology in Parkinson’s disease related to antimuscarinic drugs,” *Annals of Neurology*, vol. 54, no. 2, pp. 235–238, 2003.

[23] C.-J. Lu and L. E. Tune, “Chronic exposure to anticholinergic medications adversely affects the course of Alzheimer disease,” *The American Journal of Geriatric Psychiatry*, vol. 11, no. 4, pp. 458–461, 2003.

[24] K. Konishi, K. Hori, H. Tomioka et al., “Donepezil abolishes anticholinergic activity in a patient with amnesia,” *Pharmacology*, vol. 91, no. 1-2, pp. 86–91, 2013.

[25] J. Young and S. K. Inouye, “Delirium in older people,” *The British Medical Journal*, vol. 334, no. 7598, pp. 842–846, 2007.

[26] E. Nederhof, K. Marceau, E. A. Shirtcliff, P. D. Hastings, and A. J. Oldehinkel, “Autonomic and adrenergic interactions predict mental health in late adolescence: The TRAILS Study,” *Journal of Abnormal Child Psychology*, vol. 43, no. 5, pp. 847–861, 2015.

[27] G. S. Masson, A. R. Nair, R. B. Dange, P. P. Silva-Soares, L. C. Michelin, and J. Francis, “Toll-like receptor 4 promotes autonomic dysfunction, inflammation and microglia activation in the hypothalamic paraventricular nucleus: role of endoplasmic reticulum stress,” *PLoS ONE*, vol. 10, no. 3, Article ID e0122850, 2015.

[28] V. M. Miller, R. A. Kenny, A. E. Oakley, R. Hall, R. N. Kalaria, and L. M. Allan, “Dorsal motor nucleus of vagus protein aggregates in Lewy body disease with autonomic dysfunction,” *Brain Research*, vol. 1286, pp. 165–173, 2009.

[29] I. G. McKeith, D. W. Dickson, J. Lowe et al., “Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium,” *Neurology*, vol. 65, no. 12, pp. 1863–1872, 2005.

[30] K. Hori, K. Konishi, H. Tanaka et al., “Pharmacotherapy for behavioral and psychological symptoms in Alzheimer’s disease,” *Brain Disorders and Therapies*, vol. 2, no. 2, article 106, 2013.

[31] L. Han, J. V. Agostini, and H. G. Allroe, “Cumulative anticholinergic exposure is associated with poor memory and executive function in older men,” *Journal of the American Geriatrics Society*, vol. 56, no. 12, pp. 2203–2210, 2008.

[32] M. S. Yilmaz, B. Isık, M. Öngar et al., “Delirium due to *Datura stramonium* ingestion: a case report,” *Advances in Research*, vol. 2, no. 10, pp. 523–527, 2014.

[33] P. T. Trzepacz, “Is there a final common neural pathway in delirium? Focus on acetylcholine and dopamine,” *Seminars in Clinical Neuropsychiatry*, vol. 5, no. 2, pp. 132–148, 2000.

[34] T. T. Hsieh, T. G. Fong, E. R. Marcantonio, and S. K. Inouye, “Cholinergic deficiency hypothesis in delirium: a synthesis of current evidence,” *Journals of Gerontology, Series A: Biological Sciences and Medical Sciences*, vol. 63, no. 7, pp. 764–772, 2008.

[35] A. Sfera, M. Cummings, and C. Osorio, “Non-neuronal acetylcholine: the missing link between sepsis, cancer, and delirium?” *Frontiers in Medicine*, vol. 2, article 56, 2015.
[36] J. R. Mach Jr., M. W. Dysken, M. Kuskowski, E. Richelson, L. Holden, and K. M. Jilk, “Serum anticholinergic activity in hospitalized older persons with delirium: a preliminary study,” *Journal of the American Geriatrics Society*, vol. 43, no. 5, pp. 491–495, 1995.

[37] C. Mussi, R. Ferrari, S. Ascari, and G. Salvioli, “Importance of serum anticholinergic activity in the assessment of elderly patients with delirium,” *Journal of Geriatric Psychiatry and Neurology*, vol. 12, no. 2, pp. 82–86, 1999.

[38] L. E. Tune, “Serum anticholinergic activity levels and delirium in the elderly,” *Seminars in Clinical Neuropsychiatry*, vol. 5, no. 2, pp. 149–153, 2000.

[39] A. Baraka and S. Harik, “Reversal of central anticholinergic syndrome by galanthamine,” *The Journal of the American Medical Association*, vol. 238, no. 21, pp. 2293–2294, 1977.

[40] S. P. Wengel, W. H. Eoccaforte, and W. J. Burke, “Donepezil improves symptoms of delirium in dementia: implications for future research,” *Journal of Geriatric Psychiatry and Neurology*, vol. 11, no. 3, pp. 159–161, 1998.

[41] L. Stocker, L. Jellestad, J. Jenewein, and S. Boettger, “Challenges in the management of delirium: a case of augmentation with donepezil following inadequate response and adverse effects with risperidone,” *Psychiatria Danubina*, vol. 27, no. 1, pp. 64–66, 2015.
Research Article

5-HT\textsubscript{2A} Receptor Binding in the Frontal Cortex of Parkinson’s Disease Patients and Alpha-Synuclein Overexpressing Mice: A Postmortem Study

Nadja Bredo Rasmussen,\textsuperscript{1} Mikkel Vestergaard Olesen,\textsuperscript{1} Tomasz Brudek,\textsuperscript{1} Per Plenge,\textsuperscript{2} Anders Bue Klein,\textsuperscript{3} Jenny E. Westin,\textsuperscript{4} Karina Fog,\textsuperscript{4} Gitta Wörtwein,\textsuperscript{5} and Susana Aznar\textsuperscript{1}

\textsuperscript{1}Research Laboratory for Stereology and Neuroscience, Bispebjerg and Frederiksberg Hospitals, Copenhagen University Hospital, 2300 Copenhagen, Denmark
\textsuperscript{2}Department of Neuroscience and Pharmacology, University of Copenhagen, 2200 Copenhagen, Denmark
\textsuperscript{3}Department of Drug Design and Pharmacology, University of Copenhagen, 2200 Copenhagen, Denmark
\textsuperscript{4}Department of Neurodegeneration, Lundbeck A/S, Ottiliaævej 9, 2500 Valby, Denmark
\textsuperscript{5}Laboratory of Neuropsychiatry, Department of Neuroscience and Pharmacology, University of Copenhagen and Mental Health Center Copenhagen, 2200 Copenhagen, Denmark

Correspondence should be addressed to Susana Aznar; susana.aznar.kleijn@regionh.dk

Received 20 April 2016; Revised 24 June 2016; Accepted 5 July 2016

Academic Editor: Per Odin

Copyright © 2016 Nadja Bredo Rasmussen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The 5-HT\textsubscript{2A} receptor is highly involved in aspects of cognition and executive function and seen to be affected in neurodegenerative diseases like Alzheimer’s disease and related to the disease pathology. Even though Parkinson’s disease (PD) is primarily a motor disorder, reports of impaired executive function are also steadily being associated with this disease. Not much is known about the pathophysiology behind this. The aim of this study was thereby twofold: (1) to investigate 5-HT\textsubscript{2A} receptor binding levels in Parkinson’s brains and (2) to investigate whether PD associated pathology, alpha-synuclein (AS) overexpression, could be associated with 5-HT\textsubscript{2A} alterations. Binding density for the 5-HT\textsubscript{2A} specific radioligand \textsuperscript{3}H-MDL 100.907 was measured in membrane suspensions of frontal cortex tissue from PD patients. Protein levels of AS were further measured using western blotting. Results showed higher AS levels accompanied by increased 5-HT\textsubscript{2A} receptor binding in PD brains. In a separate study, we looked for changes in 5-HT\textsubscript{2A} receptors in the prefrontal cortex in 52-week-old transgenic mice overexpressing human AS. We performed region-specific 5-HT\textsubscript{2A} receptor binding measurements followed by gene expression analysis. The transgenic mice showed lower 5-HT\textsubscript{2A} binding in the frontal association cortex that was not accompanied by changes in gene expression levels. This study is one of the first to look at differences in serotonin receptor levels in PD and in relation to AS overexpression.

1. Introduction

Parkinson’s disease (PD) is clinically characterized by motor symptoms consisting of bradykinesia, resting tremor, rigidity, and postural instability. One of the leading hypotheses for PD pathogenesis focuses on alterations in alpha-synuclein (AS) expression, neuronal accumulation, and aggregation of AS—including formation of Lewy bodies—as a main causative factor in the pathological cascade [1]. Though PD principally is classified as a movement disorder, it has now become recognised that PD features a complex burden of different motor and nonmotor symptoms (NMS) [1, 2]. NMS covers a range of symptoms including hyposmia, visual hallucinations, sleep disturbances, a variety of dysautonomic symptoms, depression and other mood disorders, and impairment of cognition and consequently affected executive function [3].

The key brain area involved in cognition and executive function is the prefrontal cortex (PFC). The serotonin
5-HT₂A receptor is highly expressed in PFC areas, playing an important role in executive function [4] and in modulating the cognitive control of our emotional responses during decision-making [5], making them essential for inhibitory the cognitive control of our emotional responses during inhibition [4] and modulating the cognitive control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making they essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5], making them essential for inhibitory control of our emotional responses during decision-making [5].

2. Materials and Methods

2.1. 5-HT₂A Receptor Binding Assay in Human Frontal Cortex Tissue. Postmortem frontal cortex brain tissue (BA9 region) from PD patients and controls, deceased from nonneurological causes, was used for the binding assay. All brain samples were fresh-frozen, stored at minus 80°C until further use. Brains were acquired from the Harvard Brain Tissue Resource Center, USA, and the Brain Bank at Bispebjerg University Hospital (Copenhagen, Denmark) (BBH-2010-06, 1-suit number 00971). The initial total number of samples was n = 8 for PD and n = 7 for controls, but one sample from each group was excluded from further analysis as they showed significance in a Grubbs outlier test. The PD and control samples included in the statistical analysis are listed in Table 1.

Brain tissue samples from PD patients were provided by the Harvard Brain Tissue Resource Center, USA. The mean age at death for the patients was 75.4 years and mean postmortem index (PMI)—time from death to autopsy—was 20.2 hours. Brain tissue samples for the controls were provided by the Brain Bank of the Research Laboratory for Stereology and Neuroscience, Bispebjerg University Hospital, Denmark. The causes of death in the control group were gastrointestinal (GI), cardiopulmonary (CP), and/or cancer related, and mean age at death was 68.5 years and mean PMI was 43.0 hours. There was no significant difference between the PD and control group in terms of age of death (Student’s t-test, p > 0.05) and gender distribution (chi-squared test, p > 0.05), while there was a significant difference in PMI (Student’s t-test, p < 0.05).

For the brain membrane preparation, 450 mg frontal cortex tissue of each sample was homogenized in HEPES-buffer (25 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM CaCl₂, and 1.2 mM MgSO₄, pH = 7.5). The homogenate was spun down for 5 minutes, resuspended, and spun down again for 10 minutes at 4700 rpm and again resuspended in buffer in order to obtain a 5% membrane suspension (MS). Three aliquots of the MS were stored at minus 20°C until further use. Three independent but identical saturation binding assays were performed on three different days for each sample. Brain samples were blinded. For each assay, 100 μL of the MS was added to each well in a 24-well plate together with 100 μL [³H]-MDL 100.907 (at six different concentrations: 0.14; 0.28; 0.53; 1.07; 2.12; 4.63 nM) and HEPES-buffer until reaching a total volume of 300 μL. For the nonspecific binding (NSB) 10 μM unlabeled MDL 100.907 was added to each of the concentrations. Three total binding (TB) and NSB measurements were obtained for each concentration of [³H]-MDL 100.907. Incubation time was 1 hour at room temperature.

Before filtration 1 mL ice-cold HEPES-buffer was added to each well. Membranes were filtered on printed Filtermat B90–120 mm from Wallac covered with polyethylenemine.
(PEI) 0.05% using a Connectorate filtration machine fitted with a 24-pin head (Connectorate AG, Dietikon, Switzerland). The filters were washed in ice-cold HEPES-buffer for 30 seconds and left to dry on a heating plate at 98 °C. Once dried, filters were covered with MeltiLex scintillation gel and counted on the scintillation counter (6-channel micro beta counter, Perkin Elmer). The three measurements for each concentration point were averaged into one measurement and specific binding (SB) was determined as the difference between TB and NSB and plotted against ligand concentration in GraphPad. $K_d$ and $B_{max}$ values for each sample were calculated according to a nonfit linear analysis curve. Protein concentration was determined from weight.

2.2. AS Protein Levels in Human Frontal Cortex Tissue Measured by Western Blotting. For the tissue lysate preparation, 50 mg of tissue was dissected out from the 7 PD and 6 normal controls brains from equivalent area as above and placed immediately in ice-cold Tissue Extraction Reagent II (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The tissue samples were homogenized using the MagNA Lyser Instrument (2x 6000 rpm, 25 s) and related MagNA Lyser Green Beads (Roche), in an appropriate volume of tissue extraction reagent (10 mL/g). The homogenates were incubated on ice for 10 minutes and centrifuged at 16 000 × g for 20 minutes at 4 °C. The supernatants were collected, separated into aliquots, and stored at minus 80 °C until use. A portion of the supernatant was reserved for protein determination using the Bradford reagent (Sigma-Aldrich) and subsequent measurement of absorbance was done at 595 nm using bovine serum albumin as standard.

Next, samples were prepared for electrophoresis by diluting each 20 μg of protein lysate with a 4x NuPAGE® sample buffer containing 2 mM of cross-linker dithiobis(succinimidylpropionate) (DSP) (Pierce, Thermo Fisher Scientific, Waltham, MA, USA). DSP was dissolved in dimethyl sulfoxide to a 50x stock concentration prior to addition to the protein samples. Samples were incubated with cross-linker for 30 minutes at 37 °C, followed by addition of 5% β-mercaptoethanol (βME) and incubation at 70 °C for 10 minutes. The application of reducible amine-reactive cross-linker DSP followed by reductive cleavage (5% βME) prior to sodium dodecyl sulfate polyacrylamide gel electrophoresis and electroblotting improves significantly immunodetection of alpha-synuclein monomers [10].

The samples were electrophoresed on NuPAGE 4–12% Bis-Tris Gels with NuPAGE 2-(N-morpholino)ethanesulfonic acid-sodium dodecyl sulfate (Life Technologies) running buffer and Novex® Sharp Pre-Stained Protein Standard (Life Technologies). As standards, we used 10 μg of human recombinant full-length alpha-synuclein (rPeptide) treated and loaded on gels simultaneously with the tissue lysates. After electrophoresis, gels were blotted onto Odyssey® nitrocellulose membranes 0.22 μm (LI-COR Biosciences, Cambridge, UK) using the semidyrid Bio-Rad apparatus (Bio-Rad Laboratories, Hercules, CA, USA) for 60 minutes, using a 200 mA/membrane constant current in NuPAGE transfer buffer (Life Technologies) containing 20% methanol. Post-transfer membranes were treated with 0.4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 minutes at 21 °C, rinsed with Milli-Q water, and then blocked in Odyssey blocking buffer (PBS) (LI-COR) for 1 hour at 21 °C. Treatment with low concentrations of paraformaldehyde prevents washing off alpha-synuclein from nitrocellulose membranes [11].

Blots were then incubated overnight at 4 °C with rabbit monoclonal anti-alpha-synuclein antibody MJFRI (Abcam # ab138501, 1: 5000) in Odyssey blocking buffer with 0.1% Tween-20. All membranes were at the same time incubated with an anti-GAPDH antibody (clone FL-335) (# SC-25778, 1: 1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a loading control. Membranes were then washed 3x 15 minutes in PBS with 0.1% Tween-20 and incubated in secondary antibody IRDye® 800CW Goat anti-rabbit IgG (# 926-32211; LI-COR Biosciences) 1:15 000 and IRDye 680LT Goat anti-mouse IgG1-specific (# 926-68050; LI-COR Biosciences) 1: 20 000 in PBS + 0.1% Tween + 0.01% sodium dodecyl sulfate at 21 °C in the dark. Subsequently, the membranes were washed 3x 15 minutes in PBS, rinsed in Milli-Q water, air dried, and developed on LI-COR Bioscience Odyssey 9120 Infrared Imaging System.

Scanned western blots were analyzed with Image Studio Lite software v5.2 (LI-COR Biosciences). The infrared signals after background subtraction were normalized to the loading control signal.

2.3. Transgenic Animals, h-SNCA Mice, Overexpressing Human Alpha-Synuclein. Mice used in the study were male, 52-week-old, transgenic (tg) F28SNCA mice on a C57BL/6 background ($n = 10$) overexpressing wild-type human SNCA (an AS coding gene) under the control of mouse AS promoter [12, 13] and C57BL/6 wild-type (wt) mice ($n = 10$) (Taconic, H. Lundbeck A/S, Denmark). All animal experiments were approved by the Danish Veterinary and Food Administration (DVFA) and were conducted in accordance with European standards on animal welfare.

2.4. Ligands. The 5-HT$_{2A}$ receptor antagonist, $[^3H]$-MDL 100.907, [R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)-ethyl]-4-piperidine-methanol], was synthesized and radiolabelled by NOVANDI Chemistry AB, Sweden. The specific activity of $[^3H]$-MDL 100.907 was determined to be 75 676 Ci/mmol (2.8 Tbq/mmol). Radiochemical purity was greater than 97%.

2.5. 5-HT$_{2A}$ Receptor Autoradiography. Mice were decapitated and brains rapidly removed and frozen on powdered dry ice and kept at minus 80 °C until sectioning. Prefrontal cortex regions were cut into 15 μm coronal sections on a cryostat, thaw-mounted onto Superfrost Plus slides, and stored at minus 20 °C until further processing.

Autoradiography was performed using 1 nM $[^3H]$-MDL 100.907 for 5-HT$_{2A}$ receptor TB, with addition of 10 μM ketanserin to determine NSB. Briefly, sections were thawed at room temperature for a minimum of 30 minutes prior to the experiment. For preincubation, sections were submerged in...
5°C tris-buffer (50 mM tris-HCl buffer, pH = 7.4, with 0.01% ascorbic acid) for 15 minutes—with or without ketanserin. Sections were incubated for 120 minutes at 5°C in tris-buffer with the ligand, with or without ketanserin. Concentrations of radioligand were determined using a scintillation counter. After incubation, slides were dipped in ice-cold tris-buffer for 5 seconds. Slices were then washed again 2x 15 minutes in ice-cold tris-buffer and dipped in ice-cold dH2O for 20 seconds. Sections were left to dry under a fume hood overnight. After drying, sections were exposed for 5 weeks to a BAS IP-TR2040 Fuji Imaging Plate (Science Imaging, Sweden) together with [3H] specific microscales at 4°C. Imaging plates were scanned on the BAS-2500 Phosphor Image Scanner: FLA-9000, Stariion. ImageJ was used for densitometric analysis of signal intensity. Concentrations were expressed as nCi/mg. Quantification was performed blindly in separate regions of interest (ROIs) according to Paxinos and Franklin’s atlas of the mouse brain [14].

2.6. RT-qPCR Quantification of 5-HT2A-R Expression. RNA was extracted from fresh frontal cortex sections that were scraped off from parallel slides to the ones allocated for the receptor autoradiography analysis. For each animal, a total of 12 coronal frontal sections of 15 μm thickness were pooled together. Total RNA was purified using the PureLink RNA Mini Kit (Ambion) following the manufacturer’s instructions. All RNA samples were treated once with DNase using TURBO DNA-free Kit (Ambion) according to the manufacturer’s instructions. RNA samples were suspended in RNase-free water and were quantified using Agilent 2100 Bioanalyzer (Agilent Technologies). Only samples with RIN ≥ 6 were included in the analysis. RNA samples were stored at minus 80°C until further use.

A two-step real-time PCR was subsequently performed: RNA samples matched on concentration were reverse transcribed into cDNA with qScript cDNA SuperMix Kit (Quanta BioSciences) according to manufacturer’s instructions. SuperMix reaction mixture consisted of 5x reaction buffer containing optimized concentrations of MgCl2, dNTPs (dATP, dCTP, dGTP, and dTTP), recombinant RNase inhibitor protein, qScript reverse transcriptase, random primers, oligo(dT) primers, and stabilizers. cDNA synthesis step was performed following incubation times of 5 minutes at 25°C, 30 minutes at 42°C, 5 minutes at 85°C, and 5 minutes at 25°C. Thereafter cDNA product was diluted 5-fold in nuclease-free water and kept at minus 20°C until further use.

To check RNA samples for contamination with nuclear DNA a negative control PCR was done on all samples prior to cDNA synthesis omitting the reverse transcription step.

Real-time PCR reactions were performed using PerfeCTa SYBR Green FastMix (2x) Kit (Quanta BioSciences) with forward and reverse primers specific for the 5-HT2A receptor (mouse), Rpl13a (mouse reference gene), and GAPDH (mouse reference gene) (TAG Copenhagen). Primer sequences were as follows: 5-HT2A F: 5’-GCA GTA GTC CAT CAG CAA TGA GC-3’ and R = 5’-GCA GTG GCT TTC TGT TCT CC-3’; Rpl13a: F: 5’-GGA GGG GCA GGT TCT GGT AT-3’ and R = 5’-TGT TGA TGC TTT CAC AGC GT-3’; GAPDH: F = 5’-CAT CAA GAA GGT GGT GAA GCA-3’ and R = 5’-CTG TTG AAG TCA CAG GAG ACA-3’. qPCR was performed with 30 seconds for initial activation at 95°C, then 40 cycles of 5 seconds at 95°C for denaturation, 15 seconds at 60°C for annealing (END read), and 10 seconds at 72°C for elongation. To verify product a melting curve analysis between 55 and 95°C was performed after each run. Relative quantification (target/reference) was made using the ΔΔCt method [15].

2.7. Statistical Analyses. All statistical analyses were performed in GraphPad Prism 6. Significance level was set at a p value ≤ 0.05. All values are presented as mean ± standard error of the mean (SEM). Student’s t-test was used for comparing mRNA levels for the receptors analyzed, AS protein levels, and Kd and Bmax values between the PD and control brain samples. Multiple t-tests were used for comparing receptor binding levels in h-SNCA tg and wt mice for the different ROIs.

3. Results

3.1. Higher 5-HT2A Receptor Binding in Postmortem Prefrontal Cortex of PD Patients. The binding assay, performed on human brain tissue from the frontal cortex, showed a significant difference in Bmax of the 5-HT2A receptor between PD (n = 7) and control (n = 6) brain samples, with higher maximum binding level in the PD group (PD: 5.7 ± 1.0; controls: 3.2 ± 0.35; Student’s test p < 0.05) (Figure 1(a)). There was no significant difference between the PD and control group when comparing Kd (affinity) (PD: 0.5 ± 0.07; controls 0.3 ± 0.05; p > 0.05). Mean PMI (postmortem interval) was different between the two groups (PD and controls). We controlled for a potential effect of the PMI on binding results, and no correlation between Bmax and PMI (r = −0.52, p > 0.05) or between Kd and PMI (r = −0.32, p > 0.05) was found.

3.2. Increased AS Protein Levels in the Prefrontal Cortex Region of PD Brains. Detergent soluble monomeric AS protein levels, as measured by electrophoresis and immunoblotting, were increased in the PD brains compared to controls (PD: 2.56 ± 0.27; controls 1.56 ± 0.3; Student’s test p < 0.05) (Figure 1(b)). No correlation was observed between AS protein and 5-HT2A receptor binding levels in the PD brains (Spearman r = −0.48; p > 0.05).

3.3. Region-Specific Differences in 5-HT2A and 5-HT1A Receptor Binding in AS Overexpressing Mice. The regions of interest (ROIs) included FrA (frontal association cortex), PrL + Cg1/2 (prelimbic cortex and cingulate cortex), MO (medial orbital cortex), DLO (dorsolateral orbital cortex), M1 and M2 (primary and secondary motor cortex), and AI (anterior insular cortex). Results from the [3H]-MDL 100.907 autoradiography binding analysis showed significantly lower 5-HT2A receptor binding in FrA of h-SNCA mice (h-SNCA: 102.2 ± 6.5 fmol/mg; wt 126.7 ± 8.0 fmol/mg; p < 0.05). There were no significant differences in 5-HT2A receptor binding in the other ROI measured (Figure 2(a)).
3.4. No Difference in 5-HT_{2A} Receptor Gene Expression Levels. Results from RT-qPCR show no difference in 5-HT_{2A} (h-SNCA: 0.01 ± 0.003; wt 0.007 ± 0.001; \( p > 0.05 \)) receptor gene expression in the frontal cortex of h-SNCA mice when compared to wt mice (Figure 2(b)).

4. Discussion

In this study we looked at alterations in 5-HT_{2A} receptor binding levels in the frontal cortex from PD patients, and using a tg h-SNCA mouse model, we furthermore looked for whether AS overexpression could be associated with changes in 5-HT_{2A} receptor binding and gene expression. *In vitro* receptor binding studies on postmortem brain tissue from PD patients with increased AS levels indeed revealed significantly higher 5-HT_{2A} receptor binding levels, as indicated by higher maximum binding (\( B_{\text{max}} \)) in the PD brains. Results obtained using the well validated human AS overexpressing mouse model [12, 13] showed lower 5-HT_{2A} receptor binding levels in FrA cortex in the h-SNCA transgenic mice. Differences in 5-HT_{2A} receptor binding levels were not accompanied by decreased gene expression.
To our knowledge this is one of the first studies directly looking at 5-HT$_{2A}$ receptor binding levels in postmortem tissue samples from the frontal cortex of PD brains in relation to Parkinson’s disease associated pathology of AS overexpression. Previously, at least two studies had addressed the question of 5-HT$_{2A}$ receptor alterations in the frontal cortex of PD brains. A study from 1998 examined postsynaptic 5-HT$_{2A}$ receptors in the neocortex of eight PD patients [16]. It too found an increase in 5-HT$_{2A}$ receptor binding in PFC of postmortem PD tissue, using ligands other than those in this study (8-OH-DPAT and ketanserin). More recently, a Single-Positron-Emission-Tomography (SPECT) study, using the [123I]-5-I-R91150 ligand, reported lower 5-HT$_{2A}$ receptor binding in the anterior striatum and premotor cortex and increased 5-HT$_{2A}$ receptor binding in the occipital cortex of untreated PD patients [17]. However, no significant differences in 5-HT$_{2A}$ receptor binding were detected in the prefrontal and parietal cortices. This discrepancy with our findings could be explained by the fact that patients included in the latter study were in early stages of the disease and therefore not directly comparable to our patient group consisting of end stage PD patients. According to Braak staging of PD, the raphe nuclei, the origin of the serotonergic projection to the substantia nigra, striatum, globus pallidus, subthalamic nucleus, thalamus, and neocortex with connections to cortical regions including frontal cortex and PFC areas—core structures of the cortico-basal ganglia-thalamo-cortical loop compromised in PD [9]—become increasingly affected in stage 2 and are completely affected in stage 3 [18, 19]—hence, the serotonergic system is mostly affected at later stages of the disease. Another explanation could be that, contrary to the study by Melse et al. [17], which included unmedicated patients, we cannot exclude an effect of the medical treatment on the receptor upregulation observed in our PD patients.

In addition to higher binding levels of 5-HT$_{2A}$ receptor in the frontal cortex of PD brains, our study found higher concentration of AS in PD compared to controls, which corresponds well with the view that accumulation of AS plays a significant role in PD pathology. Overall, it is important to take into consideration that both in the study by Melse et al. [17] and Chen et al. [16] and in our study relatively small sample sizes were used with risk of inconclusive results. With that in mind, we can nevertheless agree that the joint results point towards disease related changes in 5-HT$_{2A}$ receptor levels that could be associated with some of the cognitive and executive dysfunction seen in PD. More studies are needed to pursue this idea further.

In the second part of our study, our aim was to investigate whether alterations in 5-HT$_{2A}$ receptor binding and expression could be associated with AS overexpression. Here decreased binding levels of 5-HT$_{2A}$ receptor were found in FrA in AS overexpressing mice. This region is part of the PFC area, and even though its exact function is still unknown, FrA is most likely involved in some of the cognitive processes related to executive function. The FrA has been proposed in a human study to be important for go/no-go task performance [20]. The go/no-go task measures attention and response inhibition control [21], and interestingly these are functions impaired in newly diagnosed drug naïve PD patients [6].
The differences in receptor binding levels observed in the AS overexpressing mice were not accompanied by differences in gene expression. The lower receptor binding in the h-SNCA mice could therefore be due to regulatory effects at the posttranslational level.

Why the changes in 5-HT\(_{2A}\) receptor binding levels display opposite directions in the PD brains and in the transgenic animal model of AS overexpression is puzzling. In the mouse model we isolate a specific segment of the underlying pathology behind PD—that is AS overexpression. In the human tissue all aspects of PD pathology along with AS overexpression, that is, dopaminergic degeneration, play in, thus adding to the complexity of mechanisms that may influence the 5-HT\(_{2A}\) receptor. Striatal degeneration also results in decreased 5-HT\(_{2A}\) binding in PFC, as seen in a 6-hydroxydopamine-induced parkinsonian rat model [22]. Changes in 5-HT\(_{2A}\) receptor levels are probably the result of a more multifaceted pathology behind PD, not directly related to AS overexpression. We cannot exclude that the receptor upregulation observed in the PD brains can also be due to compensatory drug effects administered to this group of patients, that is, antidepressants or antipsychotics; as we do not have access to this information from the patient material used for this study we cannot determine this.

In summary, in PD brains we find higher levels of 5-HT\(_{2A}\) receptor binding together with higher levels of AS. An association is not supported by transgenic mouse model results, thus pointing towards a more complex and multidimensional explanation for 5-HT\(_{2A}\) changes found in PD. Nevertheless, the most important finding from the present study is that the 5-HT\(_{2A}\) receptor seems to be dysregulated in PD. Progress in the area of 5-HT\(_{2A}\) targeting treatment is already being manifested in management of PD psychosis for example [23]. Addressing this receptor in future studies is relevant for understanding and treating some of the cognitive and executive dysfunction seen in PD.

### Competing Interests

The authors have no competing interests to declare.

### Acknowledgments

This work was supported by the Lundbeck Foundation. The authors thank the Harvard Brain Tissue Resource Center, USA, for the donation of the PD brains.

### References

[1] P. M. A. Antony, N. J. Diederich, R. Krüger, and R. Balling, “The hallmarks of Parkinson's disease,” FEBS Journal, vol. 280, no. 23, pp. 5981–5993, 2013.

[2] R. B. Postuma, D. Aarsland, P. Barone et al., “Identifying prodromal Parkinson's disease: pre-Motor disorders in Parkinson's disease,” Movement Disorders, vol. 27, no. 5, pp. 617–626, 2012.

[3] T. Simuni and K. Sethi, “Nonmotor manifestations of Parkinson’s disease,” Annals of Neurology, vol. 64, supplement 2, pp. S65–S80, 2008.

[4] S. Aznar and M. E. Hervig, “The 5-HT\(_{2A}\) serotonin receptor in executive function: implications for neuropsychiatric and neurodegenerative diseases,” Neuroscience & Biobehavioral Reviews, vol. 64, pp. 63–82, 2016.

[5] S. Aznar and A. B. Klein, “Regulating prefrontal cortex activation: an emerging role for the 5-HT\(_{2A}\) serotonin receptor in the modulation of emotion-based actions?” Molecular Neurobiology, vol. 48, no. 3, pp. 841–853, 2013.

[6] J. P. M. Van Der Vergt, O. J. Hulme, S. Zietz et al., “Attenuated neural response to gamble outcomes in drug-naive patients with Parkinson's disease,” Brain, vol. 136, no. 4, pp. 1192–1203, 2013.

[7] L. Marner, V. G. Frokjaer, J. K. Lager et al., “Loss of serotonin 2A receptors excess loss of serotonergic projections in early Alzheimer's disease: a combined [\(^{18}\)F]altanserin-PET study,” Neurobiology of Aging, vol. 33, no. 3, pp. 479–487, 2012.

[8] P. Holm, A. Eitstrup, A. B. Klein et al., “Plaque deposition dependent decrease in 5-HT\(_{2A}\) serotonin receptor in aβPPswe/PS1E9 amyloid overexpressing mice,” Journal of Alzheimer's Disease, vol. 20, no. 4, pp. 1201–1213, 2010.

[9] P. Huot, S. H. Fox, and J. M. Brotchie, “The serotonin system in Parkinson’s disease,” Progress in Neurobiology, vol. 95, no. 2, pp. 163–212, 2011.

[10] A. J. Newman, D. Selkoe, and U. Dettmer, “A new method for quantitative immunoblotting of endogenous α-synuclein,” PLoS ONE, vol. 8, no. 11, Article ID e8134, 2013.

[11] B. R. Lee and T. Kamitani, “Improved immunodetection of endogenous α-synuclein,” PLoS ONE, vol. 6, no. 8, Article ID e23939, 2011.

[12] G. H. Petit, E. Berkovich, M. Hickery et al., “Rasagiline ameliorates olfactory deficits in an α-synuclein mouse model of Parkinson’s disease,” PLoS ONE, vol. 8, no. 4, Article ID e60691, 2013.

[13] M. Westerlund, C. Ran, A. Borgkvist et al., “Lrk2 and α-synuclein are co-regulated in rodent striatum,” Molecular and Cellular Neuroscience, vol. 39, no. 4, pp. 586–591, 2008.

[14] G. Paxinos and K. B. J. Franklin, The Mouse Brain in Stereotaxic Coordinates, Academic Press, 2nd edition, 2001.

[15] T. D. Schmittgen and K. J. Livak, “Analyzing real-time PCR data by the comparative CT method,” Nature Protocols, vol. 3, no. 6, pp. 1101–1108, 2008.

[16] C. P. L.-H. Chen, J. T. Alder, L. Bray, A. E. Kingsbury, P. T. Francis, and O. J. F. Foster, “Post-synaptic 5-HT\(_{2A}\) and 5-HT\(_{2A}\) receptors are increased in Parkinson’s disease neocortex,” Annals of the New York Academy of Sciences, vol. 861, pp. 288–289, 1998.

[17] M. Melse, S. K. Tan, Y. Temel, M. J. van Kroonenburgh, and A. F. Leentjens, “Changes in 5-HT\(_{2A}\) receptor expression in untreated, de novo patients with Parkinson’s disease,” Journal of Parkinson's Disease, vol. 4, no. 2, pp. 283–287, 2014.

[18] H. Braak, K. Del Tredici, U. Rüb, R. A. I. De Vos, E. N. H. Jansen Steur, and E. Braak, “Staging of brain pathology related to sporadic Parkinson’s disease,” Neurobiology of Aging, vol. 24, no. 2, pp. 197–211, 2003.

[19] H. Braak, E. Ghebremedhin, U. Rüb, H. Bratzke, and K. Del Tredici, “Stages in the development of Parkinson's disease-related pathology,” Cell and Tissue Research, vol. 318, no. 1, pp. 121–134, 2004.

[20] K. Sasaki, H. Gemb, A. Nambu, and R. Matsuzaki, “No-go activity in the frontal association cortex of human subjects,” Neuroscience Research, vol. 18, no. 3, pp. 249–252, 1993.
[21] Y. Chudasama and T. W. Robbins, “Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans,” *Biological Psychology*, vol. 73, no. 1, pp. 19–38, 2006.

[22] Y. Li, X.-F. Huang, C. Deng et al., “Alterations in 5-HT$_2A$ receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats,” *Synapse*, vol. 64, no. 3, pp. 224–230, 2010.

[23] I. Yasue, S. Matsunaga, T. Kishi, K. Fujita, and N. Iwata, “Serotonin 2A receptor inverse agonist as a treatment for Parkinson’s disease psychosis: a systematic review and meta-analysis of serotonin 2A receptor negative modulators,” *Journal of Alzheimer’s Disease*, vol. 50, no. 3, pp. 733–740, 2016.
Quantitative EEG and Cognitive Decline in Parkinson’s Disease

Vitalii V. Cozac, Ute Gschwandtner, Florian Hatz, Martin Hardmeier, Stephan Rüegg, and Peter Fuhr

Universitätsspital Basel, Abteilung Neurophysiologie, Petersgraben 4, 4031 Basel, Switzerland

Correspondence should be addressed to Peter Fuhr; peter.fuhr@usb.ch

Received 16 December 2015; Accepted 14 March 2016

Cognitive decline is common with the progression of Parkinson’s disease (PD). Different candidate biomarkers are currently studied for the risk of dementia in PD. Several studies have shown that quantitative EEG (QEEG) is a promising predictor of PD-related cognitive decline. In this paper we briefly outline the basics of QEEG analysis and analyze the recent publications addressing the predictive value of QEEG in the context of cognitive decline in PD. The MEDLINE database was searched for relevant publications from January 01, 2005, to March 02, 2015. Twenty-four studies reported QEEG findings in various cognitive states in PD. Spectral and connectivity markers of QEEG could help to discriminate between PD patients with different level of cognitive decline. QEEG variables correlate with tools for cognitive assessment over time and are associated with significant hazard ratios to predict PD-related dementia. QEEG analysis shows high test-retest reliability and avoids learning effects associated with some neuropsychological testing; it is noninvasive and relatively easy to repeat.

1. Introduction

(1) Background. Cognitive decline is common with the progression of Parkinson’s disease (PD) [1]. Several studies have shown that the point prevalence of dementia in patients with PD (PD-D) is about 30% and that the incidence rate of dementia in PD is 4–6 times higher than in control subjects [2–4]. The cumulative prevalence of PD-D in patients surviving more than ten years after diagnosis was estimated at more than 75% [5]. Thus, prediction and early diagnosis of cognitive decline in PD are a current challenge in neurosciences as well as patient care and counselling. Various markers have been studied for early identification of PD-D and mild cognitive impairment related to PD (PD-MCI) [6–8]. Quantitative EEG (QEEG) has shown good potential in identification of cognitive deterioration in patients with PD [9]. QEEG is advancing fast, and various new methods have been introduced and applied in QEEG research. In this review, we briefly discuss the basics of QEEG and recent publications addressing its predictive value for detecting of PD-related worsening of cognition.

(2) Methods of Literature Search. References for this review were identified through search of the MEDLINE database (Supplement 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/9060649). The following search strategy was used: (((eeg) AND parkin∗)) AND (“2005” [Date - Publication]: “2015” [Date - Publication]). We identified 739 potentially eligible publications with this search query on March 2, 2015. The titles and abstracts were examined for selection criteria:

(a) full text available in English;
(b) original research studies;
(c) subjects of the study: patients with PD, who were assessed by QEEG (spectral or/and connectivity analysis) and had not undergone deep brain stimulation;
(d) QEEG variables acquired through conventional EEG machines or magnetoencephalography (MEG) in resting state eyes-closed conditions in “ON” or/and “OFF” levodopa medication condition;
(e) studies focusing on comparison between groups of PD patients with different states of cognition.
Sixty-one original research papers were identified after analysis of the titles and abstracts and subject to full text analysis. After analysis of the full text, 23 original research publications in peer-reviewed journals were selected for the final analysis. Details summarizing the profiles of the included publications are shown in Table 1. Profiles of the excluded papers are shown in Supplement 2.

(3) Analysis of the Findings. These 23 selected studies were performed by nine independent research groups. Independence of the authors was analyzed by reviewing the affiliations of the first and the corresponding authors.

Full meta-analysis was not performed because of the following reasons: firstly, in spite of a common concept, applying QEEG methods to investigate cognition of patients with PD, these studies were too heterogeneous in terms of the applied methods. The researchers use different methods of mathematical processing of the EEG, different approaches (such as spectral or connectivity analysis), and different settings. Secondly, while there is a more or less common consensus regarding diagnostic criteria of an advanced cognitive deterioration, PD-dementia (PD-D), such a consensus regarding diagnostic criteria for intermediate (between normal cognition and PD-D) cognitive disorder, mild cognitive impairment (MCI), is still under discussion [10–12].

However, the effect sizes of the reported variables were calculated in order to compare the relevant results. The effect size is a statistical measure, reflecting how much the standardized means are different between two populations [13]. The larger the effect size is, the more the two populations are distinct in a studied parameter. Similarly, correlation coefficients were analyzed by Fisher’s Z transformation [14]. In this case, the larger the Fisher Z is, the stronger the correlation is.

2. Background on QEEG

2.1. Basics of Quantitative Analysis of EEG. QEEG is a mathematical processing of EEG data to extract relevant information for subsequent analysis or comparison with other kinds of data [15, 16]. In contrast to conventional EEG, where electrical activity of the brain cells is visually analyzed, QEEG provides derivative parameters, which are generated from EEG “raw” data using computational methods. QEEG includes several procedural steps (Figure 1). The first step consists of EEG signal acquisition itself, performed with the use of various EEG machines and electrode systems. Alternatively, MEG may be used. MEG is the recording of the magnetic fields, generated by the ionic currents at the brain cellular level; thus, both EEG and MEG are methodologically similar and relevant in neuroscience [17]. The second step includes preprocessing, eliminating the following artifacts: muscle movements, sleepiness, eye blinks, heartbeat, and other types of EEG “noise.” Preprocessing is performed by selecting “clean” EEG segments for analysis. The last stage is mathematical processing of the “clean” (artifact-free) EEG signal to extract a parameter, which denotes best the process of interest (e.g., cognitive decline). Various mathematical approaches are used for the processing; they are generally classified in linear and nonlinear techniques. Linear methods are based on the concept that electric activity of the brain is a stationary process [18]. Nonlinear methods are based on the concept that EEG activity is a dynamic and irregular phenomenon [19]. Each of these methods has its advantages and disadvantages [20, 21].

2.2. Spectral Analysis. Spectral analysis is a linear technique of EEG processing. It is a process by which a complex EEG signal is decomposed into its component frequencies, and the amplitude of oscillations at each frequency bin is calculated. Since oscillations around zero (like an EEG trace) would add up to 0, amplitudes are represented by their squares, called power. The totality of powers at each frequency band is called power spectrum and could be represented as a graph (Figure 2). Thus, a power spectrum reflects “the amount of activity” in frequency bands. The frequency bands are the same as those used in conventional EEG, generally consisting of delta (0.1–3.5 Hz), theta (4–7.5 Hz), alpha (8–13 Hz), beta (14–30 Hz), and gamma (>30 Hz) [22]. However, different researchers may select slightly different frequency intervals for their analyses. Additionally, the bands could be divided into subbands, for example, alpha 1 (8–10 Hz) and alpha 2 (10–13 Hz), for the purpose of a thorough analysis.

Spectral power can be absolute or relative. Absolute power in a given frequency band, for example, in the alpha band, corresponds to the integral of all power values as measured, while relative power is the power in a given frequency band divided by the sum of all power measurements of all frequencies. Additionally, power could be global and regional. Global power reflects the average power over the whole cortex, while regional power characterizes the power in certain cortex regions. Mainly, 5 regions in each hemisphere are analyzed: frontal, temporal, parietal, occipital, and central, giving a total of 10 regions.

Additionally, some average parameters of EEG frequency can be obtained in spectral analysis [23]. Mean frequency (also referred to as mean “power frequency” or “spectral center of gravity”) is calculated as the sum of the product of the power spectrum and the frequency divided by the total sum of the power spectrum. Median frequency is the 50% quantile of the power spectrum; in other words, it is the frequency at which the power spectrum is divided into two regions with equal amplitude. Finally, peak frequency is the frequency which corresponds to the maximum of the power spectrum.

2.3. Functional Connectivity Analysis. The other type of information obtained by QEEG (apart from spectral analysis) is functional brain connectivity. Functional connectivity in the context of neuronal activity may be briefly defined as a coordinated interplay between specialized brain regions [24]. Cognitive functions (e.g., attention, memory) arise from neuronal activity, which is distributed over the brain anatomically and temporally, forming complex networks [25]. These
Table 1: Profiles of the studies, which met the inclusion criteria.

| Number | Author(s) | Type of the study/setting | Analyzed parameter(s) | Affiliation of the corresponding author |
|--------|-----------|----------------------------|------------------------|-----------------------------------------|
|        |           | Studies with EEG with 10-20 international system |                        |                                         |
| 1      | Caviness et al. 2007 [35] | Comparison of 8 PD-D patients versus 16 PD-MCI patients versus 42 PD-NC patients | Relative spectral power | Mayo Clinic, Scottsdale, USA |
| 2      | Bonanni et al. 2008 [36] | Observation of 36 LBD patients, 19 PD-D patients without cognitive fluctuations, 16 PD-D patients with cognitive fluctuations, 17 AD patients, and 50 HC | Compressed spectral arrays and relative spectral power | G. d’Annunzio University of Chieti-Pescara, Pescara, Italy |
| 3      | Fonseca et al. 2009 [37] | Comparison of 7 PD-D patients versus 10 PD-MCI patients versus 15 PD-NC patients versus 26 HC | Relative and absolute amplitudes | Pontificia Universidade Catolica de Campinas, Campinas, Brazil |
| 4      | Kamei et al. 2010 [38] | Comparison of PD patients with executive dysfunction versus 25 PD patients without executive dysfunction | Absolute spectral power | Nihon University School of Medicine, Tokyo, Japan |
| 5      | Babiloni et al. 2011 [39] | Comparison of 13 PD-D patients versus 20 AD patients versus 20 HC | Spectral and source analyses | Casa di Cura San Raffaele Cassino, Italy |
| 6      | Klassen et al. 2011 [9] | Observation of 106 PD-wD patients versus 100 PD patients: 43 with MMSE 28–30 versus 35 with MMSE 24–27 versus 22 with MMSE <24 | Relative spectral power | Mayo Clinic, Scottsdale, USA |
| 7      | Morita et al. 2011 [40] | Comparison of 12 PD-NC patients versus 31 PD-wD patients versus 38 AD patients versus 37 HC | Absolute spectral power | Nihon University School of Medicine, Tokyo, Japan |
| 8      | Pugnietti et al. 2010 [41] | Comparison of 21 PD-wD patients versus 7 PD-D patients versus 10 LBD patients versus 14 HC | Global field synchronization | Scientific Institute of S. Maria Nascente, Milan, Italy |
| 9      | Fonseca et al. 2013 [42] | Comparison of 12 PD-D patients versus 31 PD-wD patients versus 38 AD patients versus 37 HC | Absolute spectral power and coherence | Pontificia Universidade Catolica de Campinas, Campinas, Brazil |
| 10     | Gu et al. 2016 [43] | Observation of 9 PD-D patients and 17 PD-MCI patients | Relative and absolute spectral power | Nanfang Hospital, Guangzhou, China |
| 11     | Caviness et al. 2015 [44] | Observation of 71 PD-wD patients | Relative spectral power | Mayo Clinic, Scottsdale, USA |
| 12     | Fonseca et al. 2015 [45] | Comparison of 31 PD-wD patients versus 28 AD patients versus 27 HC | Absolute spectral power and coherence | Pontificia Universidade Catolica de Campinas, Campinas, Brazil |
|        |           | Studies with EEG with 256 channels |                        |                                         |
| 13     | Bousleiman et al. 2014 [46] | Comparison of 12 PD-NC patients versus 41 PD-MCI patients | Relative spectral power | Hospital of the University of Basel, Basel, Switzerland |
| 14     | Zimmermann et al. 2014 [47] | Analysis of 48 PD-wD patients | Median background frequency | Hospital of the University of Basel, Basel, Switzerland |
|        |           | Studies with 151-channel whole-head MEG |                        |                                         |
| 15     | Bosboom et al. 2006 [48] | Comparison of 13 PD-D patients versus 13 PD-wD patients versus 13 HC | Relative spectral power | VU University Medical Center, Amsterdam, the Netherlands |
| 16     | Stoffers et al. 2007 [49] | Comparison of 70 PD-wD patients versus 21 HC | Relative spectral power | VU University Medical Center, Amsterdam, the Netherlands |
| 17     | Stoffers et al. 2008 [50] | Comparison of 70 PD-wD patients versus 21 HC | Synchronization likelihood | VU University Medical Center, Amsterdam, the Netherlands |
Table 1: Continued.

| Number | Author(s)                  | Type of the study/setting                                         | Analyzed parameter(s)                        | Affiliation of the corresponding author       |
|--------|---------------------------|------------------------------------------------------------------|----------------------------------------------|------------------------------------------------|
| 18     | Bosboom et al. 2009 [27]  | Comparison of 13 PD-D patients versus 13 PD-wD patients          | Synchronization likelihood                   | VU University Medical Center, Amsterdam, the Netherlands |
| 19     | Ponsen et al. 2013 [51]   | Comparison of 13 PD-D patients versus 13 PD-wD patients          | Relative spectral power and phase lag index  | VU University Medical Center, Amsterdam, the Netherlands |
| 20     | Olde Dubbelink et al. 2013 [52] | Observation of 49 PD-wD patients and 14 HC                  | Relative spectral power                       | VU University Medical Center, Amsterdam, the Netherlands |
| 21     | Olde Dubbelink et al. 2013 [53] | Observation of 43 PD-wD patients and 14 HC                  | Phase lag index                              | VU University Medical Center, Amsterdam, the Netherlands |
| 22     | Olde Dubbelink et al. 2014 [33] | Observation of 43 PD-wD patients and 14 HC                  | Weighted graph and minimum spanning tree     | VU University Medical Center, Amsterdam, the Netherlands |
| 23     | Olde Dubbelink et al. 2014 [54] | Observation; 63 PD-wD patients                                  | Relative spectral power                       | VU University Medical Center, Amsterdam, the Netherlands |

AD: Alzheimer’s disease; DLB: dementia with Lewy bodies; HC: healthy controls; PD-D: Parkinson’s disease with dementia; PD-MCI: Parkinson’s disease with mild cognitive impairment; PD-NC: Parkinson’s disease with normal cognition; PD-wD: Parkinson’s disease without dementia.

Figure 1: Outlines of the QEEG process. (a) Main steps of the processing; (b) spectral and functional connectivity measures.
networks function on the basis of anatomical connections (white matter tracts connecting brain regions), functional connections (temporal correlations between brain regions, even anatomically unconnected), and effective connections (causal influences between networks) [26]. Thus, functional connectivity analysis is a measure, which enables quantifying the level of the functional connections between brain regions.

As discussed by Bosboom et al. (2009), when performing connectivity analyses, we assume that two dynamically active neural networks are designated "A" and "B" [27]. Time series \(a_i\) and \(b_j\), using EEG signals from both networks, are recorded. The main purpose is to analyze the functional relation between "A" and "B" from \(a_i\) and \(b_j\) and to quantify the level of this relation. This quantification is performed with both linear and nonlinear methods.

Linear approaches in connectivity analysis assume that the more \(a_i\) and \(b_j\) correspond to each other, the stronger the relation between "A" and "B" is. In this way, for instance, the coherence is calculated as an estimate of a function of frequency between two signals [28]. In contrast to coherence, where the stability of the phase relation between two signals is assessed and taken as an indicator of synchronization between the brain regions, the global field synchronization (GFS) makes no assumption about the spatial location of the activity [29, 30]. GFS is calculated as a function of all frequency bands.

Figure 2: Power spectra of a healthy person (a), a patient with PD-MCI (b), and a patient with PD-D (c); band power: 8–13 Hz. Images computed from our own EEG data using TAPEEG toolbox.
However, there can be a functional relation between the structures "A" and "B" even if time series "a_i" and "b_i" do not correspond to each other; in this case nonlinear methods of analysis are applied. One of these methods is synchronization analysis, which implies that "the state of A is a function of the state of B" [31]. Synchronization likelihood (SL) is an estimate of synchronization, which reflects dynamic interactions of the chaotically active coupled networks. SL denotes how strongly a signal channel at a given time is synchronized to other channels. Another estimate of synchronization is phase lag index (PLI). PLI is calculated from the asymmetry of the distribution of instantaneous signal phase differences between two brain regions and has the advantage of being free of effects of volume conduction as opposed to the methods mentioned before [31]. In other words, PLI reflects the degree of synchronization between couples of signals.

After characterization of single connections, the next level of connectivity analysis consists in description of the whole network, applying graph theory method. In this method functional connections between brain structures are described as graphs (networks) [32]. These graphs consist of vertices (nodes) and corresponding sets of edges (connections). There are different approaches to assess the obtained graph, for example, weighted graph analysis and minimum spanning tree. The two fundamental measures of weighted graph are clustering coefficient (CC) and path length (PL). Olde Dubbelink et al. (2014) describe CC as an estimate of "the likelihood that neighbors of a vertex are also connected to each other, and characterizes the tendency to form local clusters" [33]. In other words CC describes local "connectedness." The same authors described PL as a "measure for global integration of the network. It is defined as the harmonic mean of the shortest path between any two vertices in the network, where the shortest path between two vertices is defined as the path with the largest total weight." Thus PL describes global "connectedness."

Graphs may be very complex and large, forming a variety of nodes and paths. A subgraph can be developed which connects all nodes through the shortest paths without forming cycles; such subgraph is referred to as minimum spanning tree of a weighted graph [34]. The following measures are used for minimum spanning tree estimation: leaf number (the number of nodes with only one edge), eccentricity of a node (the length of the longest connection from this node to any other node), betweenness centrality of a node (the fraction of all connections in the tree that include, but do not stop at, that node), and tree hierarchy (a quotient of the leaf number to the product of twice the number of edges to the highest betweenness centrality of any node in the tree). These measures estimate the complexity of connections in the topographical brain network [34]. There are other various types of connectivity analysis, but we briefly described only those, which will be referred to further in the text of this review.

3. Reliability of the QEEG Analysis

3.1. Individual Variability. According to Näpflin et al. (2007) interindividual variability of absolute power of the traditional frequency bands in healthy humans is large, while intraindividually the power spectrum remains stable over a period of 12 to 40 months in healthy individuals [61].

However, interpretation of a change in relative power in an individual is ambiguous and requires knowledge of more information than a change in absolute power. For example, a decrease of the relative alpha power can be due to either a decrease of absolute alpha power but also to an increase of the absolute power in one or more of the other frequency bands without any change in the absolute alpha power or to a combination of both. In cross-sectional comparisons of small groups of individuals, alterations in relative power are more easily detected than changes in absolute power, while absolute power is a good measure for longitudinal, intraindividual changes or cross-sectional comparisons of very large populations. Derived indices were proposed as a possible solution for the problem that exists in relative power relationship between frequency bands: spectral ratio (sum of alpha and beta powers divided by the sum of delta and theta powers) [40] or alpha/theta ratio [43].

3.2. Test-Retest Effect. According to consecutive reports EEG frequency parameters are stable over time. Gasser et al. (1985) were amongst the first to address the issue of test-retest reliability of EEG parameters [62]. They reported that alpha electrical activity of the brain cortex showed the best reliability and delta and beta activity had the worst reliability. Dustman et al. (1999) investigated the variability of absolute and relative powers in five frequency bands, delta, theta, alpha, beta, and gamma, over the interval of 6 months in a sample of 222 males aged from 4 to 90 years [63]. Age-related dependence of the parameters was identified, but the frequency markers, especially power in the alpha band, showed a satisfactory reliability over time. Later, Näpflin et al. (2007), in the above-mentioned study, replicated these results in healthy adults [61].

Additionally, the EEG frequency markers are not influenced by cognitive activity. Grandy et al. (2013) investigated the modifiability of the alpha frequency of healthy subjects before and after a series sessions of cognitive tasks [64]. Cognitive tasks had no significant effects on the resting state peak alpha frequency 7.5–12.5 Hz.

3.3. Influence of Dopamine-Replacement Therapy on QEEG Parameters. The effects of levodopa and dopaminergic medication on the EEG activity of the patients yielded ambiguous results: while some researchers reported that patients in a medicated and a nonmedicated state revealed no influence of dopamine-replacement therapy on frequency characteristics [49, 65], various other studies reported that levodopa treatment of PD induces an increase in alpha and beta bands and a decrease of theta and delta bands. These latter changes are referred to as "activation" of EEG [66].

George et al. (2013) analyzed the EEG power spectra and connectivity in nondemented PD patients in ON- and OFF-medication state, in both resting state and during a cognitive task [67]. These results were compared to those of a group of healthy controls. No significant changes in powers were identified in relation to medication. Despite that fact, the authors showed that dopaminergic medication reduced...
the pathological synchronization in the beta band in the resting state and induced task-related increase of beta power. These findings were consistent with the previous reports [50, 68]. According to other researchers levodopa treatment has influence on functional brain connectivity assessed by MEG and these changes were mostly identified in beta frequency range [69]. Therefore, studies of beta activity require adjustments according to dopaminergic stimulation while data with alpha and theta activity is probably largely independent from dopaminergic influence.

4. Spectral Characteristics of Cognitive States in PD

4.1. Global Power Spectra. Seventeen studies focused on spectral features of cognitive states in PD. Six of these 17 studies focused on the capacity of discrimination between better and worse states of cognition in PD (e.g., group of patients with PD-MCI versus group with PD patients with normal cognition (PD-NC) or group with PD-MCI versus group with PD-D) [35, 36, 42, 43, 46, 48] (Table 2). Global delta and theta powers (these variables were increased in PD-D patients) and peak background frequency (decreased in PD-D patients) had the largest effect sizes to discriminate PD-NC versus PD-D. Global delta power (increased in PD-D patients), peak background frequency, and global alpha power (decreased in PD-D patients) had the largest effect sizes to distinguish PD-MCI versus PD-D. Additionally, beta peak frequency was significantly increased ($p < 0.01$), and global alpha power and alpha/theta ratio were significantly decreased ($p < 0.01$ and $p < 0.01$) in PD-D versus PD-MCI in one report (although original data was not available) [43]. Global alpha power, peak background frequency (decreased in PD-MCI patients), and global theta power (increased in PD-MCI patients) had the largest effect sizes to discriminate PD-NC versus PD-MCI.

Patients with PD-D were compared to PD patients without dementia in two studies [42, 48]. The latter group might include both PD-NC and PD-MCI. However, global delta and theta powers (increased in PD-D patients) had the largest effect sizes. In one study, two groups of patients with PD-D, with cognitive fluctuations (CF) and without CF, were compared by the analysis of the compressed spectral arrays (CSA) [36]. CF are described as disorders of consciousness ranging from reduced arousal to stupor; CF indicate a worse state of dementia [56]. CSA is a method of epoch-to-epoch QEEG representation for each derivation, CSA provide information on various QEEG parameters like spectral powers, dominant frequency (DF), mean frequency where the maximal power is represented in the sum of all epochs, DF variability (DFV) across all analyzed epochs, and other parameters. Global alpha and prealpha (5.6–7.9 Hz) powers had the largest effect sizes: alpha was decreased and “prealpha” was increased in patients with PD-D and CF.

4.2. Topographic Distribution of Power Spectra. Topographic distribution of spectral powers was addressed in 7 studies [36–38, 40, 46, 48, 51]. Theta and alpha powers in temporal and parietal regions bilaterally had the largest effect sizes to distinguish between PD-NC and PD-D patients. Theta power was increased and alpha power decreased in PD-D patients. Spectral ratio (sum of alpha and beta powers divided by the sum of delta and theta powers) in frontal regions and delta and alpha powers in posterior derivations had the largest effect sizes to distinguish between PD-MCI and PD-D. Delta power was increased and alpha power and spectral ratio were decreased in PD-D patients. Theta and beta powers and spectral ratio in posterior derivations had the largest effect sizes to distinguish between PD-NC and PD-MCI. Theta power was increased and alpha power was decreased in PD-MCI patients. In one study PD patients with executive dysfunction were compared to PD patients without executive dysfunction [38]. The largest effect size had spectral ratio in frontal derivations; spectral ratio was decreased in patients with executive dysfunction. Additionally, in one study PD-D patients were compared with PD without dementia [48]. The largest effect sizes had alpha and delta powers in temporal, parietal, and occipital regions and beta and delta powers in central regions, and beta, alpha, and delta powers in frontal regions. Delta power was increased, and alpha and beta powers were decreased in PD-D patients. Finally, prealpha, DF, and DFV in frontal, temporal, and parietooccipital derivations had the largest effect size for distinguishing PD-D patients without CF from PD-D patients with CF [36]. Prealpha and DFV were increased and DF was decreased in patients with PD-D and CF.

4.3. Correlation of Power Spectra with Cognitive Assessment Tools. Correlation of spectral powers with different cognitive assessment tools and tests was analyzed in 7 studies [35, 39, 40, 45, 47, 48, 50]. The details are presented in Table 3. The mostly used tool for cognitive assessment in these studies was the MMSE. Positive Fisher’s $Z$ was observed for Mini-Mental State Examination (MMSE) and spectral ratios at all scalp locations, relative power in the range 8–13 Hz (alpha), and peak background frequency, while negative Fisher’s $Z$ was observed for MMSE and relative power in the range 0–4 Hz (delta). Negative Fisher’s $Z$ was observed for Cambridge Cognitive Examination (CAMCOG) and relative power in the range 4–8 Hz (theta) in bilateral occipital and right temporal regions. Additionally, in one study, correlation of median frequency with cognitive domains was investigated [47]. Significant correlations were observed for “episodic and long term memory domain,” followed by “overall cognitive score,” “fluency domain,” “attention domain,” and “executive functions domain.” In one study no correlation of absolute power spectra with neuropsychiatric inventory was reported in nondemented PD patients [45]. Additionally, longitudinal correlation of frequency results with cognitive states in PD using tools for cognitive assessment was assessed in 3 studies [36, 44, 52]. In the first study [36], correlation with Frontal Assessment Battery scores was investigated: negative Fisher’s $Z$ was observed for power in the range 8–12 Hz (alpha) and positive Fisher’s $Z$ for powers in the range 4–8 Hz (theta), over 2 years [36]. In another study [52], various tools for cognitive assessment correlated with power spectra over 7 years of observation: negative Fisher’s $Z$ was observed: for global relative powers (GRP) in the range
Table 2: EEG and MEG spectral markers which significantly discriminated between cognitive states in PD.

| Author(s)          | Diagnostic groups of patients with PD (N) | Mean age (years) | Evaluative tests: cognitive pathology (criteria) | Parameter(s) showed significant difference between the groups with PD | Effect size (95% CI) |
|--------------------|-------------------------------------------|------------------|-------------------------------------------------|---------------------------------------------------------------------|---------------------|
| Bosboom et al. 2006⁴⁸ | PD-D (13) PD-wD (13)                       | 74.4 71.7        | Dementia (DSM-IV)                              | GRP delta (0.5–4 Hz) and GRP theta (4–8 Hz)                         | PD-wD versus PD-D 1.47 (0.60, 2.34) |
|                    |                                           |                  |                                                 | GRP alpha (8–13 Hz) and GRP beta (13–30 Hz)                        | PD-wD versus PD-D −1.47 (−2.34, −0.60) |
|                    |                                           |                  |                                                 | GRP gamma (30–48 Hz)                                               | PD-wD versus PD-D −1.47 (−2.34, −0.60) |
| Caviness et al. 2007³⁵ | PD-D (8) PD-MCI (16) PD-NC (42)           | 78.0 80.4 74.6   | Dementia (DSM-IV); MCI (Petersen 2004 [55])    | GRP delta (1.5–3.9 Hz)                                             | PD-NC versus PD-MCI 0.11 (−0.47, 0.68) |
|                    |                                           |                  |                                                 | GRP theta (4–7.9 Hz)                                               | PD-MCI versus PD-D 1.27 (0.35, 2.19) |
|                    |                                           |                  |                                                 | GRP alpha (8–12.9 Hz)                                             | PD-NC versus PD-MCI 0.75 (0.16, 1.34) |
|                    |                                           |                  |                                                 | GRP beta1 (13–19.9 Hz)                                            | PD-MCI versus PD-D 0.38 (−0.46, 1.24) |
|                    |                                           |                  |                                                 | GRP beta2 (20–30 Hz).                                             | PD-NC versus PD-D 1.37 (0.57, 2.17) |
|                    |                                           |                  |                                                 | Peak frequency at locations P3, P4, and Oz                        | PD-MCI versus PD-D 1.21 (0.57, 1.91) |
| Bonanni et al. 2008³⁶ | PD-DnF (19) PD-DF (16)                     | 70.0⁴⁸           | PD-D (history of PD preceded dementia for at least 24 months); cognitive fluctuations (CAF, Walker et al. 2000 [56]) | GRP theta (4.0–5.5 Hz)                                             | PD-DnF versus PD-DF 2.82 (1.88, 3.75) |
|                    |                                           |                  |                                                 | GRP prealpha (5.6–7.9 Hz)                                         | PD-DnF versus PD-DF 5.26 (3.86, 6.67) |
|                    |                                           |                  |                                                 | GRP alpha (8.0–12.0 Hz)                                           | PD-DnF versus PD-DF −8.40 (−10.47, −6.32) |
|                    |                                           |                  |                                                 | Mean frequency                                                    | PD-DnF versus PD-DF −0.93 (−1.64, −0.24) |
|                    |                                           |                  |                                                 | DF in parietooccipital derivations                                | PD-DnF versus PD-DF −1.18 (−1.90, −0.46) |
|                    |                                           |                  |                                                 | DFV in parietooccipital derivations                               | PD-DnF versus PD-DF 1.19 (0.47, 1.91) |
field synchronization (GBS) was addressed in one study and coherence in another one. Patients with PD-D were compared with PD patients without dementia in both studies. PD-D patients had significantly higher GBS in theta frequency range ($\rho < 0.02$) and lower GBS in the alpha 1 range ($\rho < 0.02$) [41]; higher frontal interhemispheric (F3-F4) and higher frontooccipital intrahemispheric (F3-O1; F4-O2) coherence in the beta frequency band was observed in another study [42].

In two studies SL was investigated. In one study correlation of connectivity markers with cognitive tests in PD patients without dementia and with varying disease duration was investigated [50]. Higher level of perseveration executive task in patients with recently diagnosed PD (in the last 6 months before participation in the study) was associated with increased interhemispheric SL in alpha 1 band. In an exploratory study by Bosboom et al. (2009) PD-D patients were compared to nondemented PD patients [27]. Patients with PD-D had lower interhemispheric SL between temporal regions (frequency ranges: 0.5–4 Hz, 4–8 Hz and 8–10 Hz) and parietal regions (30–48 Hz); lower intrahemispheric SL between frontal and temporal and frontal and parietal regions in the left hemisphere (8–15 Hz) and frontal and temporal regions in the right hemisphere (8–15 Hz and 13–30 Hz). At the same time, higher intrahemispheric SL was found between occipital and temporal and occipital and parietal regions in the left hemisphere (15–30 Hz) and between parietal and occipital regions in the right hemisphere (8–10 Hz).

Phase lag index (PLI) was investigated in two studies. A comparison of PD-D patients with nondemented PD patients showed weaker PLI in frontotemporal (0.5–4 Hz) and parietotemporooccipital (8–13 Hz) couplings in demented

### Table 2: Continued.

| Author(s) | Diagnostic groups of patients with PD (N) | Mean age (years) | Evaluative tests: cognitive pathology (criteria) | Parameter(s) showed significant difference between the groups with PD | Effect size (95% CI) |
|-----------|------------------------------------------|------------------|-----------------------------------------------|-------------------------------------------------|----------------------|
| Fonseca et al. 2013 [42] | PD-D (12) | 70.3 | Dementia (Dubois et al. 2007 [57]) | Mean absolute power delta (0.8–3.9 Hz) | PD-D versus PD-D 0.85 (0.16, 1.54) |
| | PD-wD (31) | 68.1 | | Mean absolute power theta (4.29–7.8 Hz) | PD-wD versus PD-D 1.23 (0.52, 1.94) |
| Bousleiman et al. 2014 [46] | PD-MCI (41) | 67.2 | MCI (Litvan et al. 2012 [58]). | GRP alpha 1 (8–10 Hz) | PD-MCI versus PD-MCI 0.82 (−0.131, −0.001) |
| | PD-NC (12) | | | Beta (13–30 Hz) peak frequency | PD-MCI versus PD-D 1.10 (0.27, 1.92) |
| Ku et al. 2016 [43] | PD-D (9) | 56.7 | Dementia (DSM-IV); MCI (Petersen 2004 [55]) | GRP alpha (8–13 Hz) | PD-MCI versus PD-D 1.10 (−1.92, −0.27) |
| | PD-MCI (17) | 62.1 | | alpha/theta ratio: alpha (8–13 Hz) divided by theta (4–7 Hz) | PD-MCI versus PD-D 1.10 (−1.92, −0.27) |

a Original data not available, effect size and confidence intervals estimated using $p$ value conversion.

b The study is longitudinal; only assessment on admission is shown in this table.

c Age for groups of the patients is not available; age of the combined sample is shown.

d Mean age not available, mean age calculated from median and range (Hozo et al. 2005 [59]).

EAF: Clinical Assessment of Fluctuations; DF: dominant frequency; DFV: dominant frequency variability; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders IV; GRP: global relative power; MCI: mild cognitive impairment; PD: Parkinson’s disease; PD-NC: Parkinson’s disease without cognitive impairment; PD-MCI: Parkinson’s disease with mild cognitive impairment; PD-D: Parkinson’s disease with dementia; PD-wD: Parkinson’s disease without dementia; PD-DnF: Parkinson’s disease with dementia without cognitive fluctuations; PD-DF: Parkinson’s disease with dementia with cognitive fluctuations.

0.5–4 Hz (delta) and CAMCOG and Spatial Span Test (SSP); for GRP in the range 4–8 Hz (theta) and CAMCOG, Pattern Recognition Memory (PRM), Semantic Fluency Test, and Spatial Span Test; for GRP in the range 8–10 Hz (alpha 1) and Spatial Working Memory (SWM), Positive Fisher’s Z was observed: for powers in the range 8–13 Hz (alpha 1 and alpha 2) and 30–48 Hz (gamma) and CAMCOG, PRM, and SSP; for powers in the range 4–8 Hz (theta) and SWM [45]. In the third study [44], correlation with power in the range 2.5–4 Hz (delta) was investigated: negative Fisher’s Z was observed for MMSE, Rey Auditory Verbal Learning, Controlled Oral Word Association Test and Stroop, while positive Fisher’s $Z$ was observed for Clinical Dementia Rating Sum of Boxes and Functional Assessment Staging Tool.

#### 4.4. Hazard of Conversion to PD-D.

The relation of power spectra to conversion to PD-D was examined in 3 studies [9, 43, 54]. The details are presented in Table 4. Hazard ratios of conversion to PD-D were analyzed in 2 studies. The hazard ratio of conversion to PD-D was significantly higher for patients with background EEG frequency below the median value of the entire sample at baseline [9] and the theta power above the median value of the entire sample at baseline [54]. In one study, patients with PD-MCI who converted to PD-D over two years had increased beta peak frequency and decreased alpha relative power and alpha/theta ratio at baseline [43].

#### 5. Brain Functional Connectivity and Cognitive States in PD

Seven studies focused on functional connectivity features of cognitive states in PD [27, 33, 41, 42, 50–52]. Global
| Author(s)                  | Age, mean | N                  | Correlation                                                                 | Fisher’s Z (95% CI) |
|---------------------------|-----------|--------------------|------------------------------------------------------------------------------|--------------------|
| Bosboom et al. 2006 [48]   | 71.7      | 13 PD-wD patients | Left occipital theta (4–8 Hz) versus CAMCOG                               | −0.70 (−1.32, 0.08) |
|                           |           |                    | Right occipital theta (4–8 Hz) versus CAMCOG                               | −0.67 (−1.29, 0.05) |
|                           |           |                    | Right temporal theta (4–8 Hz)                                               | −0.68 (−1.30, 0.06) |
| Caviness et al. 2007 [35]  | 76.4      | 66 PD-wD patients  | GRP delta (1.5–3.9 Hz) versus MMSE                                         | −0.51 (−0.76, −0.26) |
|                           |           |                    | GRP alpha (8–12.9 Hz) versus MMSE                                          | 0.34 (0.10, 0.59)  |
|                           |           |                    | Peak background frequency versus MMSE                                       | 0.42 (0.18, 0.67)  |
| Stoffers et al. 2008 [50]  | 59.4      | 18 de novo PD patients | Relative low alpha (8–10 Hz) versus redundancy of the second order         | −0.11 (−0.19, −0.01) |
|                           |           |                    | (Vienna perseveration) in bilateral central and parietal regions             |                    |
|                           |           |                    | Spectral ratio (SR) at Fp location (electrode positions Fp1 and Fp2) versus| 0.30 (0.10, 0.50)  |
|                           |           |                    | MMSE                                                                         |                    |
|                           |           |                    | SR at F location (electrode positions F3, F4, F7, and F8) versus MMSE       | 0.32 (0.12, 0.52)  |
|                           |           |                    | SR at C location (electrode positions C3 and C4) versus MMSE                | 0.28 (0.08, 0.48)  |
|                           |           |                    | SR at P location (electrode positions P3 and P4) versus MMSE                | 0.32 (0.12, 0.52)  |
|                           |           |                    | SR at T location (electrode positions T3, T4, T5, and T6) versus MMSE       | 0.32 (0.12, 0.52)  |
|                           |           |                    | SR at O location (electrode positions O1 and O2) versus MMSE                | 0.35 (0.16, 0.55)  |
| Morita et al. 2011 [40]    | 67.6      | 100 PD patients    | Relative alpha1 (8–10.5 Hz) in parietal regions (Brodmann areas 5, 7, 30, 40,43) versus MMSE | 0.35 (−0.27, 0.97) |
|                           |           |                    | Relative alpha1 (8–10.5 Hz) in occipital regions (Brodmann areas 5, 7, 30, 40,43) versus MMSE | 0.44 (−0.18, 1.05) |
|                           |           |                    | No significant correlation with any marker                                   |                    |
| Babiloni et al. 2011 [39]  | 72.0      | 13 PD-D patients   | Absolute power: delta (0.8–3.9 Hz), theta (4.29–7.8 Hz), alpha (8.2–12.5 Hz), and beta (12.9–36.3 Hz) versus neuropsychiatric inventory |                    |
|                           |           |                    | Median frequency versus episodic and long term memory cognitive domain (CD$^b$) | 0.60 (0.31, 0.90)  |
|                           |           |                    | Median frequency versus overall cognitive score$^c$                        | 0.51 (0.22, 0.80)  |
|                           |           |                    | Median frequency versus fluency CD                                          | 0.41 (0.12, 0.70)  |
|                           |           |                    | Median frequency versus attention CD                                       | 0.39 (0.10, 0.68)  |
|                           |           |                    | Median frequency versus executive functions CD                            | 0.35 (0.06, 0.65)  |

Original data not available in the publications. Fisher’s Z calculated from correlation coefficient and sample size (Lipsey and Wilson, 2001 [60]).

$^a$Sum of absolute power values for alpha (8.20–12.89 Hz) and beta (13.28–30.8 Hz); waves divided by the sum of absolute power values for delta (1.17–3.91 Hz) and theta (4.3–7.81 Hz).

$^b$Parameter, which includes a set of cognitive tests from a specific cognitive category, for example, memory and attention.

$^c$Parameter, which includes an average of 26 cognitive tests from all cognitive domains.

CAMCOG: Cambridge Cognition Examination; GRP: global relative power; MMSE: Mini-Mental State Examination; PD-D: Parkinson’s disease with dementia; PD-wD: Parkinson’s disease without dementia.

In this study, general region-to-region connectivity was stronger in theta band and weaker in delta, alpha, and beta bands in PD-D. A longitudinal observation of initially nondemented PD patients showed correlation of worsening of CAMCOG performance with a decrease of PLI in frontal and temporal regions in frequency range 8–10 Hz [53]. Finally, a graph theory analysis of longitudinal connectivity changes of nondemented PD patients was performed in one study [33]. Worsening of cognitive performance over time correlated with increase in eccentricity in the frequency range 8–10 Hz and decrease of clustering coefficient and path length in the frequency range 4–8 Hz.

### 6. Conclusions

The results of this review support the idea that spectral and connectivity markers have a significant impact in discriminating PD patients with different levels of cognitive decline.
Table 4: Prediction of progression to dementia in Parkinson's disease with spectral EEG markers.

| Author(s)                  | Number of subjects, duration of observation after baseline EEG/MEG | Incidence of PD-D                                                                 | Significant QEEG risk factor(s)                                                                 |
|----------------------------|------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Klassen et al. 2011 [9]    | $N = 106$ PD-wD patients, 0.3 to 8.8 (mean 3.3) years            | Incidence within 5 years by Kaplan-Meier method was 34%                         | Hazard ratios: background rhythm frequency $<$ median (8.5) was 13.0; theta power $>$ median (19.0) was 3.0 |
| Gu et al. 2016 [43]        | $N = 17$ PD-MCI and 9 PD-D patients, 2 years                     | 35% (6 PD-MCI patients progressed to PD-D patients)                              | Increase of the beta peak frequency and decrease of alpha relative power and alpha/theta ratio correlated with progression to PD-D; PPV of the combined marker was 62, and PLR was 4.4 |
| Olde Dubbelink et al. 2014 [54] | $N = 63$ PD-wD patients, 7 years                               | 30% (19 patients)                                                              | Hazard ratios: beta power $<$ median (27.96) was 5.21; peak frequency $<$ median (8.39) was 3.97; theta power $>$ median (22.85) was 2.82 |

PD-D: Parkinson’s disease with dementia; PD-MCI: Parkinson’s disease with mild cognitive impairment; PD-wD: Parkinson’s disease without dementia; PPV: positive predictive value; PLR: positive likelihood ratio.

regardless of the variety of approaches to calculate these markers. To summarize, a slowing of EEG frequencies correlates with a decline of cognition. Accordingly, an increase of spectral powers in the “slow” frequency bands $<$8 Hz (delta and theta) and a decrease in the “fast” frequency bands $>$8 Hz (alpha, beta, and, less significantly, gamma) are spectral markers of PD-related cognitive decline. Topographically, occipital, parietal, and temporal regions show the higher significance.

Additionally, the above-mentioned spectral markers showed significant hazard ratio in predicting conversion of nondemented PD patients to PD-D. Patients with spectral powers in “fast” waves below and in “slow” waves above the median values have significantly higher risk of developing PD-D within 2 to 7 years.

The connectivity patterns of the PD patients with cognitive impairment show changes in the same frequency ranges, where spectral markers of cognitive decline are identified: mostly in theta (4–8 Hz), alpha 1 (8–10 Hz), and beta (13–30 Hz) ranges. The connectivity patterns of PD patients with cognitive decline changed in frontal, temporal, parietal, and occipital regions. However, the number of connectivity studies focusing on cognitive states of PD patients is still very small; by the same token the studies had different setting and various connectivity markers were investigated. A common trend of cognitive decline in PD seems to be a decrease of connectivity in parietotemporooccipital regions.

In sum, changes in spectral powers, delta and theta, have the highest significance to discriminate between PD-D and dementia-free patients with PD, while changes in spectral powers, theta and alpha, have the highest significance to separate MCI from normal cognition in PD. Findings regarding discrimination between MCI and dementia in PD are less consistent within reports, though delta and beta powers showed good discriminative capacity. With regard to connectivity measures, PLI has the highest significance to discriminate between PD-D and nondemented patients with PD.

Importantly, changes of spectral QEEG markers precede the clinical manifestation of cognitive decline in PD, as was shown in longitudinal studies. Thus, these markers may become a valuable aid for timely selection of patients prone to pharmacological and nonpharmacological interventions of prevention at a very early stage of PD and thereby potentially improve clinical results.

Prospective studies with larger cohorts investigating topographical scalp distribution of QEEG changes as well as connectivity and its association with cognitive decline in PD are warranted. These studies will result in biomarkers that are likely to contribute to individualized counselling and treatment of patients.

7. Limitations of This Review

This review has several limitations. First, there is no common opinion regarding which certain markers can be used to predict cognitive decline in PD. By virtue of various fast developing methods and approaches, different research groups investigate different methods: spectral markers, connectivity markers, or their combination. In these conditions a thorough comparison of QEEG markers remains a challenge. However, future methods might further improve the validity of QEEG biomarkers of cognitive decline in PD.

Second, criteria for the diagnosis of PD-MCI are changing over time [12, 55]. In some studies a simple cognitive screening is performed using Mini-Mental State Examination tool; in other cases a full cognitive assessment is performed with
many cognitive tests. Since 2012 the Movement Disorders Society Task Force guidelines set a common criteria for PD-MCI [58]; however, the Diagnostic and Statistical Manual of Mental Disorders fifth edition has replaced the term MCI by “neurocognitive impairment” in 2013 [70].

In sum, while differentiation between patients with PD with an intact cognitive state and patients with PD-D could be performed more or less clearly using QEEG markers, identification of the borderline level of cognition is relatively difficult.

Competing Interests
Vitalii V. Cozac received grant from Camelia Botnar Foundation; Ute Gschwandtner received grants from the Parkinson Schweiz, Jacques and Gloria Gossweiler Foundation, Freiwillige Akademische Gesellschaft Basel, Gottfried und Julia Bangerter-Rhyner Foundation, the Swiss National Science Foundation, Camelia Botnar Foundation, and Hedwig Widmer Foundation and unrestricted grants from UCB Pharma AG, Abbvie AG, and General Electrics; Florian Hatz received grant from Freiwillige Akademische Gesellschaft Basel; Martin Hardmeier has nothing to disclose; Stephan Rüegg has nothing to disclose; Peter Fuhr received grants from Parkinson Schweiz, Jacques and Gloria Gossweiler Foundation, Freiwillige Akademische Gesellschaft Basel, Gottfried und Julia Bangerter-Rhyner Foundation, the Swiss National Science Foundation, the Swiss Multiple Sclerosis Society, Camelia Botnar Foundation, and Hedwig Widmer Foundation and unrestricted grants from UCB Pharma AG, Roche AG, Abbvie AG, General Electrics, and Advisory Board: Biogen Inc.

References

[1] R. L. Rodnitzky, “Cognitive impairment and dementia in Parkinson disease,” in UpToDate, S. T. DeKosky and A. F. Eichler, Eds., 2015, http://www.uptodate.com/contents/cognitive-impairment-and-dementia-in-parkinson-disease.

[2] D. Aarsland, J. Zaccai, and C. Brayne, “A systematic review of prevalence studies of dementia in Parkinson’s disease,” Movement Disorders, vol. 20, no. 10, pp. 1255–1263, 2005.

[3] O. Riedel, J. Klotsche, A. Spottek et al., “Cognitive impairment in 873 patients with idiopathic Parkinson’s disease. Results from the German Study on Epidemiology of Parkinson’s Disease with Dementia (GEPAD),” Journal of Neurology, vol. 255, no. 2, pp. 253–264, 2008.

[4] J. W. Kim, S. M. Cheon, M. J. Park, S. Y. Kim, and H. Y. Jo, “Cognitive impairment in Parkinson’s disease without dementia: subtypes and influences of age,” Journal of Clinical Neurology, vol. 5, no. 3, pp. 133–138, 2009.

[5] M. A. Hely, W. G. J. Reid, M. A. Adena, G. M. Halliday, and J. G. L. Morris, “The Sydney multicenter study of Parkinson’s disease: the inevitability of dementia at 20 years,” Movement Disorders, vol. 23, no. 6, pp. 837–844, 2008.

[6] C. H. Williams-Gray, A. Goris, M. Saiki et al., “Apolipoprotein e genotype as a risk factor for susceptibility to and dementia in Parkinson’s disease,” Journal of Neurology, vol. 256, no. 3, pp. 493–498, 2009.

[7] B. Mollenhauer, L. Rochester, A. Chen-Plotkin, and D. Brooks, “What can biomarkers tell us about cognition in Parkinson’s disease?” Movement Disorders, vol. 29, no. 5, pp. 622–633, 2014.

[8] C.-H. Lin and R.-M. Wu, “Biomarkers of cognitive decline in Parkinson’s disease,” Parkinsonism and Related Disorders, vol. 21, no. 5, pp. 431–443, 2015.

[9] B. T. Klassen, J. G. Hentz, H. A. Shill et al., “Quantitative EEG as a predictive biomarker for Parkinson disease dementia,” Neurology, vol. 77, no. 2, pp. 118–124, 2011.

[10] B. Winblad, K. Palmer, M. Kivipelto et al., “Mild cognitive impairment—beyond controversies, towards a consensus: report of the international working group on mild cognitive impairment,” Journal of Internal Medicine, vol. 256, no. 3, pp. 240–246, 2004.

[11] K. Palmer and B. Winblad, “Mild cognitive impairment: continuing controversies,” Nature Clinical Practice. Neurology, vol. 3, no. 2, article E1, 2007.

[12] M. Ganguli, B. E. Snitz, J. A. Saxton et al., “Outcomes of mild cognitive impairment by definition: a population study,” Archives of Neurology, vol. 68, no. 6, pp. 761–767, 2011.

[13] K. Kelley and K. J. Preacher, “On effect size,” Psychological Methods, vol. 17, no. 2, pp. 137–152, 2012.

[14] N. J. Cox, “Speaking Stata: correlation with confidence, or Fisher’s z revisited,” Stata Journal, vol. 8, no. 3, pp. 413–439, 2008.

[15] M. Nuwer, “Assessment of digital EEG, quantitative EEG, and EEG brain mapping: report of the American Academy of Neurology and the American Clinical Neurophysiology Society,” Neurology, vol. 49, no. 1, pp. 277–292, 1997.

[16] N. V. Thakor and S. Tong, “Advances in quantitative electroencephalogram analysis methods,” Annual Review of Biomedical Engineering, vol. 6, pp. 453–495, 2004.

[17] F. Lopes da Silva, “EEG and MEG: relevance to neuroscience,” Neuron, vol. 80, no. 5, pp. 1112–1128, 2013.

[18] N. K. Al-Qazzaz, S. H. B. M. Ali, S. A. Ahmad, K. Chellappan, M. S. Islam, and J. Escudero, “Role of EEG as biomarker in the early detection and classification of dementia,” The Scientific World Journal, vol. 2014, Article ID 906038, 16 pages, 2014.

[19] H. Kantz and T. Schreiber, Nonlinear Time Series Analysis, Cambridge University Press, Cambridge, UK, 2nd edition, 2004.

[20] B. Schelter, M. Winterhalder, and J. Timmer, Eds., Handbook of Time Series Analysis: Recent Theoretical Developments and Applications, Wiley-VCH, Weinheim, Germany, 2006.

[21] K. J. Blinowska, “Review of the methods of determination and classification of dementia,” The Scientific World Journal, vol. 2014, Article ID 906038, 16 pages, 2014.

[22] E. Niedermeyer and F. H. Lopes da Silva, Eds., Electroencephalography: Basic Principles, Clinical Applications, and Related Fields, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 5th edition, 2005.

[23] K. A. Otto, “EEG power spectrum analysis for monitoring depth of anaesthesia during experimental surgery,” Laboratory Animals, vol. 42, no. 1, pp. 45–61, 2008.

[24] A. A. Fingelkurts, A. A. Fingelkurts, and S. Kähkönen, “Functional connectivity in the brain—is it an elusive concept?” Neuroscience and Biobehavioral Reviews, vol. 28, no. 8, pp. 827–836, 2005.

[25] S. Palva and J. M. Palva, “Discovering oscillatory interaction networks with M/EEG: challenges and breakthroughs,” Trends in Cognitive Sciences, vol. 16, no. 4, pp. 219–229, 2012.
[26] M. Rubinov and O. Sporns, "Complex network measures of brain connectivity: uses and interpretations," *NeuroImage*, vol. 52, no. 3, pp. 1059–1069, 2010.

[27] J. L. W. Bosboom, D. Stoffers, E. C. Wolters, C. J. Stam, and H. W. Berendse, "MEG resting state functional connectivity in Parkinson's disease related dementia," *Journal of Neural Transmission*, vol. 116, no. 2, pp. 193–202, 2009.

[28] J.-M. Schoffelen and J. Gross, "Source connectivity analysis with MEG and EEG," *Human Brain Mapping*, vol. 30, no. 6, pp. 1857–1865, 2009.

[29] T. Koenig, D. Lehmann, N. Saito, T. Kugiumi, T. Kinoshita, and M. Koukkou, "Decreased functional connectivity of EEG theta-frequency activity in first-episode, neuroleptic-naïve patients with schizophrenia: preliminary results," *Schizophrenia Research*, vol. 50, no. 1-2, pp. 55–60, 2001.

[30] T. Koenig, D. Studer, D. Hubl, L. Mele, and W. K. Strik, "Brain connectivity at different time-scales measured with EEG," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 360, no. 1457, pp. 1015–1023, 2005.

[31] C. J. Stam, G. Nolte, and A. Daffertshofer, "Phase lag index: assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources," *Human Brain Mapping*, vol. 28, no. II, pp. 1178–1193, 2007.

[32] D. J. Watts and S. H. Strogatz, "Collective dynamics of 'small-world' networks," *Nature*, vol. 393, no. 6684, pp. 404–406, 1998.

[33] K. T. E. Olde Dubbelink, A. Hillebrand, D. Stoffers et al., "Disrupted brain network topology in Parkinson's disease: a longitudinal magnetoencephalography study," *Brain*, vol. 137, no. 1, pp. 197–207, 2014.

[34] C. J. Stam, P. Tewarie, E. Van Dellen, E. C. W. van Straaten, A. Hillebrand, and P. Van Mieghem, "The trees and the forest: characterization of complex brain networks with minimum spanning trees," *International Journal of Psychophysiology*, vol. 92, no. 3, pp. 129–138, 2014.

[35] J. N. Caviness, J. G. Hentz, V. G. Evidente et al., "Both early and late cognitive dysfunction affects the electroencephalogram in Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 13, no. 6, pp. 348–354, 2007.

[36] L. Bonanni, A. Thomas, P. Tiraboschi, B. Perfetti, S. Varanese, and M. Onofri, "EEG comparisons in early Alzheimer’s disease, dementia with Lewy bodies and Parkinson's disease with dementia patients with a 2-year follow-up," *Brain*, vol. 131, no. 3, pp. 690–705, 2008.

[37] L. C. Fonseca, G. M. A. S. Tedrus, G. H. Letro, and A. S. Bossoni, "Dementia, mild cognitive impairment and quantitative EEG in patients with Parkinson's disease," *Clinical EEG and Neuroscience*, vol. 40, no. 3, pp. 168–172, 2009.

[38] S. Kamei, A. Morita, K. Serizawa, T. Mizutani, and K. Hirayanagi, "Quantitative EEG analysis of executive dysfunction in Parkinson disease," *Journal of Clinical Neurophysiology*, vol. 27, no. 3, pp. 193–197, 2010.

[39] C. Babiloni, M. F. De Pandis, F. Vecchio et al., "Cortical sources of resting state electroencephalographic rhythms in Parkinson's disease related dementia and Alzheimer's disease," *Clinical Neurophysiology*, vol. 122, no. 12, pp. 2355–2364, 2011.

[40] A. Morita, S. Kamei, and T. Mizutani, "Relationship between slowing of the EEG and cognitive impairment in Parkinson disease," *Journal of Clinical Neurophysiology*, vol. 28, no. 4, pp. 384–387, 2011.

[41] L. Pugnetti, F. Baglio, E. Farina et al., "EEG evidence of posterior cortical disconnection in PD and related dementias," *International Journal of Neuroscience*, vol. 120, no. 2, pp. 88–98, 2010.

[42] L. C. Fonseca, G. M. A. S. Tedrus, P. N. Carvas, and E. C. F. A. Machado, "Comparison of quantitative EEG between patients with Alzheimer’s disease and those with Parkinson’s disease dementia," *Clinical Neurophysiology*, vol. 124, no. 10, pp. 1970–1974, 2013.

[43] Y. Gu, J. Chen, Y. Lu, and S. Pan, "Integrative frequency power of EEG correlates with progression of mild cognitive impairment to dementia in Parkinson’s disease," *Clinical EEG and Neuroscience*, vol. 47, no. 2, pp. 113–117, 2016.

[44] J. N. Caviness, J. G. Hentz, C. M. Belden et al., "Longitudinal EEG changes correlate with cognitive measure deterioration in Parkinson’s disease," *Journal of Parkinson’s Disease*, vol. 5, no. 1, pp. 117–124, 2015.

[45] L. C. Fonseca, G. M. A. S. Tedrus, A. L. R. A. Rezende, and H. F. Giordano, "Coherence of brain electrical activity: a quality of life indicator in Alzheimer's disease?" *Arquivos de Neuro-Psiquiatria*, vol. 73, no. 5, pp. 396–401, 2015.

[46] H. Bousleiman, R. Zimmermann, S. Ahmed et al., "Power spectra for screening parkinsonian patients for mild cognitive impairment," *Annals of Clinical and Translational Neurology*, vol. 1, no. 11, pp. 884–890, 2014.

[47] R. Zimmermann, U. Gschwandtner, F. Hatz et al., "Correlation of EEG slowing with cognitive domains in nondemented patients with Parkinson's disease," *Dementia and Geriatric Cognitive Disorders*, vol. 39, no. 3-4, pp. 207–214, 2014.

[48] J. L. W. Bosboom, D. Stoffers, C. J. Stam et al., "Resting state oscillatory brain dynamics in Parkinson's disease: an MEG study," *Clinical Neurophysiology*, vol. 117, no. 11, pp. 2521–2531, 2006.

[49] D. Stoffers, J. L. W. Bosboom, J. B. Deijen, E. C. Wolters, H. W. Berendse, and C. J. Stam, "Slowing of oscillatory brain activity is a stable characteristic of Parkinson's disease without dementia," *Brain*, vol. 130, no. 7, pp. 1847–1860, 2007.

[50] D. Stoffers, J. L. W. Bosboom, E. C. Wolters, C. J. Stam, and H. W. Berendse, "Dopaminergic modulation of cortico-cortical functional connectivity in Parkinson's disease: an MEG study," *Experimental Neurology*, vol. 213, no. 1, pp. 191–195, 2008.

[51] M. M. Ponsen, C. J. Stam, J. L. W. Bosboom, H. W. Berendse, and A. Hillebrand, "A three dimensional anatomical view of oscillatory resting-state activity and functional connectivity in Parkinson's disease related dementia: an MEG study using atlas-based beamforming," *NeuroImage: Clinical*, vol. 2, no. 1, pp. 95–102, 2013.

[52] K. T. E. Olde Dubbelink, D. Stoffers, J. B. Deijen, J. W. R. Twisk, C. J. Stam, and H. W. Berendse, "Cognitive decline in Parkinson's disease is associated with slowing of resting-state brain activity: a longitudinal study," *Neurobiology of Aging*, vol. 34, no. 2, pp. 408–418, 2013.

[53] K. T. E. Olde Dubbelink, D. Stoffers, J. B. Deijen et al., "Resting-state functional connectivity as a marker of disease progression in Parkinson's disease: a longitudinal MEG study," *NeuroImage: Clinical*, vol. 2, no. 1, pp. 612–619, 2013.

[54] K. T. E. Olde Dubbelink, A. Hillebrand, J. W. R. Twisk et al., "Predicting dementia in Parkinson disease by combining neurophysiologic and cognitive markers," *Neurology*, vol. 82, no. 3, pp. 263–270, 2014.

[55] R. C. Petersen, "Mild cognitive impairment as a diagnostic entity," *Journal of Internal Medicine*, vol. 256, no. 3, pp. 183–194, 2004.
[56] M. P. Walker, G. A. Ayre, J. L. Cummings et al., “The clinician assessment of fluctuation and the one day fluctuation assessment scale: two methods to assess fluctuating confusion in dementia,” British Journal of Psychiatry, vol. 177, pp. 252–256, 2000.

[57] B. Dubois, D. Burn, C. Goetz et al., “Diagnostic procedures for Parkinson’s disease dementia: recommendations from the Movement Disorder Society Task Force,” Movement Disorders, vol. 22, no. 16, pp. 2314–2324, 2007.

[58] I. Litvan, J. G. Goldman, A. I. Tröster et al., “Diagnostic criteria for mild cognitive impairment in Parkinson’s disease: Movement Disorder Society Task Force guidelines,” Movement Disorders, vol. 27, no. 3, pp. 349–356, 2012.

[59] S. P. Hozo, B. Djulbegovic, and I. Hozo, “Estimating the mean and variance from the median, range, and the size of a sample,” BMC Medical Research Methodology, vol. 5, article 13, 2005.

[60] M. W. Lipsey and D. B. Wilson, Practical Meta-Analysis (Applied Social Research Methods), Sage, Thousand Oaks, Calif, USA, 2001.

[61] M. Näpflin, M. Wildi, and J. Sarnthein, “Test–retest reliability of resting EEG spectra validates a statistical signature of persons,” Clinical Neurophysiology, vol. 118, no. 11, pp. 2519–2524, 2007.

[62] T. Gasser, P. Bächer, and H. Steinberg, “Test-retest reliability of spectral parameters of the EEG,” Electroencephalography and Clinical Neurophysiology, vol. 60, no. 4, pp. 312–319, 1985.

[63] R. E. Dustman, D. E. Shearer, and R. Y. Emmerson, “Life-span changes in EEG spectral amplitude, amplitude variability and mean frequency,” Clinical Neurophysiology, vol. 110, no. 8, pp. 1399–1409, 1999.

[64] T. H. Grandy, M. Werkle-Bergner, C. Chicherio, F. Schmiedek, M. Lövden, and U. Lindenberger, “Peak individual alpha frequency qualifies as a stable neurophysiological trait marker in healthy younger and older adults,” Psychophysiology, vol. 50, no. 6, pp. 570–582, 2013.

[65] M. Moazami-Goudarzi, J. Sarnthein, L. Michels, R. Moukhtieva, and D. Jeanmonod, “Enhanced frontal low and high frequency power and synchronization in the resting EEG of parkinsonian patients,” NeuroImage, vol. 41, no. 3, pp. 985–997, 2008.

[66] A. Gironell, M. Barbanoj, P. Anderer et al., “EEG effects of levodopa in Parkinson’s disease,” Electroencephalography and Clinical Neurophysiology, vol. 103, article 199, 1997.

[67] J. S. George, J. Strunk, R. Mak-McCully, M. Houser, H. Poizner, and A. R. Aron, “Dopaminergic therapy in Parkinson’s disease decreases cortical beta band coherence in the resting state and increases cortical β band power during executive control,” NeuroImage: Clinical, vol. 3, pp. 261–270, 2013.

[68] P. Brown, ”Abnormal oscillatory synchronisation in the motor system leads to impaired movement,” Current Opinion in Neurobiology, vol. 17, no. 6, pp. 656–664, 2007.

[69] C. J. Stam, “Use of magnetoencephalography (MEG) to study functional brain networks in neurodegenerative disorders,” Journal of the Neurological Sciences, vol. 289, no. 1-2, pp. 128–134, 2010.

[70] J. R. Simpson, “DSM-5 and neurocognitive disorders,” Journal of the American Academy of Psychiatry and the Law, vol. 42, no. 2, pp. 159–164, 2014.