PRODROMAL PARKINSON'S DISEASE

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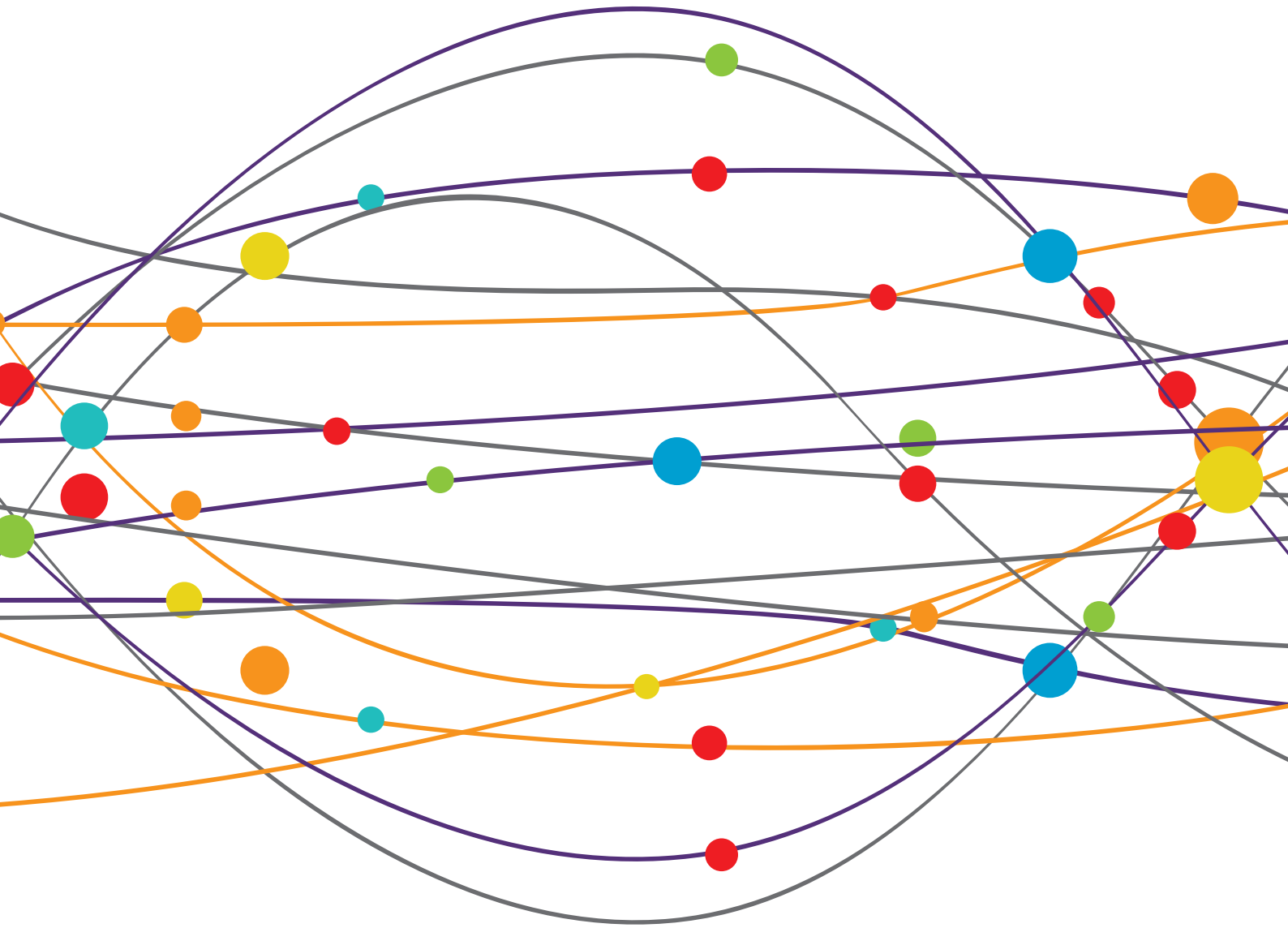

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PRODROMAL PARKINSON'S DISEASE

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EDITORIAL
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Editorial: Prodromal Parkinson's Disease

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Editorial on the Research Topic

Prodromal Parkinson's Disease

In the prodromal stage of Parkinson's disease (PD), cardinal motor symptoms (bradykinesia, rigidity, and resting tremor) have not yet developed and hence clinical diagnostic criteria for PD are not met (1, 2). However, neurodegeneration is already ongoing, and a variety of motor and non-motor symptoms may be identified. In addition to these clinical prodromal markers, recent studies have demonstrated a potentially important role of imaging, biofluid, and tissue markers in the characterization and identification of the prodromal stage of PD (3). An extensive and up-to-date overview of the status of these prodromal markers was included in the recent publication of the MDS research criteria for prodromal PD (2).

In this special issue of Frontiers in Neurology – Movement Disorders, a total of seven contributions add to the clinical and non-clinical aspects of prodromal PD, each targeting specific issues that need further exploration, but sometimes promising as potential biomarkers in the identification of prodromal PD or as treatment targets to tackle disease progression. Hustad and Aasly provide an overview of clinical, fluid, tissue, genetic, and imaging markers of prodromal PD. Clinical prodromal markers can include non-motor symptoms (hyposmia, REM sleep behavior disorder, constipation, excessive daytime sleepiness, depression, cognitive symptoms, autonomic nervous system dysfunction) of varying likelihood ratio to predict PD. Subtle motor signs in prodromal PD can be observed by the clinician or by using quantitative motor testing, however future wearable technologies will probably contribute to more detailed insights in these motor markers. Voice changes such as modulations in volume, pitch and tone can be detected in the early stage of PD, but it is still not entirely clear how vocal abnormalities could be used as a prodromal marker (4, 5). Similarly, alterations in auditory processing have been described in early stage PD, including difficulties with tone discrimination, and with perception of loudness and emotional aspects of speech. De Groote et al. provide a literature review of central auditory processing in early PD and point out which audiological and electrophysiological techniques could be explored to identify auditory prodromal markers.

The loss of muscle atonia during rapid-eye movement sleep (REM sleep without atonia or RSWA) and dream-enactment behaviors are the two characteristic features of REM sleep behavior disorder (RBD) (6). Idiopathic RBD is a highly specific marker for future development of a synucleinopathy (7, 8). Roguski et al. discuss the epidemiology, etiology, diagnostic considerations, and management of (idiopathic) RBD in a narrative literature review. The authors also provide ethical reflections and practical suggestions to guide the clinician in the difficult discussion of the prognosis of idiopathic RBD to asymptomatic, or potentially prodromal PD patients. Providing sufficient information, counseling and offering clinical follow-up visits to these individuals, without

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overloading them with extensive details about the possible risks, remains a balancing act for the clinician and researcher (9).

Different imaging modalities can contribute to define imaging biomarkers for prodromal PD, including single-photon-emission computed tomography, positron-emission tomography, magnetic resonance imaging (MRI) (10). Ellmore et al. have performed resting-stage functional MRI (fMRI) in RBD patients, PD patients, and control individuals to explore the substantia nigra functional connectivity and its relation to the serum uric acid levels. Lower levels of serum uric acid, are indeed associated with higher risk for PD in men. Catecholamine biofluid markers are interesting candidate markers for prodromal PD, given the noradrenergic, serotonergic and dopaminergic dysfunctions in prodromal and early PD as well as the recent "body first" vs. "brain first" concept of PD pathogenesis. Vermeiren et al. have reviewed the available evidence for the role of catecholamine biofluid markers in prodromal PD and provide recommendations for further exploration of extracellular vesicles as a potential novel biomarker.

Asymptomatic individuals carrying the G2019S mutation in *LRRK2* are at-risk to develop PD, with disease penetrance ranging from 25 to 42.5% by the age of 80 years, with a variable expression of non-motor symptoms including sleep dysfunction (11, 12). Longitudinal follow-up of these asymptomatic mutation carriers is helpful for defining the early stages of prodromal PD, as in a proportion of these individuals a phenoconversion to PD will be observed. Crown et al. have investigated potential prodromal features in a LRRK2-G2019S knock-in mouse model. The LRRK2 kinase inhibitor MLi-2 was administered in a subgroup of the mice. The results of this study, comparing the wild-type and G2019S knock-in populations, suggest alterations in behavioral and physiological aspects of sleep in the G2019S mice.

The ultimate goal of the accurate identification of individuals with prodromal PD is the administration of a neuroprotective treatment to these individuals to slow down or halt disease progression. Sportelli et al. discuss the association between diabetes mellitus type 2 and PD, and highlight the role of metformin as a potential disease-modifying treatment for PD. Preclinical evidence in animal models and clinical evidence in other age-related diseases suggest that metformin might delay aging-related symptoms and manifestations as well as offer anti-inflammatory activity.

Research in the field of prodromal PD is a fascinating and rapidly developing area, involving fundamental and translational as well as clinical and paraclinical aspects. Validation of biomarkers that allow to identify individuals at risk for developing clinical PD is a currently unmet need. Further developments in this area are urgently needed in order to manage the growing burden of PD.

AUTHOR CONTRIBUTIONS

DC, PS, and KRC wrote the first draft of the manuscript and critically revised the content of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Clinical and Imaging Markers of Prodromal Parkinson's Disease

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The diagnosis of Parkinson's disease (PD) relies on the clinical effects of dopamine deficiency, including bradykinesia, rigidity and tremor, usually manifesting asymmetrically. Misdiagnosis is common, due to overlap of symptoms with other neurodegenerative disorders such as multiple system atrophy and progressive supranuclear palsy, and only autopsy can definitively confirm the disease. Motor deficits generally appear when 50–60% of dopaminergic neurons in the substantia nigra are already lost, limiting the effectiveness of potential neuroprotective therapies. Today, we consider PD to be not just a movement disorder, but rather a complex syndrome non-motor symptoms (NMS) including disorders of sleep-wake cycle regulation, cognitive impairment, disorders of mood and affect, autonomic dysfunction, sensory symptoms and pain. Symptomatic *LRRK2* mutation carriers share non-motor features with individuals with sporadic PD, including hyposmia, constipation, impaired color discrimination, depression, and sleep disturbance. Following the assumption that the pre-symptomatic gene mutation carriers will eventually exhibit clinical symptoms, their neuroimaging results can be extended to the pre-symptomatic stage of PD. The long latent phase of PD, termed prodromal-PD, represents an opportunity for early recognition of incipient PD. Early recognition could allow initiation of possible neuroprotective therapies at a stage when therapies might be most effective. The number of markers with the sufficient level of evidence to be included in the MDS research criteria for prodromal PD have increased during the last 10 years. Here, we review the approach to prodromal PD, with an emphasis on clinical and imaging markers and report results from our neuroimaging study, a retrospective evaluation of a cohort of 39 participants who underwent DAT-SPECT scan as part of their follow up. The study was carried out to see if it was possible to detect subclinical signs in the preclinical (neurodegenerative processes have commenced, but there are no evident symptoms or signs) and prodromal (symptoms and signs are present, but are yet insufficient to define disease) stages of PD.

Keywords: Parkinson's disease, prodromal markers, LRRK2, DAT-SPECT, olfaction

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INTRODUCTION

Parkinson's disease (PD) is clinically defined by the presence of cardinal motor symptoms, bradykinesia in combination with at least one of rest tremor or rigidity (1). The cardinal motor symptoms depend upon progressive degeneration of the dopamine-containing neurons in the substantia nigra pars compacta (SNpc) (2). The histopathological

hallmark of PD is the presence of Lewy bodies (LBs), fibrillar aggregates in which α-synuclein is a major constituent (3). Pathological studies have shown a strong correlation between the extent of Lewy Body related cell loss in the Substantia Nigra (SN) and the severity of bradykinesia (4). Nigrostriatal dopaminergic damage can be monitored by functional neuroimaging techniques, such as positron emission tomography (PET) or single photon emission tomography (SPECT) (2).

During the last 25 years the clinical-pathological concept of PD has been challenged. Pathological studies estimate 40–60% loss of dopaminergic cells and reduction of synaptic function by up to 80% before the appearance of motor symptoms meeting current PD criteria appear (4). The Braak hypothesis posit the spread of Lewy pathology in a caudal to rostral pattern, suggesting early involvement of the peripheral autonomic nervous system (5).

According to current diagnostic criteria, PD is clinically diagnosed when disease progression is already advanced. This latent phase, which can vary from 5 to more than 20 years is called the prodromal phase of PD (6, 7). In this phase symptoms or signs of PD neurodegeneration are present, but a classic clinical diagnosis based on fully evolved motor parkinsonism is not yet possible (8). This phase represents an opportunity for earlier diagnosis, investigation of the pathophysiological cascade and when disease- modifying treatment become available, to possibly slow or prevent the onset of motor symptoms in PD (9–11). Patients in the prodromal phase constitute the ideal candidates to participate in trials of neuroprotective therapies because of their wide therapeutic window and lack of symptomatic therapies (6). Thus, identification of individuals in this phase is a clinical and research priority (6, 12). We are in need of biomarkers for the early diagnosis of PD. NIH Biomarkers Definitions Working Group defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" (13).

Ten years ago there were six known prodromal markers of PD present, rapid eye movement (REM) sleep behavior disorder, olfactory loss, constipation, depression and anxiety, erectile dysfunction and somnolence, none of which had more than two studies documenting diagnostic value (14). Today, because of an extensive research into prodromal PD, the number of markers with the sufficient level of evidence to be included in the MDS research criteria for prodromal PD, have increased and includes as well orthostatic hypotension, urinary dysfunction, possible subthreshold parkinsonism (UPDRS >3 excluding action tremor) / abnormal quantitative motor testing and clearly abnormal dopaminergic PET/SPECT (8, 14).

CLINICAL MARKERS

Non-motor Markers (Table 1)

Olfactory Loss

Hyposmia is one of the most common and best-characterized non-motor features and is often one of the earliest prodromal features to emerge (15, 16). The association of hyposmia with PD is widely accepted. About 80% of PD patients have impaired olfaction, which is in line with Braak's hypothesis of Lewy pathology in the olfactory bulb (Braak stage 1) (17). Hyposmia can be objectively quantified with standard tests such as 12-item Brief Smell Identification Test (B-SIT) (4, 18). However the likelihood of developing PD is unclear. In a recent review and meta-analysis investigating the association between hyposmia and PD, hyposmia was associated with a 3.84-fold risk of developing PD (16). In the Honolulu-Asia Aging Study, olfactory dysfunction was associated with an increased risk of PD; however, the association was significant only for the first 4 years of follow-up because of the lack of systematic measurements of smell in epidemiological studies (19, 20). Other investigators noted that, among first-degree relatives of patients with PD, olfactory dysfunction significantly correlated with development of PD within the subsequent 2–5 years (19). Ponsen et al. (21) found in their prospective cohort study of 361 non-parkinsonian, non-demented first-degree relatives of PD patients, that a two-step approach of initial olfactory testing followed by dopamine transporter (DAT)-SPECT scanning in individuals with hyposmia strongly increases specificity while retaining the high sensitivity associated with olfactory testing alone.

TABLE 1 | Clinical non-motor markers of prodromal Parkinson's disease.

Marker	References
Olfactory loss	(15–21)
Constipation	(8, 19, 22–27)
Rem sleep behavior disorder	(28–34)
Excessive daytime somnolence	(13, 36, 37)
Depression/anxiety	(38–45)
Global cognitive deficit	(46–49)
Orthostatic hypotension	(46, 50, 51)
Erectile dysfunction	(52–54)
Urinary dysfunction	(54, 55)

Constipation

Characterized by infrequent stools, difficult stool passage, or both, is one of the first, most common and disabling NMS to develop during the prodromal phase (22, 23). Recently constipation was included in both the research criteria for prodromal PD diagnostics as one of the risk factors for future development of PD (8, 22). Pathological alpha-synuclein inclusions can be detected in the entire gastrointestinal tract as early as 20 years before the diagnosis of PD, supporting the Braak proposed model for the pathophysiology of alpha-synuclein aggregates in PD (Braak stage 1) and making constipation one of the earliest recognizable prodromal features (22, 24). In a recent review and meta-analysis estimating the magnitude of association between premorbid constipation and later diagnosis of PD, constipation was associated with a 2.27-fold increased risk of developing PD, compared with someone without, and the increase in risk persists over a decade prior to diagnosis (25). Abbot et al. (26) found that after adjustment for confounders

men with fewer than 1 bowel movement per day had a 2.7-fold higher odds of developing PD compared to men with more frequent bowel movements In the Honolulu-Asia Aging Study cohort, the mean interval from bowel-movement abnormality to PD symptoms was 10 years (and it was 12 years to PD diagnosis) (19, 26). Gao et al. (27) found in their Health Professionals Follow-up Study and Nurse Health Study that infrequent bowel movements were associated with a higher future risk of PD in the next 6 years.

REM-Sleep Behavior Disorder

REM sleep behavior disorder (RBD) is a parasomnia characterized by dream-enacting behavior typically involving vocalizations or movements of the upper extremities, related to unpleasant dreams and loss of normal REM-sleep muscle atonia (28–30). RBD can be classified into an idiopathic form (iRBD) and a marker of prodromal neurodegeneration or a secondary form which occurs in patients already diagnosed with PD (31). RBD was first described in 1986 by Schenck et al. (29). Patient-reported questionnaires have been developed for identification of individuals with RBD, but similarly to patients with hyposmia, patients with RBD are often not aware of their symptoms. Accurate collateral history from a bed partner is usually necessary to make the diagnosis. In questionable cases or for individuals without bed partners, polysomnography can be obtained (29). Cohort studies indicate that iRBD convert to PD and other synucleinopathies such as dementia with Lewy bodies and multiple systems atrophy (28, 32). Pheno-conversion risk between 2 and 5 years is about 15–35%, and the risk may increase to 41% to 90.9% if extending the follow-up period up to 12–25 years, thus making iRBD to date the most specific clinical prodromal marker of PD (29, 33). When examining prodromal criteria as well as the independence of prodromal markers to predict conversion to PD or dementia with Lewy bodies, Fereshtehnejad et al. (34) found that diagnostic accuracy of the MDS research criteria for prodromal PD was high in the RBD population.

Excessive Daytime Somnolence

Excessive daytime somnolence (EDS) consists in the inability to maintain wakefulness during the day, with sleep occurring unintentionally or at inappropriate times (13). EDS is a well-known feature of advanced PD with a prevalence of 30–40% (35). Two published population-based studies looked at EDS as a potential prodromal symptom in PD. The first was the Honululu-Asia Aging study which report a 2.8-fold increased relative risk of developing PD in the future in men who reported a subjective sense of daytime sleepiness (36). The second a population based study (220,000 participants) found that those who reported having daytime napping of $\geq$1 h had a 1.5-fold increase risk of developing PD (37). In a recent study Abbot et al. (36) stained for α-synuclein (Lewy pathology) in multiple brain regions in a sample of 211 men and found that EDS was more common in the presence vs. absence of Lewy pathology ($p = 0.034$) and the association became stronger 36.7% [11/30], $p = 0.023$ when LP reached the anterior cingulate gyrus, insula mesocortex, and midfrontal, midtemporal, and inferior parietal neocortex (Braak stage 5) and 3-fold increase [51.9% [14/27], $p < 0.001$] with further infiltration into the primary motor and sensory neocortices (Braak stage 6) (36).

Depression/Anxiety

Depression and anxiety are relatively common features of PD. Descriptive studies as early as 1913 noted a personality type, described as particularly industrious, devoted to hard work, inflexible, punctual, cautious, and moralist to be associated with PD (38). This anecdotal concept of premorbid -parkinsonian personality is supported by the Minnesota Multiphasic Personality Inventory (MMPI) long-term historical cohort study, suggesting that an anxious personality trait may predict an increased risk of PD developing many years later (39).

Clinically significant depressive disturbances are found to occur in 40–50% of patients with PD (40, 41). The onset of depressive syndromes and their natural history do not parallel the course of the motor symptoms (40, 42). A higher incidence of depression in patients who were later diagnosed with PD, supports the hypothesis of there being a biological risk factor for depression in these patients (43). Depression in PD has been related to multiple neurotransmitter dysfunctions, including dopamine (SNpc), serotonin (raphe nuclei), and noradrenaline (locus coeruleus). The involvement of both raphe nuclei and locus coeruleus at Braak stage 2, might indicate depression as a prodromal symptom of PD (44). The relationship between depression and subsequent PD appears to be strongest in the immediate "premotor" years before diagnosis of PD. Retrospective case-control analysis of a population-based study from Rotterdam suggests that both anxiety and depression become significantly more common in patients only about 1–2 years before PD diagnosis (45).

Global Cognitive Deficit

Cognitive deficits was associated with increased PD risk in two prospective studies investigating global cognition and cognitive decline (46). Darweesh et al. (47) found in a population-based cohort study including 7,386 participants of the Rotterdam Study with median 8.3 years of follow-up, poor baseline cognitive functioning indicated the probable onset of parkinsonism and probable Parkinson disease (47). Schrag et al. (48) analyzed data from 8,166 patients aged older than age 50 years with incident diagnosis of PD and 46,755 controls looking at likelihood ratios, sensitivity, specificity, and positive and negative predictive values for individual symptoms and combinations of presentations including cognitive decline. They found that cognitive decline was significantly associated with PD within 5 years before diagnosis. Based on those two studies and the study from Weintraub et al. (49) who found that global cognition was numerically, but not statistically worse in individuals with hyposmia and incident PD compared with those who remained PD free, global cognitive deficit was recently added as a prodromal marker in the MDS research criteria for prodromal PD (46).

Orthostatic Hypotension

Neurogenic orthostatic hypotension (nOH), the hallmark feature of degeneration of the autonomic nervous system,

refers to clinically diagnosed orthostatic hypotension (OH) with confirmation based on quantitative assessments of supine/sitting and standing blood pressure drop with alternative causes of OH (dehydration, cardiac disease, autonomic neuropathy, medication, etc.) eliminated after comprehensive clinical assessment (46, 50). Symptomatic OH is based on clinical OH diagnosis or a positive orthostatic hypotension questionnaire without comprehensive diagnostic investigation regarding the cause (46). The work by the US Autonomic Disorders Consortium showed that nOH in the presence of rapid eye movement sleep behavior disorder and reduced olfaction carries a 10 percent annual cumulative risk of developing Parkinson disease and dementia with Lewy bodies (51). The MDS Research Criteria for Prodromal PD consider nOH as one of the key features of prodromal PD (8). Recently new levels of diagnostic certainty for neurogenic and symptomatic orthostatic hypotension have been added to the criteria (46).

Erectile Dysfunction

Dysautonomia is common among all synucleinopathies and limited dysautonomia may predate the motor symptoms by up to 20 years (52). Although erectile dysfunction (ED) is an autonomic symptom only a few studies have documented the frequencies of ED in PD. In a large-scale cohort with 32,616 US men, Gao et al. (53) observed that erectile dysfunction was prevalent among PD patients and that ED antedates PD diagnosis by many years. Postuma et al. (54) reported from a prospective follow up in a RBD cohort that ED was significantly abnormal up to 5 years before the development of a defined neurodegenerative disease. However, Hasan et al. (52) did not find ED to be a premotor symptom among PD cases.

Urinary Dysfunction

Schrag et al. (55) found in a case control study a relative risk of 1.9 for urinary dysfunction at 5 years before PD diagnosis compared with controls ($n = 25\,544$). Among patients with idiopathic RBD, symptoms of urinary frequency were documented up to 7 years before conversion to PD, with an extrapolated prodromal interval of 13 years (54). The specificity of this marker is, however, relatively low.

Motor Markers

The UPDRS was developed as a rating scale within PD (56). According to MDS prodromal PD criteria, possible subthreshold parkinsonism on expert examination defined as a UPDRS score >3 excluding action tremor or MDS-UPDRS score >6, excluding postural and action tremor, is a clinical motor marker for prodromal PD (8).

UPDRS first becomes abnormal 4.5 years before diagnosis. Voice and face akinesia seem to be the first signs to develop, followed by rigidity, gait abnormalities, limb bradykinesia and finally tremor (68). Simple quantitative motor tests, may be able to identify parkinsonism earlier than subjective examination (68). Wearable or smartphone-based sensor technologies have been considered for continuous monitoring. However, sensor-based quantitative motor and non-motor markers such as cardiac and/or autonomous dysfunction in prodromal PD require further prospective evidence and standardization of methods (46).

Fluid, Tissue and Genetic Markers (Table 2)

Altered α-synuclein metabolism in the central nervous system has a central role in the pathogenesis of PD and several studies have focused on determining α-synuclein species in different fluids and tissues. The α-synuclein is mainly expressed by neuronal cells as a cytoplasmic protein in its native form or in the oligomeric, phosphorylated form. However, because of its access to the extracellular space, it can be detected in cerebrospinal fluid (CSF) (13). Longitudinal changes in CSF α-synuclein and other biomarkers in PD have been examined in different cohorts with different results (69–72). In a recent study by Mollenhauser et al. (61) CSF-α-synuclein in drug-naïve PD, healthy controls, and prodromal PD in the Parkinson's Progression Markers Initiative (PPMI) up to 36-month follow-up was analyzed. According to the results, CSF α-synuclein decreases early in the disease, preceding motor PD. However, CSF- α-synuclein does not correlate with progression and therefore does not reflect ongoing dopaminergic neurodegeneration. Blood has been a disappointing target to-date because red cells contain large quantities of α-synuclein, obscuring any theoretical difference in levels between patients and controls (73).

In large prospective studies, low plasma urate levels in men have repeatedly been shown to be associated with higher PD risk and are recently proved sufficiently sensitive and specific to be included as a risk marker in the MDS Research Criteria for Prodromal PD (46, 57–59).

In recent years there has been an increasing interest in Neurofilament light chain (NfL) as a biofluid biomarker for PD. Oosterweld et al. (60) found that CSF and serum NfL levels in combination with CSF α-synuclein species(phosphorylated-/total α-synuclein, and oligomeric-/total α-synuclein) may serve as a biomarker panel for discrimination of PD patients compared with controls.

The GI tract harbors the largest nervous system outside the CNS accessible for biopsy-taking by endoscopy. However, recent studies show conflicting results regarding α-synuclein detection in GI tract as a potential biomarker of PD. Schneider et al. (74) conclude in their review that data retrieved so far on alpha synuclein aggregations in the GI tract/salivary glands are still unsatisfactory in terms of specificity and sensitivity and are therefore not suitable to serve as a robust diagnostic biomarker (75).

Phosphorylated α-synuclein in skin biopsy has been shown to be sensitive (55–100%) as well as highly specific (>90%) for PD and prodromal PD (idiopathic RBD). Similarly, biopsy of the submandibular gland shows considerable promise. However, sensitivity of this marker depends on the number and location of tissue samples and the specificity may vary between biopsy techniques. Prospective studies proving predictive value are still lacking (46, 62–66).

Although there are promising approaches in fluid and tissue biomarker research, no biofluid or histological marker has proven sufficiently sensitive and specific to be included as a prodromal marker in the MDS research criteria for prodromal

TABLE 2 | Fluid, tissue, and genetic markers of prodromal Parkinson's disease.

Method	Biomarker	Value	References
Fluid			
Blood			
	Urat	Low plasma urate levels in men are associated with higher PD risk	(46, 57–59)
	NfL	Biomarker panel in combination with CSF α-synuclein species	(60)
CSF			
	α-synuclein	No correlate with PD progression	(61)
	NfL	Biomarker panel in combination with CSF α-synuclein species	(60)
Tissue			
Skin			
	α-synuclein	Phosphorylated α-synuclein in skin biopsy are sensitive (55–100%) and highly specific (>90%) for PD and prodromal PD (idiopathic RBD)	(46, 62–64)
Submandibular gland			
	α-synuclein	Sensitivity of the marker depends on the number and location of tissue samples and specificity may vary between biopsy techniques	(65, 66)
Genetic			
	G2019S LRRK2 mutation	Mutation carriers without motor symptoms of PD represents a unique opportunity for studying the prodromal stage of PD	(67)

PD. Currently there are no validated biomarkers to assist in diagnosing PD or determining its neuropathological progression (76). So far, the prodromal criteria are composed of clinical and imaging signs (77).

Genetic Cohorts

Evidence from family and twin studies in addition to advances in molecular genetics have indicated important genetic contributions to the pathogenesis of PD (78). Although, monogenic causes of PD, such as autosomal dominant mutations in the *SNCA*, *LRRK2,* or *VPS35* genes, is limited to small minority of individuals, asymptomatic carriers of mutations that cause monogenic forms of PD provide the clearest information on the development of prodromal features (10, 17, 79).

The most common monogenic cause of PD is mutation of the autosomal dominant Leucine Rich Repeat Kinase (*LRRK2*) gene, a complex gene whose role in neurodegeneration is not completely understood. The G2019S mutation in the *LRRK2* gene, represents the most common pathogenic mutation identified in PD worldwide, accounting for up to 1–6% of sporadic and 3–19% of familial PD with even higher frequencies in Ashkenazi Jews (11, 80). At present there are no sensitive methods to identify those likely to develop the disease. Non-manifesting carriers (NMC) are considered to have an increased risk, G2019S penetrance range between 30 and 80% at age 80, for future development of the disease (11). Phenoconversion from a motorically asymptomatic to an affected state probably reflects an age-associated failure to compensate for kinase dysfunction (81). Once manifest, the motor features of *LRRK2*- PD are largely indistinguishable from idiopathic PD (10). The identification and follow-up of carriers of the *LRRK2*- G2019S mutation who still have not developed motor symptoms of PD represents a unique opportunity for studying the prodromal stage of PD (67). Mirelman et al. (82) has been the first to evaluate the MDS Research Criteria for Prodromal PD in carriers of the *LRRK2*-G2019S mutation and the first among Ashkenazi Jews. According to their results, the criteria had high sensitivity and specificity in identifying prodromal PD in this high- risk unique cohort.

Glucocerebrosidase (*GBA*) mutations are together with *LRRK2* variants, the most common genetic risk factors for late-onset PD. About 5–10% of PD patients have mutations in the *GBA1* gene and *GBA* mutation raises as high as nearly 7-fold of odds ratio for PD in its carriers.

IMAGING MARKERS

Neuroimaging of genetic PD can provide unique opportunities to investigate changes occurring in the pre-symptomatic period in asymptomatic carriers (83). Although dopamine levels cannot be measured directly by using imaging, various methods can be used to assess altered function of nigrostriatal dopaminergic neurons terminals. The most easily accessible approach is the use of markers for the dopamine transporter (DAT). Functional cerebrum imaging using tracers, that can penetrate the blood-brain barrier, can identify diseased areas in the cerebrum with either positron emission tomography (PET) or single photon emission computed tomography (SPECT) (84).

Dopamine transporter single photon emission tomography (DAT-SPECT) is a neuroimaging method providing a semiquantitative assessment of striatal dopaminergic deafferentation and is a well-established method for the assessment and investigation of PD (11, 85). In patients with PD, DAT-SPECT shows decreased striatal DAT uptake, indicating substantia nigra dopaminergic dysfunction that is more marked in the putamen than in the caudate nucleus (12). Studies in unaffected subjects with PD mutations, hyposmia, and a first-degree relative with PD or RBD demonstrate abnormal dopaminergic imaging in advance of motor symptoms (86).

Cohort studies comparing RBD subjects to healthy controls have demonstrated that around 20–40% of RBD patients have abnormal DAT imaging (17). In one of the largest studies using DAT- SPECT, a prospective study of 43 iRBD patients, Iranzo et al. (12) found that decreased striatal DAT uptake (^{123}I-FP-CIT binding) and substantia nigra hyperechogenicity might be useful markers to identify individuals at increased risk for developing synucleinopathies. After a follow up of 2.5 years, there was a pathologically reduced ^{123}I-FP-CIT binding in 17 (40%) of 43 participants and substantia nigra hyperechogenicity in 14 (36%) of 39 participants. A total of 63% of the participants had reduced ^{123}I-FP-CIT binding or substantia nigra hyperechogenicity at baseline. Of these, 30% developed a neurodegenerative disorder (five PD, two dementia with Lewy bodies, and one multiple system atrophy). When examining iRBD patients with serial ^{123}I-FP-CIT SPECT Iranzo et al. (87) found a decline in striatal tracer uptake reflecting a progressive nigrostriatal dopaminergic dysfunction. The DAT deficit seen in RBD is less severe than in established PD suggesting that dopaminergic imaging may have the potential to quantify progression through the prodromal phase (17, 88, 89). In a recent study by Bae et al. (90) they found that 3.0-T susceptibility-weighted MR imaging showed alterations of nigral hyperintensity in patients with iRBD that corresponded to DAT SPECT findings. However, future studies with a larger number of study subjects are recommended since 36.1% of the patients with iRBD showed discordance between the findings.

The first study performing DAT-SPECT in a cohort of unaffected carriers of the G2019S mutation was made by Sierra et al. (91) were they report abnormal DAT imaging in 43.7% of the participants.

Sossi et al. (92) examined changes in dopamine turnover in the asymptomatic PD phase using PET imaging with 18F-fluorodopa and found dopamine turnover to be elevated in asymptomatic mutation carriers at increased risk of PD. Wile et al. (93) did two cross-sectional PET studies showing that LRRK2 mutation carriers without manifest Parkinson's disease had greater 18F-fluorodopa uptake and dopamine transporter binding than did individuals with sporadic Parkinson's disease increased serotonin transporter binding in the striatum, brainstem, and hypothalamus, possibly reflecting compensatory changes in serotonergic innervation preceding the motor onset of Parkinson's disease. In another study Liu et al. (94) used the PET tracer N-^{123}C-methyl-piperidin-4-yl propionate to scan for acetylcholinesterase activity in 4 patients with LRRK2 Parkinson's disease, 16 LRRK2 mutation carriers without Parkinson's disease, eight patients with idiopathic Parkinson's disease, and 11 healthy controls. They found that LRRK2 mutations are associated with significantly increased cholinergic activity in the brain in mutation carriers without Parkinson's disease compared with healthy controls.

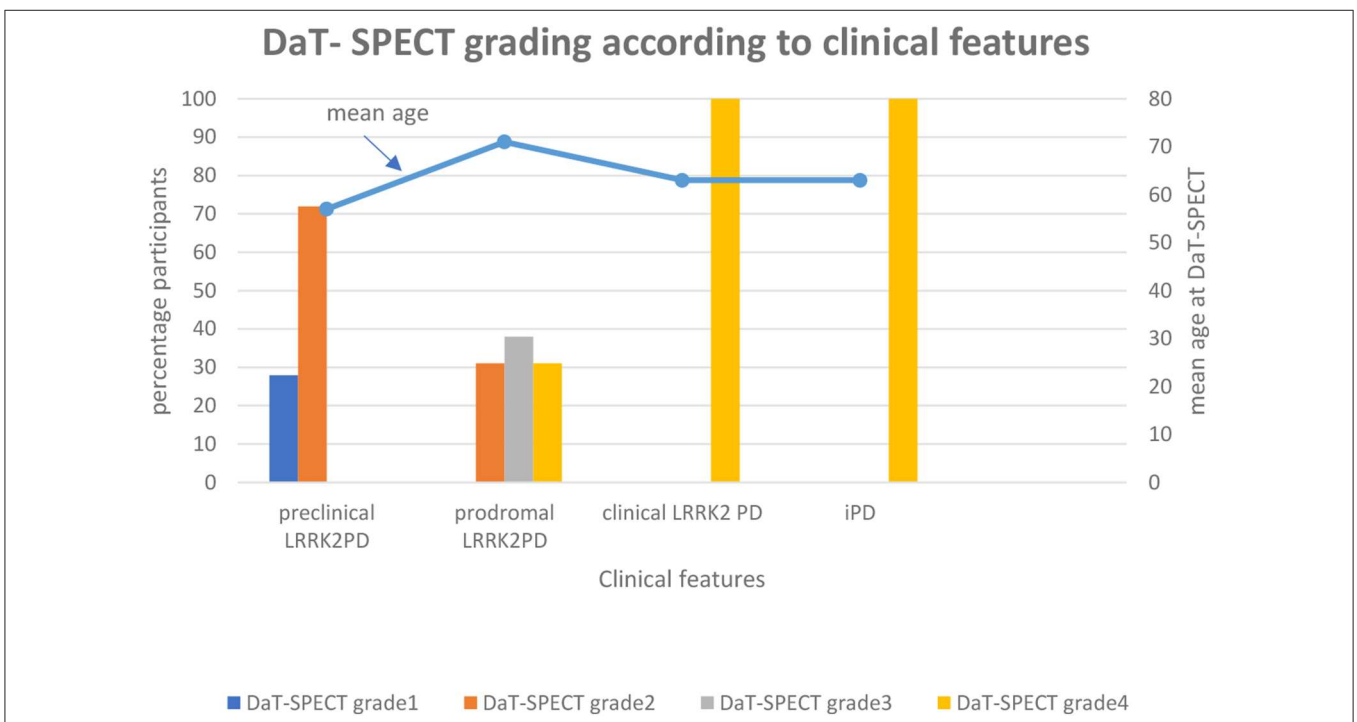

FIGURE 1 | In the preclinical *LRRK2* PD group (UPDRSIII <5), 28 percent (5/18) of the participants have normal DAT-SPECT scans and 72 percent (13/18) have abnormal DAT-SPECT scans grade 2 with an almost normal, symmetrical tracer uptake with a discrete reduction in one or both putamina. In the prodromal *LRRK2* PD group (UPDRS III 5–10), all of the participants have abnormal DAT-SPECT scans: 31 percent (4/13) grade 2, 38 percent (5/13) grade 3 with an asymmetric tracer uptake with normal or almost normal uptake in the putamen of one hemisphere and reduced uptake in the contralateral putamen and 31 percent (4/13) grade 4 with a posterior- anterior degeneration pattern. In the clinical *LRRK2* group and idiopathic PD group all of the participants have abnormal DAT-SPECT scans grade 4.

In the clinical setting, DAT- PET has several advantages over DAT- SPECT like superior DAT selectivity, shorter static imaging protocols without the need for pharmacological thyroid protection, better image resolution with PET, and possibility to obtain quantitative outcome measures with full dynamic PET acquisitions, when required (95).

According to a update of the MDS Research Criteria for Prodromal Parkinson's Disease by Heinzel et al. (46) several imaging approaches have potential as sensitive and specific markers of prodromal PD as suggested by associations with RBD, GBA, or LRRK2 mutation carriers, Dementia with Lewy Bodies, and PD. These promising neuroimaging techniques include 11C-donepezil PET/CT (cholinergic (parasympathetic) gut innervation), 123I-metaiodobenzylguanidine scintigraphy (cardiac sympathetic denervation), susceptibility-weighted and neuromelanin-sensitive MRI (dorsal nigral hyperintensity; integrity of pigmented neurons of the locus coeruleus), coeruleus), 11Cmethylreboxetine PET (noradrenergic nerve terminals originating in the locus coeruleus), structural connectivity and functional MRI (striatal or whole-brain function) (17, 46, 96–98).

In a recent study by our group, a retrospective evaluation of a cohort of 39 participants who underwent DAT-SPECT scan as part of their follow up by movement disorder expert (JOA) at the department of Neurology at St. Olav's Hospital in Trondheim were performed. The report was given prior to the imaging studies. The material has been described in previous reports (80, 99–101). Our objective was to assess whether a combination of systematic clinical testing and different imaging techniques in familial PD cases could detect subclinical signs in the preclinical and prodromal stages of PD. We characterized the cohort of 39 participants with visual analysis of DAT- SPECT imaging to assess patterns of dopaminergic degeneration. Participants were divided into five groups based on the Movement Disorders Society (MDS) Research Criteria for Prodromal PD (8, 73). (1) healthy, (2) preclinical *LRRK2* PD (*LRRK2*- mutation- carriers without clinical symptoms), (3) prodromal *LRRK2* PD (*LRRK2* mutation carriers with presence of early symptoms and signs before PD diagnosis is possible), (4) clinical *LRRK2* PD (*LRRK 2* carriers with diagnosis of PD based on the presence of classical motor signs) and (5) clinical PD (idiopathic PD).

Clinical assessment included the Unified Parkinson's Disease Rating Scale (UPDRS) part III (motor part). Participants without a PD diagnosis, were divided into preclinical *LRRK2* PD (UPDRS III < 5) or prodromal *LRRK2* PD (UPDRS III 5–10) based on the UPDRS part III score, as seen above.

The participants were distributed as follows: 1 healthy, 18 preclinical *LRRK2* PD,12 prodromal *LRRK2* PD, 5 clinical *LRRK2* –PD, and 3 clinical idiopathic PD (iPD). We assume that pre-symptomatic gene mutation carriers will eventually exhibit clinical symptoms and, thus, the imaging results can be extended to the pre-symptomatic stage of PD (92).

DAT-SPECT scans were visually categorized by 1 observer according to predefined visual patterns of dopaminergic degeneration (102). It has been suggested that diagnostic accuracy in DAT-SPECT scans might be highly dependent on the

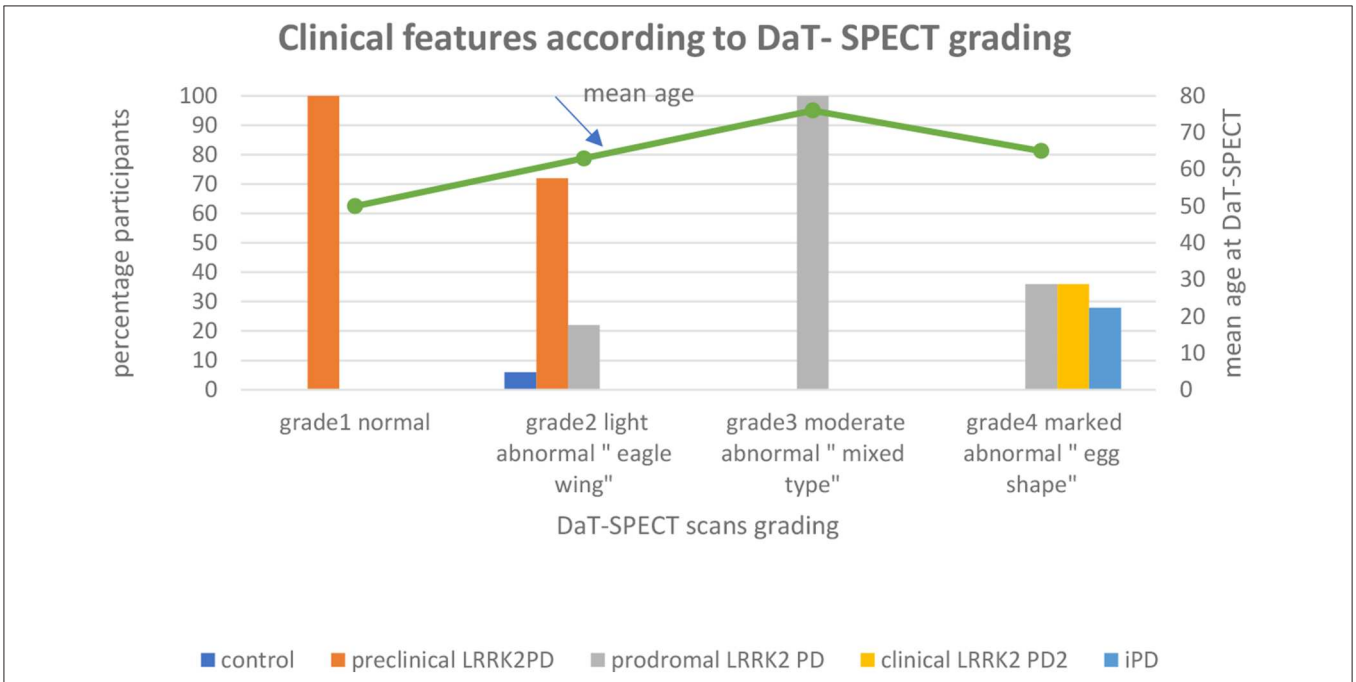

FIGURE 2 | Among the participants with normal DAT-SPECT scans, 100 percent (5/5) have preclinical *LRRK2* PD. Among the perticipants with light abnormal "eagle wing" DAT-SPECT scans, 72 percent (13/18) have preclinical *LRRK2* PD, 22 percent (4/18) have prodromal *LRRK2* PD and the one control patient accounted for 6%. Among the participants with moderate abnormal "mixed type" DAT-SPECT scans, 100% (5/5) have prodromal LRRK2 PD. Among the participants with marked abnormal "egg shape" DAT-SPECT scans, 36 percent (4/11) have prodromal *LRRK2* PD, 36 percent (4/11) have clinical *LRRK2* PD and 28 percent (3/11) have idiopathic PD.

reviewers experience as currently interpretation is mainly visual and therefore semi- quantitively and subjective (11). To avoid bias the observer was blinded to diagnosis and clinical features. The results were graded as normal (grade 1) or abnormal (grade 2–5), distinguishing an almost normal, symmetrical tracer uptake with a discrete reduction in one or both putamina (grade 2"eagle wing"), an asymmetric tracer uptake with normal or almost normal uptake in the putamen of one hemisphere and reduced uptake in the contralateral putamen (grade 3"mixed type"), a posterior-anterior degeneration pattern (grade 4 "egg shape") and severe degeneration pattern (grade 5 "burst striatum").

A correlation of the scan findings with the clinical symptoms and diagnosis was performed. Interobserver disagreement to the scan findings was considered.

In the preclinical *LRRK2* PD group, 28 percent (5/18) of the participants have normal DAT-SPECT scans and 72 percent (13/18) have abnormal DAT-SPECT scans grade 2 with an almost normal, symmetrical tracer uptake with a discrete reduction in one or both putamina.

In the prodromal *LRRK2* PD group, all of the participants have abnormal DAT-SPECT scans: 31 percent (4/13) grade 2, 38 percent (5/13) grade 3 with an asymmetric tracer uptake with normal or almost normal uptake in the putamen of one hemisphere and reduced uptake in the contralateral putamen and 31 percent (4/13) grade 4 with a posterior-anterior degeneration pattern.

In the clinical *LRRK2* PD group and idiopathic PD group, all participants have abnormal DAT -SPECT scans grade 4 (**Figure 1**).

Among the participants with normal DAT-SPECT scans, 100 percent (5/5) have preclinical *LRRK2* PD.

Among the participants with light abnormal "eagle wing" DAT -SPECT scans, 72 percent (13/18) have preclinical *LRRK2* PD, 22 percent (4/18) have prodromal *LRRK2* PD and the one control patient accounted for 6%.

Among the participants with moderate abnormal "mixed type" DAT-SPECT scans, 100% (5/5) have prodromal *LRRK2* PD.

Among the participants with marked abnormal "egg shape" DAT-SPECT scans, 36 percent (4/11) have prodromal *LRRK2* PD, 36 percent (4/11) have clinical *LRRK2* PD and 28 percent (3/11) have idiopathic PD (**Figure 2**).

CONCLUSION

New research criteria for prodromal PD are a promising tool to identify cases of incident PD over 5 years, arguing for their usefulness in defining target populations for disease-prevention trials (103). There are a wide variety of proven markers of prodromal PD with different predictive abilities and different lead times. The field of prodromal PD is rapidly expanding, with new diagnostic markers discovered each year (104). It is now possible to define with reasonable certainty the probability that a specific person has prodromal PD (104). According to the MDS research criteria for prodromal PD published in 2015, a Bayesian naive classifier approach is used to estimate the likelihood that an individual has prodromal PD by considering age and predictive information from risk and prodromal markers (8, 46). Once neuroprotective therapy has been developed, systematic screening for prodromal PD and resultant prompt treatment could even prevent clinical PD from ever becoming clinically relevant (104). However, clinical PD is a heterogeneous and complex disease with many different possible etiologies. Some evidence suggests that the presence of RBD, symptomatic hypotension, and cognitive deficits is associated with a more malignant PD phenotype, with a different prodromal state. Similarly, patients with *LRRK2* mutations often have prominent prodromal gait deficits, and *LRRK2* carriers with synuclein pathology exhibit more cognitive impairment, anxiety, and orthostatic hypotension than those without which will likely have a different prodromal state. Heterogeneity of prodromal states should be further investigated and may be important for targeted trial recruitment (46). Age and sex may impact the diagnostic accuracy of prodromal PD as well as the predictive properties of single risk and prodromal markers of PD and was taken into account when the MDS criteria of prodromal PD to improve the accuracy of PD prediction was revised (46, 105). Some PD patients suffer more from non-motor symptoms (106). Data quality of prodromal markers and their sensitivity and specificity may depend on assessment methods used. Not least, the accuracy of the PD diagnosis may vary between studies such as in register studies using medical record data (46, 107). Pillotto et al. (108) evaluated the MDS prodromal PD criteria in two independent prospective studies. They found that the criteria have low sensitivity and positive predictive values, but high specificity and negative predictive values in their cohorts. It is therefore required thorough quantitative/objective and specific diagnostic testing to yield the diagnostic accuracy necessary for selecting populations at risk for the first intervention trials in prodromal PD. Further research and refinements are needed for optimizing cut-offs and establishing appropriate means to account for the age-related normal changes, missing data, or incomplete assessment the diagnostic accuracy necessary for selecting populations at risk for the first intervention trials in prodromal PD.

AUTHOR CONTRIBUTIONS

EH designed and conceptualized the study, analyzed the data, and drafted the manuscript for intellectual content. JA designed and conceptualized the study, collected the data, analyzed the data, and revised the manuscript for intellectual content.

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BRIEF RESEARCH REPORT
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Sleep Spindles and Fragmented Sleep as Prodromal Markers in a Preclinical Model of LRRK2-G2019S Parkinson's Disease

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Sleep disturbances co-occur with and precede the onset of motor symptoms in Parkinson's disease (PD). We evaluated sleep fragmentation and thalamocortical sleep spindles in mice expressing the p.G2019S mutation of the leucine-rich repeat kinase 2 (*LRRK2*) gene, one of the most common genetic forms of PD. Thalamocortical sleep spindles are oscillatory events that occur during slow-wave sleep that are involved in memory consolidation. We acquired data from electrocorticography, sleep behavioral measures, and a rotarod-based motor enrichment task in 28 *LRRK2*-G2019S knock-in mice and 27 wild-type controls (8–10 month-old males). Sleep was more fragmented in *LRRK2*-G2019S mice; sleep bouts were shorter and more numerous, even though total sleep time was similar to controls. *LRRK2*-G2019S animals expressed more sleep spindles, and individual spindles were longer in duration than in controls. We then chronically administered the LRRK2-inhibitor MLi-2 in-diet to $n = 12$ *LRRK2*-G2019S and $n = 15$ wild-type mice for a within-subject analysis of the effects of kinase inhibition on sleep behavior and physiology. Treatment with MLi-2 did not impact these measures. The data indicate that the *LRRK2*-G2019S mutation could lead to reduced sleep quality and altered sleep spindle physiology. This suggests that sleep spindles in *LRRK2*-G2019S animals could serve as biomarkers for underlying alterations in sleep networks resulting from the *LRRK2*-G2019S mutation, and further evaluation in human *LRRK2*-G2019S carriers is therefore warranted.

Keywords: Parkinson's disease, prodromal, LRRK2, sleep spindles, sleep fragmentation, EEG, biomarker

INTRODUCTION

Mutations of the leucine-rich repeat kinase-2 (*LRRK2*) gene represent one of the most common genetic causes of Parkinson's disease (PD) (1). As with idiopathic PD, *LRRK2* PD is associated with the progressive loss of dopaminergic neurons in the substantia nigra pars compacta that ultimately results in debilitating motor symptoms such as bradykinesia, rigidity, and tremor

(2). *LRRK2*-G2019S is the most prevalent *LRRK2* mutation, accounting for 5–6% of autosomal dominant PD and ∼1% of sporadic late-onset PD (3). G2019S is a toxic gain-of-function mutation associated with a variety of cellular effects such as increased glutamatergic activity, neuronal hyper-excitability, deficits in vesicular trafficking, autophagy, and disrupted mitochondrial function (4–6). While work has begun to reveal how the G2019S mutation affects cellular and synaptic function, little is known about how this mutation affects brain circuits.

Although cardinal motor symptoms are most commonly associated with PD, ∼80% of patients report sleep problems such as sleep fragmentation, excessive daytime sleepiness, and rapid-eye-movement (REM) sleep behavior disorder (RBD) (7). These symptoms can precede motor symptoms in idiopathic PD by as much as 7 years (8–10). Although sleep disturbances and sleep-associated neurophysiology have been studied in idiopathic PD, much less is known about how sleep is altered in *LRRK2* PD, particularly during the prodromal period. Furthermore, while RBD is one of the earliest prodromal markers of idiopathic PD, it is not as common in *LRRK2* PD (11, 12). Given that sleep disturbances are a feature of *LRRK2* PD (11), there is a need to characterized and identify early sleep alterations unique to *LRRK2* PD, particularly as they relate to non-REM (NREM) sleep.

There are multiple features of the G2019S mutation suggesting that disrupted *LRRK2* expression could alter cellular activity and neural circuits involved in sleep maintenance. For example, *LRRK2* expression is high in the cortex and thalamus (13, 14), two regions involved in the maintenance of NREM sleep. The G2019S mutation is also associated with the potentiation of glutamatergic synapses (6, 15–17), an effect that could excite thalamocortical circuits involved in NREM sleep. One hallmark feature of NREM sleep is the sleep spindle. Sleep spindles are 9–16 Hz thalamocortical oscillations believed to support memory consolidation by coordinating neural activity in cortical, striatal, and limbic circuits (18–20). Spindle density is positively correlated with declarative memory performance, such as the integration of new lexical information (21) and word-pair recall (22). Spindle density is also positively correlated with the refinement of motor skills (23). Given evidence that corticothalamic circuits involved in spindle generation are altered in *LRRK2*-G2019S PD, and evidence for disrupted motor skill learning in PD (24), we hypothesized that the relationship between spindle activity and motor learning would be disrupted in *LRRK2*-G2019S mice.

In this study, we examine the effect of the G2019S mutation on sleep behavior and physiology in *LRRK2*-G2019S knock-in (KI) mice. The G2019S KI mouse is homozygous for the human *LRRK2*-G2019S mutation. Some studies report progressive dopamine-related neurodegeneration and mitochondrial abnormalities by age 12 months but not age 6 months in these mice (25, 26). G2019S KI mice do not reliably display gross motor impairments, though there have been reports of increased exploratory behavior (27), hyperkinesia at 3 months of age (28), and resiliency to social stress (29).

Given the link between sleep disturbances and PD (8–10), we hypothesized that G2019S KI mice would show disrupted sleep patterns relative to wild-type (WT) controls. Specifically, it was hypothesized that G2019S mice would express reduced measures of sleep quality and, given evidence for potentiated glutamatergic transmission with the G2019S mutation, that spindle oscillations would be enhanced.

To investigate these questions, sleep structure, behavior, and spindle oscillations were analyzed in G2019S KI mice and WT controls. Additionally, to determine whether excessive kinase activity altered sleep physiology, the LRRK2-inhibitor MLi-2 was administered to G2019S and WT mice to determine if the drug restored physiological or behavioral effects that resulted from the *LRRK2*-G2019S mutation.

METHODS

Subjects

A total of $n = 28$ *LRRK2*-G2019S KI (C57BL/6-Lrrk2tm4.1Arte) and $n = 27$ C57BL/6 WT (C57BL/6NTac) control male mice from Taconic Farms (Rensselaer, NY) were acquired between 8 and 16 weeks of age, and aged in the colony room until they reached 8–10 months. Mice were housed in a room with 12-h light/dark cycles, and experiments were performed during the light cycle. Mice had *ad-libitum* access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Arizona and conformed to the guidelines of the National Institutes of Health. Two weeks prior to surgery and until experiments began mice were handled for ∼15 min a day for 5 days/week. One week before surgery, mice were switched to a control diet [D01060501 from Research Diets Inc. (New Brunswick, NJ)]. Mice were pair-housed until 5 days before surgery after which they were individually housed to avoid damage to implanted electrode arrays. Following experimentation, mice were euthanized with CO_2 and cardiac puncture. Cortical tissue was collected, flash frozen, and sent to the Fell laboratory for analysis of LRRK2 expression, as in Fell et al. (30).

Surgical Procedure

Mice were anesthetized with 3% isoflurane, placed in the stereotactic apparatus, and then given subcutaneous carprofen or ketoprofen (5 mg/kg). Isoflurane levels were subsequently kept between 1 and 2%, the skull was cleaned, and Metabond (Parkell, Edgewood, NY) was applied to the skull surface. Two rectangular craniotomies were drilled bilaterally and centered at AP: 0 mm ML: ± 1.5 mm. Electrocorticography (ECoG) arrays consisting of three 0.4 mm diameter gold pins (Mill-Max Mfg. Corp., Oyster Bay, NY) were placed on the cortical surface in each craniotomy (**Figure 1B**). A reference gold pin was placed on the cerebellum and two stainless steel electromyography (EMG) wires were inserted into the neck muscle. The electrode arrays were secured to the skull with dental cement. Mice were allowed 10–12 days to recover before regular recording sessions began. Five days prior to the first recording session, the quality of the ECoG signals were checked and each mouse was exposed to their sleep box for 10 min, the rotarod training apparatus (Rotarod task) for 2 min, and an empty box (Box task) for 5 min in order to reduce novelty effects.

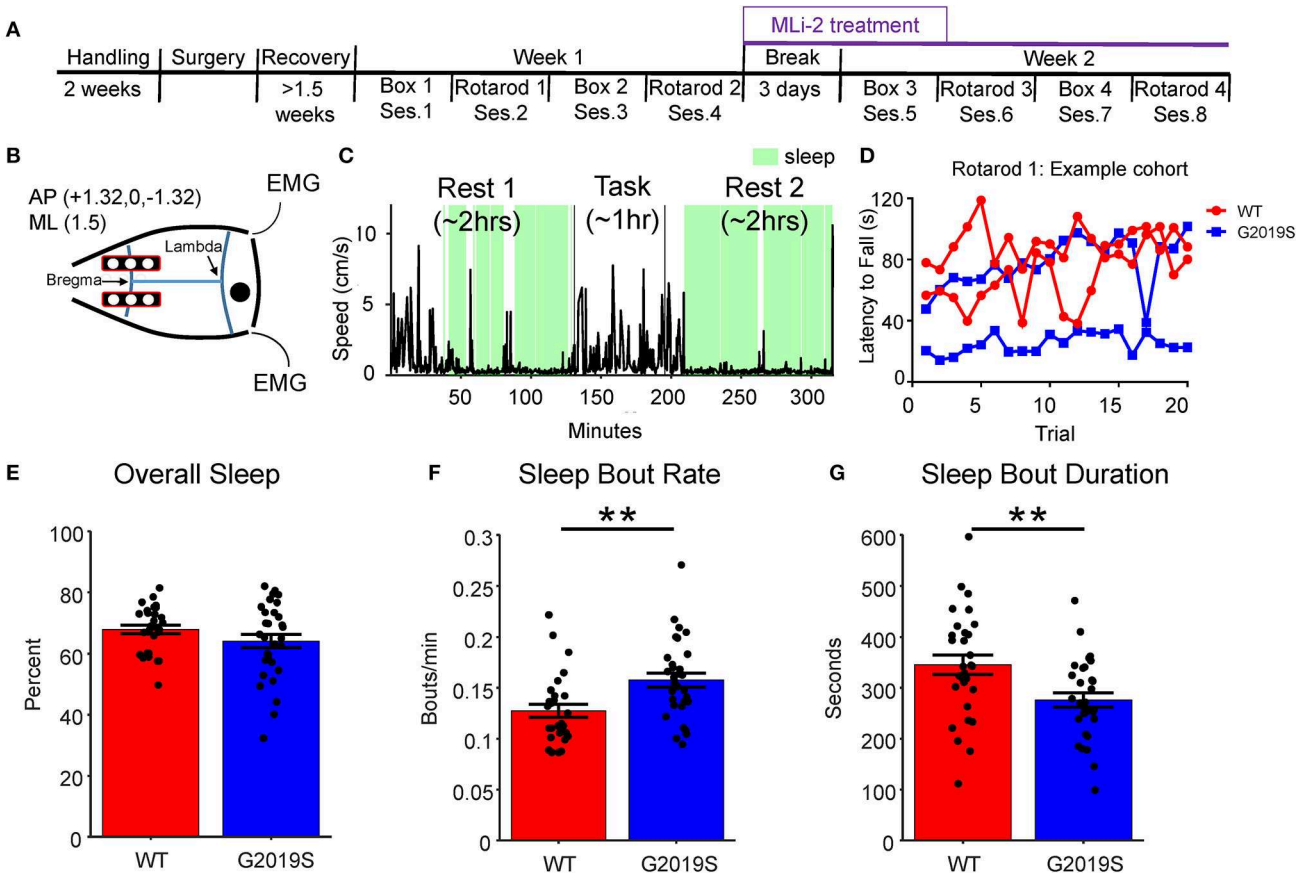

FIGURE 1 | Experimental Overview and Sleep Behavior. **(A)** The experiment took place over 2 weeks. Each week animals alternated between (2x) empty box days and (2x) rotarod days. During the second week a subset of animals were given food containing MLi-2. **(B)** Two rows of three electrodes were placed bilaterally on the cortical surface over M1 and S1 at AP +3.1, 0, −3.1 ML +/−1.5. **(C)** Sleep was identified (shown in green) during Rest 1 and Rest 2 based on movement, inertial data and EMG. **(D)** Example rotarod data obtained from one cohort of mice on Rotarod 1. Each mouse's latency to fall across 20 trials was recorded to obtain a daily average as well as a within-day learning slope. **(E)** G2019S and WT slept a similar amount of time overall as measured by percent time asleep during Rest 1 and Rest 2. **(F)** G2019S animals had more frequent sleep bouts (bouts per minute) than WT mice ($t_{58} = -3.201$, $^{**}p < 0.01$). **(G)** G2019S animals had shorter sleep bouts than WT mice ($t_{54} = 3.048$, $^{**}p < 0.01$). Error bars indicate ± SEM.

Data Acquisition

Neural, EMG, and inertial data were acquired using the Intan data acquisition system (Intan Technologies Inc., Los Angeles, CA). ECoG and EMG signals were acquired at 12.5 kHz. Overhead position tracking data was gathered at 30 frames-per second by a Manta GigE camera (Allied Vision, Exton, PA). Between 2 and 4 mice were recorded simultaneously from mixed genotype groups.

Sleep and Box Recording Days

During neural recordings of sleep, animals were housed in 18 x 18 cm polycarbonate boxes. Each box was enclosed in a metal mesh Faraday cage and sound-attenuating foam. Each sleep box contained bedding from the mouse's home cage. Recording sessions occurred 4 days a week for 2 weeks. Each session had the same structure (**Figures 1A,C**) whereby mice were plugged into the recording apparatus approximately 3 h into the beginning of their light cycle. The recording session consisted of 2 h of pre-task sleep (Rest 1) in the sleep box followed by a 1-h task condition which involved either the exploration of an empty box (Box) or the rotarod motor training task (Rotarod). Completion of the task was followed by a second 2-h sleep period (Rest 2). The task (Box or Rotarod) was switched on alternating days (**Figure 1A**). In the Box task, mice were placed in a clean and empty polycarbonate box and left undisturbed for 1 h. In the Rotarod task, the mice were unplugged from the recording apparatus and placed on the rotarod. The details of the Rotarod task are described below.

Rotarod Training Task

This task was based on the motor-learning paradigm described in Li et al. (31). The rotarod apparatus had 4 lanes, 1 per mouse. Mice were placed on the rod and allowed to rest for 1 min after which the rod began to rotate and accelerated from 0 to 79 rpm over 3 min. Once the last mouse fell off the rod, the rod was stopped, and mice were placed back on the rod in the order they fell off. This process was repeated for 20 trials (**Figure 1D**). All

mice were video recorded and latency to fall was subsequently scored by a research assistant blinded to genotype.

Drug Administration

Following the 4th recording session (Week 1), mice either continued to have *ad-libitum* access to control chow (Research Diets D01060501, 10% kcal fat and cornstarch) or *ad-libitum* access to chow containing LRRK2 inhibitor MLi-2. MLi-2 was added as powder by Research Diets and chow was otherwise identical to control. MLi-2 chow was formulated to provide concentrations of 60 mg/kg (30). In a study of chronic in-diet administration of MLi-2 over 11 days, this dosage has been shown to reduce the ratio of pS935 to total LRRK2 to < 0.1 after 4 h (30).

Drug group assignment was random, and experimenters were blind to drug condition. Each recording cohort contained at least one mouse on MLi-2. Mouse weight and food intake was recorded daily. Mice remained on MLi-2 chow until the conclusion of experiment and were euthanized after a total of 3 weeks MLi-2 exposure. Phosphorylation of residue S395 of the LRRK2 protein was used as a read-out of LRRK2 kinase activity and thus following euthanasia, extent of kinase inhibition was assessed by analysis of pSer935 LRRK2/total LRRK2 in cortex by western blot, as described prior (30). Animals identified as having insufficient kinase inhibition ($n = 7$ WT and $n = 2$ G2019S) on drug or inappropriately low kinase activity ($n = 1$ G2019S) on vehicle were removed from analysis of drug-related effects. In animals used for analysis of MLi-2-related effects, there was $> 90\%$ reduction of kinase activity after treatment evident for both WT and transgenic mice (**Supplementary Figure 1**).

Analysis

Signal Processing and Statistical Analyses

ECoG signals were analyzed using Fourier and wavelet measures of spectral power and frequency using custom Matlab™ functions. Normality of distributions were checked with the Anderson-Darling test. The Wilcoxon Rank-Sum test was used for non-parametric data. The Holm multiple comparisons correction was used for *post-hoc* comparisons. Cohen's d was used as a measure of effect size.

Inertial Measurement and EMG

Inertial data was obtained through a sensor mounted on the neural recording headstage. Inertial data has been demonstrated to provide an excellent readout of sleep/wake state (32). Motion was quantified by summing the absolute value of the first derivative of acceleration ("jerk" or $|m/s^3|$). A threshold of 1 m/s^3 was set for all datasets based on visual inspection. EMG signals were band-pass filtered (70–250 Hz) and the absolute value of the signal was smoothed using a 200 ms moving average to measure muscle tone (33).

Identification of Sleep

To be classified as sleep, two of three conditions had to be met: (1) inertial data <1 m/s^3, (2) EMG activity $<$ a session-by session visually scored threshold, and (3) speed <2 cm/s. If two of these conditions were true for > 40 s (34), the period was classified as sleep. Analyses of sleep behavior was restricted to 110-min periods beginning 5 min after the start of each Rest epoch in order to eliminate possible artifact from researcher presence at the start and end of the session.

Identification of Sleep-Spindles

Spindles were identified using a threshold-crossing approach similar to Phillips et al. (35). The analysis of spindles involved $n = 22$ G2019S and $n = 26$ WT animals. Animals were excluded if (1) data from only one hemisphere was acquired, (2) the animal did not complete the experiment, or (3) no spindles were identified on >2 days during Week 1 or >2 days during Week 2. To reduce common noise and identify local spindle events, common average re-referencing was implemented (36). A common average of the left hemisphere electrodes was subtracted from the signal of the right anterior electrode. This electrode was used for spindle identification due to its proximity to motor cortex (M1) and distance from potential hippocampal REM sleep-associated theta volume conduction.

To identify spindles, the ECoG signal was bandpass filtered to the sigma band (9–16 Hz, 12th order Butterworth filter) and smoothed with a 20-ms Hanning window. A threshold was set by calculating a trimmed (Winsorized) standard deviation (between 10 and 90th percentiles) based on sigma power during sleep. Candidate spindle events were identified when sigma power was >2.5 standard deviations and remained above 1.7 standard deviations for ≥ 500 ms and ≤ 2 s. Only events that occurred during identified sleep were included. The oscillatory frequency of each spindle was determined using Burg's method (40th order; pburg Matlab function).

Measuring the Relationship Between Spindle Activity and Behavioral Performance

Given the role of sleep spindles in memory consolidation, we evaluated the relationship between motor performance and learning with post-task spindle density. The relationship between motor learning and spindle density was measured by measuring the Pearson's correlation coefficient between the change in spindle density from Rest 1 to Rest 2 and the mean latency to fall. This was only performed on Rotarod 1 and Rotarod 2, both off-drug days for which there was within-day rotarod learning as determined by a significant positive correlation ($p < 0.05$) between trial number and the latency to fall.

RESULTS

LRRK2-G2019S Mice Expressed Fragmented Sleep

Sleep fragmentation was assessed as the number of sleep bouts per minute and the average sleep bout duration. Data from Rest 1 and Rest 2 were combined, and only Week 1 data was used in this analysis in order to identify genotypic differences in sleep quality. We observed that G2019S KI mice slept a similar amount of time as WT mice ($t_{54} = 1.656$, $p = 0.104$; **Figure 1E**); however, G2019S mice had significantly more sleep

bouts ($t_{58} = -3.201$, $p = 0.002$, $d = 0.825$; **Figure 1F**), and these sleep bouts were shorter in duration than WT mice ($t_{54} = 3.048$, $p = 0.004$, $d = 0.782$; **Figure 1G**). To assess the effect of task on sleep quality measures, Rest 2 sleep features following Rotarod and Box tasks were compared for WT and G2019S animals. WT but not G2019S animals slept more following Rotarod compared to Box ($t_{28} = 3.251$, $p = 0.006$ for WT, $d = 0.604$; **Supplementary Figure 2A**). Both groups had a greater sleep bout rate following Rotarod sessions ($t_{29} = 3.25$, $p = 0.006$, $d = 0.605$ for WT; $t_{27} = 2.192$, $p = 0.037$, $d = 0.534$ for G2019S; **Supplementary Figure 2B**) and there was no effect of task on sleep bout duration for either group ($t_{27} = -0.014$, $p = 0.989$ for WT; $t_{28} = -2.015$, $p = 0.107$ for G2019S; **Supplementary Figure 2C**).

Sleep Spindle Density and Duration Are Increased in LRRK2-G2019S Mice

Evidence for enhanced synaptic excitability in the *LRRK2*-G2019S mutation (6, 15–17) led to the hypothesis that increased cortical glutamatergic output would result in increased sleep spindle density. Example candidate spindle events, wavelet spectrograms and spindle power spectral densities are shown in **Figures 2A–F**. Accordingly, spindle density in G2019S animals during Week 1 (pre-drug) was greater than WT animals ($t_{40} = -2.17$, $p = 0.036$, $d = 0.604$; **Figure 2G**). We chose the rotarod motor learning task to induce spindles in post-task sleep (Rest 2). While Rest 2 showed a greater spindle density than Rest 1 for nearly all mice, surprisingly we found that spindle density was greater following the Box task compared to the Rotarod task. This was true both as a relative change in spindle density from Rest 1 to Rest 2 ($t_{24} = -4.131$, $p = 0.005$, $d = 0.647$ in WT; $t_{22} = -3.104$, $p = 1.64 \times 10^{-4}$, $d = 0.825$ in G2019S; **Supplementary Figure 3A**) and by comparing spindle density in Rest 2 alone, for which G2019S animals shows a greater Box relative to Rotarod spindle density difference ($t_{46} = -8.543$, $p = 4.76 \times 10^{-11}$, $d = -2.43$, **Supplementary Figure 3B**).

We also hypothesized that enhanced synaptic excitability in mice carrying the G2019S mutation would lead to higher

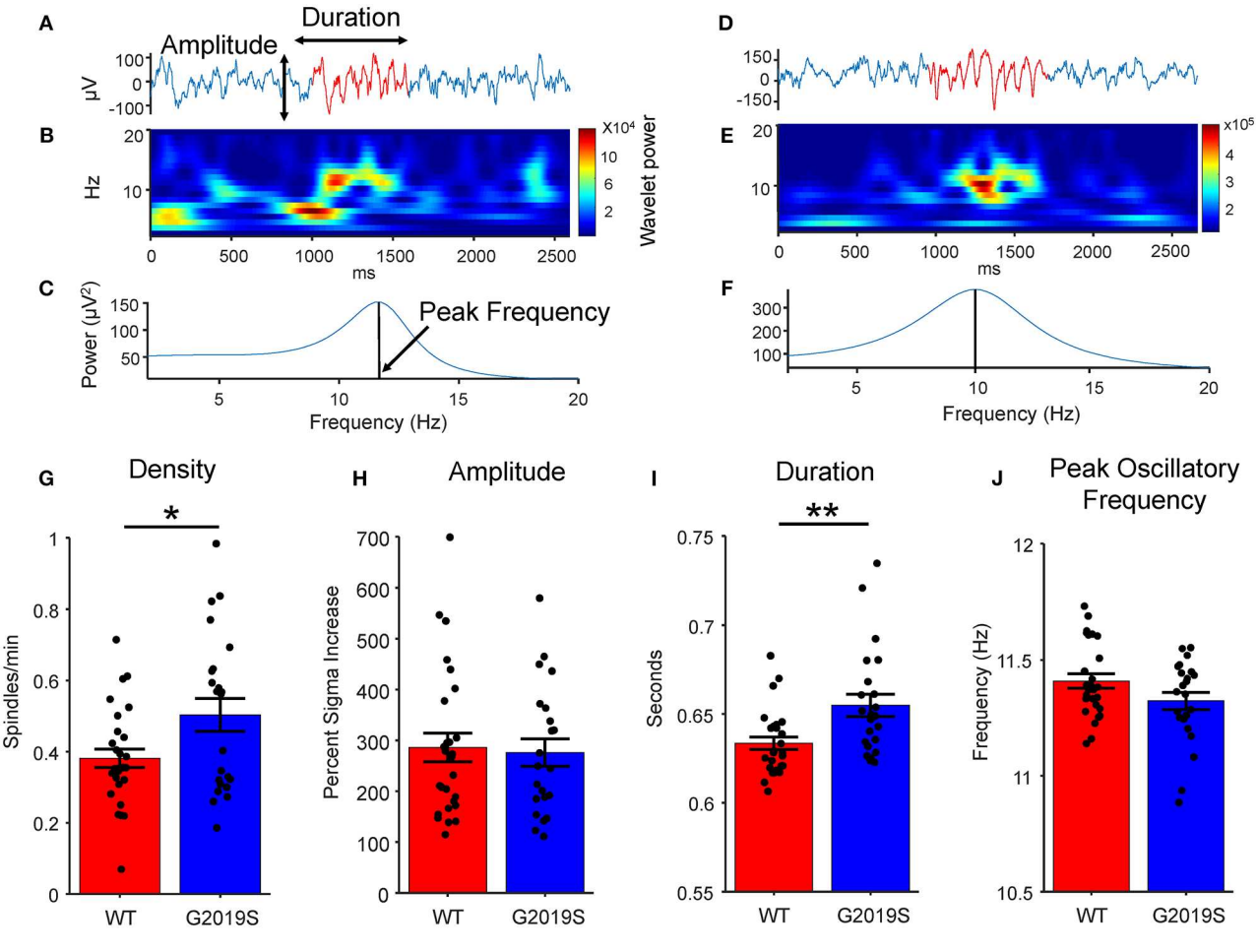

FIGURE 2 | Spindle Identification and Properties. Two example traces with common average re-referenced signal show in blue and identified putative spindle shown in red, **(A)** one from a WT mouse and **(D)** one from a G2019S mouse. **(B,E)** Wavelet spectrogram of putative spindle events. **(C,F)** Spindle power spectral density using Matlab's pburg function. **(G)** G2019S animals had a greater sleep spindle density (spindles/minute of sleep) than did WT animals ($t_{40} = -2.17$, $^{*}p < 0.05$). **(H)** There was no difference in spindle amplitude by genotype. **(I)** G2019S animals had significantly longer duration spindles than WT controls ($t_{49} = -2.862$, $^{**}p < 0.01$ Wilcoxon Rank-Sum). **(J)** There was no significant difference between WT and G2019S peak spindle oscillatory frequency. Error bars indicate ± SEM.

spindle power, frequency, and duration. Spindle amplitude was quantified as the percent increase in sigma power during a spindle from baseline sleep. No difference in peak spindle frequency ($t_{49} = 1.927$, $p = 0.060$; **Figure 2J**) or amplitude ($t_{49} = 0.507$, $p = 0.614$; **Figure 2H**) was observed between G2019S and WT mice. Spindle durations were significantly longer in G2019S mice ($t_{49} = -2.862$, $p = 0.007$, $d = 0.802$; **Figure 2I**). Rotarod performance did not differ between WT and G2019S animals.

Rotarod Performance Did Not Differ Between WT and LRRK2-G2019S Animals

Rotarod performance and learning was analyzed for WT and G2019S mice during Week 1 to assess genotypic differences. The mean latency to fall across all 20 trials of the Rotarod task was used to measure overall motor performance for each mouse (**Figures 3A–D**). The mean latency to fall did not differ significantly between G2019S and WT animals (Wilcoxon-Rank Sum, $p = 0.099$; **Figures 3E,F**). Regressing trial number against latency to fall revealed within-day learning for both WT and G2019S animals on Rotarod 1 ($t_{28} = 6.75$, $p = 2.53 \times 10^{-7}$ for WT and $t_{28} = 7.10$, $p = 9.94 \times 10^{-8}$ for G2019S; **Figure 4E**) and Rotarod 2 ($t_{28} = 3.55$, $p = 0.001$ for WT and $t_{28} = 2.73$, $p = 0.011$ for G2019S; **Figure 4F**). Both groups also showed an increased mean latency to fall from Rotarod 1 to Rotarod 2 ($t_{28} = 5.26$, $p = 1.37 \times 10^{-5}$ for WT and $t_{28} = 4.60$, $p = 8.39 \times 10^{-5}$ for G2019S; **Figure 4G**). There were no genotypic differences in these learning metrics ($t_{52} = 0.88$, $p = 0.383$).

To determine if spindle activity was related to within-session learning, we also regressed learning slopes with changes in spindle density from Rest 1 to Rest 2. This analysis did not identify any within-day effect for Rotarod 1 ($R = -0.166$, $p = 0.462$ in WT; $R = 0.055$, $p = 0.813$ in G2019S; **Supplementary Figure 4A**) or Rotarod 2 ($R = 0.340$, $p = 0.122$ in WT; $R = -0.202$, $p = 0.381$ in G2019S; **Supplementary Figure 4B**). The same analysis was done on measures of distance traveled in the Box task ($R = -0.068$, $p = 0.742$ for WT; $R = -0.063$, $p = 0.793$ for G2019S; **Supplementary Figure 4C**) and between-day learning with no significant correlations observed ($R = 0.297$, $p = 0.179$ in WT; $R = 0.336$, $p = 0.137$ in G2019S; **Supplementary Figures C,D**).

4–7 Day in-Diet Treatment of 60 mg/kg LRRK2 Inhibitor MLi-2 Did Not Alter Sleep Behavior, Physiology, or Rotarod Performance

The above analyses were repeated as a within-subject comparison (Week 2 - Week 1) of behavior and physiology for WT-vehicle, WT-drug, G2019S-vehicle, and G2019S-drug. No changes in percentage of time asleep ($F_{3,49} = 0.784$, $p = 0.508$; **Figure 4A**), mean sleep bout length ($F_{3,49} = 1.181$, $p = 0.327$; **Figure 4B**),

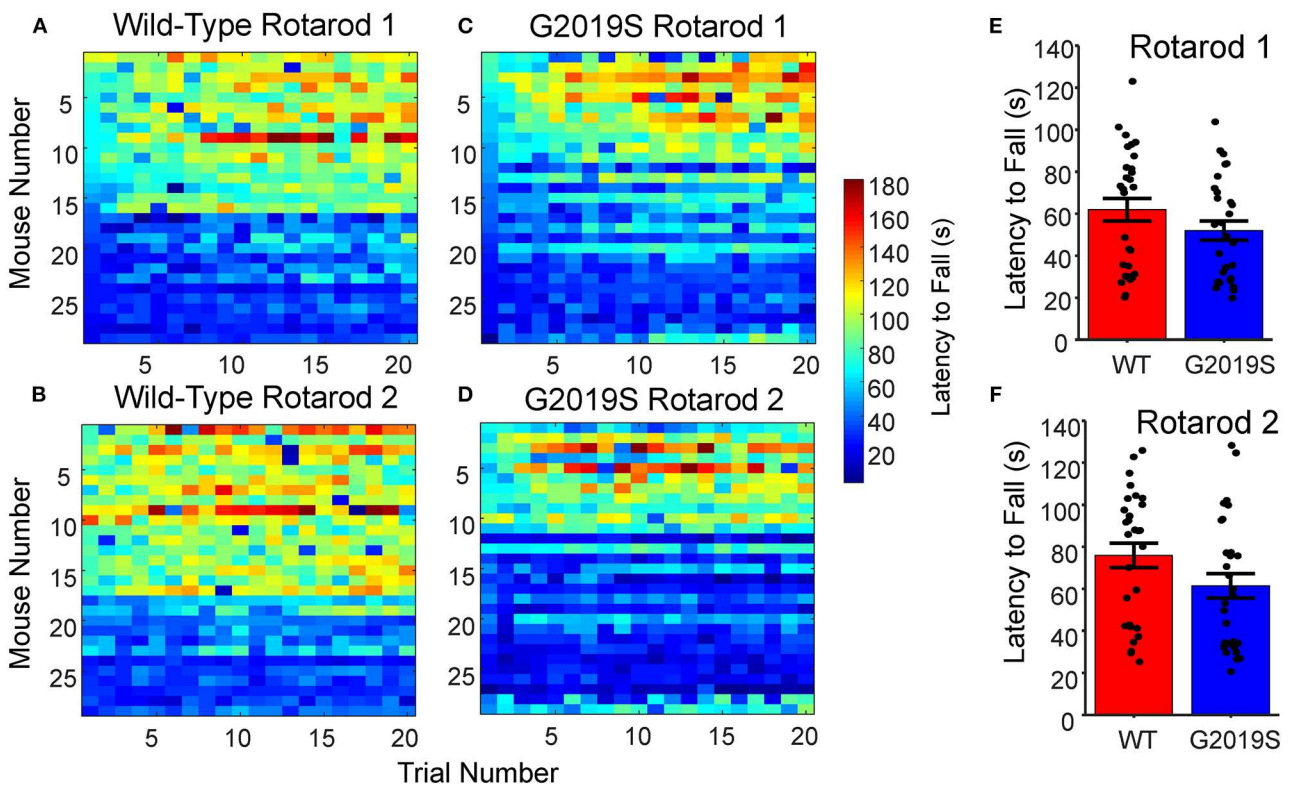

FIGURE 3 | Rotarod Motor Learning Task. **(A–D)** Raw latency to fall measures for all WT and G2019S mice sorted by performance for Rotarod 1 and 2. **(E,F)** No significant differences were present for average latency to fall between G2019S and WT for either Rotarod 1 or Rotarod 2 (*t*-test, $p > 0.05$). Error bars indicate ± SEM.

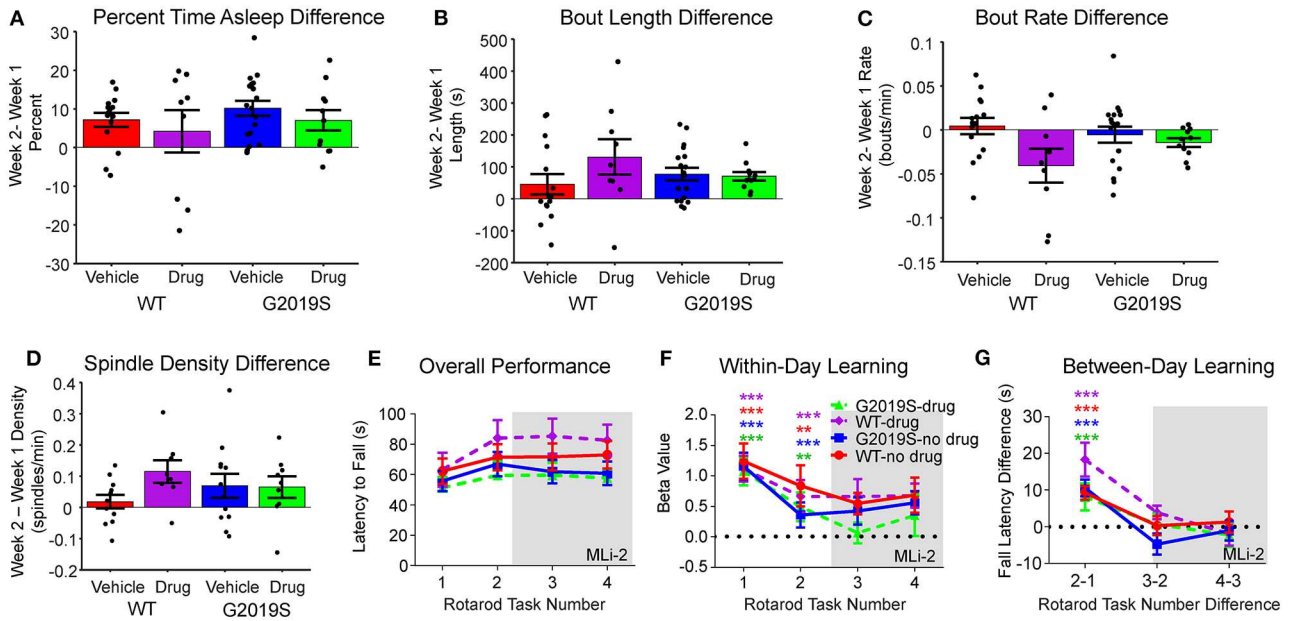

FIGURE 4 | Effect of MLi-2. There was no effect of MLi-2 on **(A)** percent time asleep, **(B)** sleep bout length, or **(C)** sleep bout rate as measured by the within-animal difference of Week 2- Week 1. **(D)** Similarly, MLi-2 had no effect on spindle density measures. **(E)** Rotarod Performance did not change with MLi-2 administration, nor were there group differences in **(F)** within-day learning or **(G)** between-day learning by drug condition.

or mean sleep bout rate ($F_{3,49} = 2.724$, $p = 0.054$; **Figure 4C**) were observed. Similarly, there were no group differences in rotarod performance during Week 2 with drug administration ($F_{3,45} = 2.71$, $p = 0.056$; **Figures 2C–E**).

It was hypothesized that if the observed increase in sleep spindle density in G2019S mice (**Figure 3J**) was the result of excessive kinase activity, MLi-2 should reduce spindle density in G2019S mice during Week 2. Subtracting Week 2 average spindle density from Week 1 average spindle density, we tested the hypothesis that G2019S-drug animals would show decreased Week 2 spindle density, but observed no effect ($F_{3,36} = 1.300$, $p = 0.290$; **Figure 4D**).

DISCUSSION

Sleep disruption is strongly associated with PD, and impaired sleep often precedes the onset of motor symptoms (9, 37, 38). Despite the close relationship between sleep disruption and PD, few studies have looked at how the G2019S mutation affects sleep behavior, and none to our knowledge have examined how sleep is altered in *LRRK2*-G2019S KI mice. Consistent with reports of sleep disturbances in *LRRK2* PD patients (11), we observed disrupted sleep in *LRRK2*-G2019S mice. Specifically, while total sleep time in G2019S and WT mice was similar, sleep bouts in G2019S mice were shorter and more frequent, indicating sleep fragmentation. These effects were not rescued by delivery of MLi-2, a potent LRRK2 inhibitor. In addition, sleep spindles were longer and more frequent in G2019S mice.

While no previous studies to our knowledge have examined sleep fragmentation in G2019S mice, sleep fragmentation (39) and insomnia (40) have been identified in other animal models of PD (41), and are reported in patients with idiopathic (38, 42) and *LRRK2* PD (11, 43). We found that G2019S mice expressed fragmented sleep (**Figure 1F**), adding validity to the *LRRK2*-G2019S KI model and suggesting that *LRRK2*-G2019S animals show prodromal PD symptoms.

A recurring concern for the study of *LRRK2* PD in mice has been the difficulty identifying motor deficits (5). Accordingly, we found no evidence for motor impairment in 8–10 month old *LRRK2*-G2019S mice. It is possible that the rotarod may have not be well-suited to identify gross motor deficits, as one study found that *LRRK2*-G2019S animals showed decreased performance on bar and drag tests at 6 months, but not the rotarod (28).

Our study is the first to identify physiological changes in sleep in the *LRRK2*-G2019S KI mouse model. Specifically, sleep-spindle density and duration were increased in *LRRK2*-G2019S mice. *LRRK2* is expressed in the thalamus and cortex, two structures crucial for the generation and maintenance of spindle oscillations (13, 14). Increased LRRK2 kinase activity resulting from the G2019S mutation has been shown to enhance neuronal excitability and glutamate release in cortical cells from *LRRK2*-G2019S KI mice (6, 44). Increased excitability could stimulate corticothalamic circuits, resulting in increased spindle density and duration. While we also predicted the oscillatory frequency and amplitude of spindles would be enhanced, no effect on these features was identified.

We observed that suppression of kinase activity through in-diet administration of MLi-2 did not alter sleep fragmentation,

spindle density, or spindle duration. This suggests that the immediate effects of the G2019S mutation did not drive the observed effect on spindle activity. It is therefore possible that long-term developmental effects from persistent increased LRRK2 activity contributed to altered spindle activity and sleep quality in G2019S mice. Furthermore, because MLi-2 was only administered for 1 week, the lack of any observed effect of MLi-2 on sleep is not necessarily indicative for the effects of long-term treatment with MLi-2.

While we observed increased spindle density in G2019S mice, there is evidence that patients with idiopathic PD express fewer spindles relative to healthy controls (38). Therefore, *LRRK2* PD may differ from idiopathic PD in its effect on spindle oscillations. Differences in sleep physiology between idiopathic and *LRRK2* PD are also suggested by the observation that while RBD is a common feature of idiopathic PD, it is less common in *LRRK2* PD (45). Future studies could test relationship between spindle density and *LRRK2*-G2019S in human *LRRK2*-G2019S patients using polysomnography.

In summary, the results of the present study suggest a link between the *LRRK2*-G2019S mutation and alterations in behavioral and physiological features of sleep in mice. None of these changes were affected by 4–7 day in-diet suppression of LRRK2 activity via MLi-2, suggesting that neural circuit and developmental changes induced by the G2019S mutation extend beyond increased kinase activity. Furthermore, the identification of increased sleep fragmentation, increased sleep spindle density, and longer sleep spindle duration may serve as early biomarkers of *LRRK2* PD.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at the University of Arizona (IACUC).

AUTHOR CONTRIBUTIONS

LC designed the study, collected the data, performed the analysis, and wrote the manuscript. MB designed the study, collected the data and assisted with review. J-PW designed the study and performed the analysis. AE collected the data and performed the analysis. EM collected the data and performed the analysis. KG collected the data. LW collected the data and performed the analysis. MF performed the histological analysis. TF designed the study and edited and reviewed the manuscript. SC designed the study, performed the analysis, and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00324/full#supplementary-material

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Metformin as a Potential Neuroprotective Agent in Prodromal Parkinson's Disease—Viewpoint

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To date, there are no clinically effective neuroprotective or disease-modifying treatments that can halt Parkinson's disease (PD) progression. The current clinical approach focuses on symptomatic management. This failure may relate to the complex neurobiology underpinning the development of PD and the absence of true translational animal models. In addition, clinical diagnosis of PD relies on presentation of motor symptoms which occur when the neuropathology is already established. These multiple factors could contribute to the unsuccessful development of neuroprotective treatments for PD. Prodromal symptoms develop years prior to formal diagnosis and may provide an excellent tool for early diagnosis and better trial design. Patients with idiopathic rapid eye movement behavior disorder (iRBD) have the highest risk of developing PD and could represent an excellent group to include in neuroprotective trials for PD. In addition, repurposing drugs with excellent safety profiles is an appealing strategy to accelerate drug discovery. The anti-diabetic drug metformin has been shown to target diverse cellular pathways implicated in PD progression. Multiple studies have, additionally, observed the benefits of metformin to counteract other age-related diseases. The purpose of this viewpoint is to discuss metformin's neuroprotective potential by outlining relevant mechanisms of action and the selection of iRBD patients for future clinical trials in PD.

Keywords: Parkinson's disease, prodromal, metformin, neuroprotection, idiopathic REM behavior disorder

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INTRODUCTION

PD is the fastest growing neurodegenerative age-related disorder with numbers of patients projected to double by 2040 globally (1). The neuropathology is complex and mainly characterized by two features, a selective degeneration of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and the presence of fibrillar aggregates referred to as Lewy bodies (LBs), mainly composed of α-synuclein, which manifest in motor and non-motor features (2–4). According to Braak's hypothesis, the progressive accumulation of α-synuclein-rich LBs begins in the medulla oblongata and anterior olfactory structures and progresses in a stereotypical bottom-up caudo-rostral direction to the neocortex (5). This concept is concordant with the now recognized prodromal state of PD, in which the ongoing pathological process confined to the lower brainstem areas are associated only with some specific non-motor/pre-motor features of PD (6). Many would therefore argue that the emergence of these symptoms along with some genetic markers in susceptible individuals could mark the beginning of PD.

Despite a massive effort involving preclinical investigations and post-mortem studies, the precise pathogenic mechanisms remains unclear and no unifying mechanism has been discovered to account for neurodegeneration in PD (7). It is likely that PD may occur as a result of multiple variable processes involving a range of pathogenic mechanisms. These include, mitochondrial dysfunction, oxidative stress, protein aggregation, abnormal protein degradation due to alterations in proteostasis mechanisms, neuroinflammation, and aging (8–10). While many of these processes might initiate the pathogenic process, by the time motor symptoms become manifest, a cascade of biochemical events leading to cell death will have become engaged. This might explain why clinical trials aimed at early intervention for neuroprotection or neuromodulation, based on preventing discrete components of this cascade, have consistently failed in the face of the already established widespread pathological change and a maelstrom of disruption of cellular functions. At present there are no clinically effective neuroprotective or disease-modifying strategies available for PD and this remains one of the defining unmet needs in the management of this challenging disorder (11, 12). Worryingly, a plethora of potential neuroprotective agents have been produced on the back of *in vitro* models of dopaminergic cell death and positive effects in *in vivo* animal models of the presumed pathogenic processes occurring in PD, but none have translated into effective treatments in man (13, 14). Over a billion US dollars have been spent by charity and industry to fund, develop and validate neuroprotective treatment strategies but to no avail.

THE FAILURE OF CURRENT CLINICAL TRIAL DESIGNS

An alternative explanation for the failure to develop neuroprotective or disease-modifying strategies may rest with the design of clinical studies as these presume that all patients with PD have identical pathogenic mechanisms underlying their disease, which is unlikely to be true. In addition, most neuroprotective trials in PD have enrolled patients either in a "*de novo* untreated stage" or "early stable treated stage" in attempts to intervene at a point where the rescue of neurons is still feasible—yet the results have been uniformly negative (15). This approach may be flawed as post-mortem studies show that by 4 years after a clinical diagnosis of PD, there is already virtually complete nigrostriatal denervation of the dorsal putamen, profound nigral cell loss and abundant Lewy pathology, which raises the question of the possibility to achieve neuroprotection even in these "early motor stages" (16). It is thus crucial to understand when to intervene, taking into account the natural history pattern of PD, which develops from a pre-prodromal and prodromal stage progressing through stable and unstable phases to a palliative stage (17) (**Supplementary Figure 1**).

A body of clinical and pathological evidence support the concept of a prodromal stage of PD existing several years, maybe decades, prior to formal PD diagnosis (6, 18). This prodromal stage theoretically represents the ideal time point during which neurodegeneration has just commenced and restoration or protection is still feasible (19). Indeed, recent observations suggest that mitochondrial dysfunction, increased glycolysis and neuroinflammation occur in the prodromal stage of PD (20). In addition, most PD studies continue to mainly focus on motor endpoints despite PD being recognized to also be a non-motor disorder, with a complex range of non-motor symptoms (NMS) that span from prodromal to advance stages of the disease (21). These include dysphagia, autonomic dysfunction, sleep disorders, mood disturbances, cognitive impairment, and dementia (22, 23). This raises the possibility that undertaking clinical trials in the prodromal phase could be an essential step for investigating disease progression and testing agents with putative neuroprotective or disease modifying effects. In addition, drug repurposing could represent an interesting source of candidates to treat or slow diseases as costs are considerably lower than those needed for designing and optimizing a new drug. Furthermore, the safety and tolerability of many repurposed molecules is likely to have been already been established, making clinical trials more cost-effective and in need of smaller samples sizes. To date, drug repurposing have shown many advantages mostly in symptom management of disease progression (24). A drug has yet to be found that can fully revert or prevent the mechanisms of neurodegeneration, however anti-diabetic drugs have been proven to be safe and potentially effective in the treatment of PD.

ASSOCIATION BETWEEN TYPE 2 DIABETES MELLITUS AND PD

An association between type 2 diabetes mellitus (T2DM) and PD has previously been reported (25), although meta-analyses of prospective cohort studies now suggest that the risk of developing PD among diabetic patients is quite low (26). Patients with both T2DM and PD present aggravated motor symptoms, higher degree of cognitive impairment and earlier onset of motor complications compared to non-diabetic subjects with PD (27–31). In addition, common pathogenic mechanisms also exist between PD and T2DM (32) and are listed in **Table 1**.

Anti-diabetic Drugs and PD Progression

The potential effect of anti-diabetic drugs on the progression of PD has been assessed by several groups. For example, the use of thiazolidinediones was associated with a decreased risk of developing PD in diabetic patients (40) and a reduction of neurodegeneration and neuroinflammation in animal models (41–44). Incretin mimetic agents such as exenatide may also confer some degree of neuroprotection in functional models of PD (45–48). Exenatide has been shown to reduce dopaminergic cell death, improve motor and cognitive functions, decrease neuroinflammation and mitochondrial dysfunction (45, 49–51). A single-center randomized, double-blind, placebo-controlled trial showed that patients with moderate PD treated with exenatide once a week for 48 weeks had a significant 3.5-point advantage compared with those given placebo in the Movement Disorders Society Unified Parkinson's Disease Rating

TABLE 1 | List of common pathogenic mechanisms that may exist between PD and T2DM.

PD link target	Possible mechanism	References
Striatum	Impaired DA function in the dorsal striatum and impaired SN iron homeostasis	(33)
Peroxisome and mitochondria	Reduced expression of PGC-1α	(34)
Inflammation	Increased neuroinflammation	(35)
Amyloid and α-synuclein	Acceleration of α-synuclein amyloid fibril formation	(36)
SNCA gene in *SNCA*-deficient mice	Association between *SNCA* and insulin resistance	(37)
DJ1 and *PINK1* genes	Dysfunction linked to insulin resistance in mouse models	(38) (39)

From (32).

Scale (52). This study also observed some improvement in cognitive decline associated with PD (53, 54). Undoubtedly these initial findings are encouraging and also provide evidence that anti-diabetic agents may have a therapeutic or potentially a neuroprotective role in the treatment of PD. Furthermore, a national multicentre study addressing the potential of exenatide and neuroprotection has now begun in the UK to explore if the findings of the single-center study can be replicated. We posit that another well-established, well-tolerated anti-diabetic drug, metformin, with a long-established safety record should be also investigated in PD. Preclinical studies have shown that metformin may target most pathological mechanisms involved in PD and improve some aging outcomes. In addition to having a pleiotropic action, metformin has the advantage of being orally administered and thus preferential for patients to use over a long period of time, compared to subcutaneous injections needed to administer exenatide.

RATIONALE FOR SELECTING METFORMIN

Metformin is a cheap yet highly effective drug which has been used for over 50 years for the management of T2DM (55, 56). It is a synthetic dimethyl biguanide, orally administrated, which has been shown to reduce total mortality compared to other diabetes agents (57, 58). Metformin has a global safety record, is well-tolerated by the majority of patients and is used by roughly 125 million people worldwide (59). Metformin does not undergo significant metabolism and is excreted unchanged via the organic cation transporter-2 in the kidney (59, 60). Metformin does not have significant adverse effects and it has low risk for hypoglycaemia, however, it may cause vitamin B-12 deficiency (61) and lactic acidosis, mainly in patients with significant renal function impairment (62, 63). It is of important note that reported incidence of lactic acidosis in patients receiving metformin is very low and in over 20,000 patients exposed to metformin in clinical trials, there were no reports of lactic acidosis (64); diarrhea, nausea and stomach upset represent more common side effects (65).

Beyond its anti-diabetic properties, metformin has a pleiotropic action and potentially slows aging by targeting mitochondrial metabolism and insulin signaling (66). Recent studies have demonstrated that metformin can rapidly penetrate the blood–brain barrier (67) and confer neuroprotection against stroke, cognitive impairment, Huntington's disease and potentially prevent dementia (68–73). Metformin can reduce α-synuclein phosphorylation and aggregation, influence cellular processes associated with age-related conditions including inflammation and autophagy, all of which are associated with PD pathogenesis. These actions are described in detail below as they may represent the potential of metformin to be neuroprotective or disease modifying in PD (74) (**Figure 1**).

MECHANISMS OF ACTION OF METFORMIN

Mitochondrial Dysfunction

Mitochondrial dysfunction is commonly accepted as a key component of the pathogenesis of PD—through the inhibition of complex I and oxidative stress in sporadic disease and the linkage to *SNCA*, *parkin*, *PINK1*, *DJ-1,* and *LRRK2* mediated genetic forms of the illness (9, 75–77). Metformin also acts on mitochondria to alter the activity of the respiratory chain and to decrease reactive oxygen species (ROS) (78–81). This may have functional significance as metformin can protect dopaminergic cells against MPP^+ toxicity *in vitro* by attenuating mitochondrial dysfunction and oxidative stress (82). Whether this translates *in vivo* is unclear as metformin has not consistently protected dopaminergic cells against toxin induced damage although it may reduce markers of oxidative stress—such as superoxide dismutase (83) and alter the expression of key mitochondrial proteins in basal ganglia (84). Metformin also restores the mitochondrial integrity of dopaminergic neurons disrupted by p*arkin* or *LRRK2* mutations in fruit flies (85). Importantly, metformin promotes the expression of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) (84), a key regulator of mitochondrial biogenesis (86). PGC-1α is downregulated in the brain in PD and it protects dopaminergic neurones in animal models of PD (87). Although not directly focused on PD, metformin can alter mitochondrial fission and fusion protein expression and mitochondrial fragmentation in experimental systems linked to diabetes-induced oxidative stress, endothelial dysfunction, atherosclerosis development and Down's syndrome (88, 89). All of this being supportive of a potential neuroprotective role.

It is of important note that most of metformin's effects are an indirect result of complex I inhibition, although its

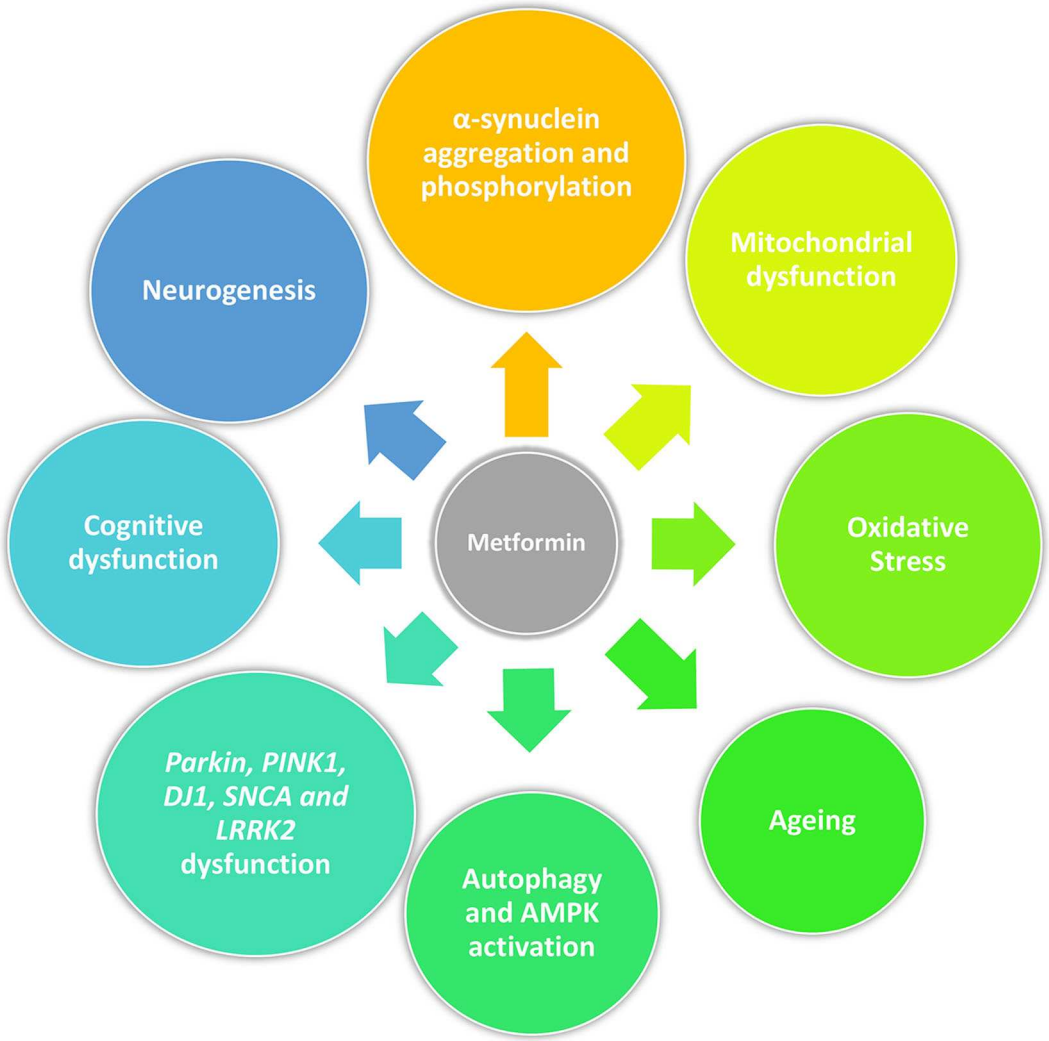

FIGURE 1 | Pleiotropic action of metformin in PD. Beyond its anti-diabetic properties, metformin may act as a neuroprotective drug by reducing α-synuclein phosphorylation and aggregation, mitigating mitochondrial dysfunction and oxidative stress, influencing cellular processes associated with age-related conditions including cellular senescence and autophagy, and promoting neurogenesis. Furthermore, it could restore physiological molecular functions disrupted by genetic mutations related with PD (*Parkin*, *PINK1*, *DJ1*, *SNCA,* and *LRRK2*) and have an effect on cognition.

exact mechanism warrants further investigation. Complex I alterations are widely associated with mitochondrial dysfunction and PD risk, as previously described in MPTP and rotenone models (90, 91). Despite this contradiction, however, sub-lethal concentrations of complex I inhibitors, such as metformin, which do not generate ROS (or produce a reduced amount), could still be beneficial and have a neuroprotective action (74).

α-Synuclein Aggregation and Phosphorylation

The relationship between α-synuclein accumulation in LBs and neuronal toxicity is strong (92, 93). The evidence from familial *SNCA* mutations and PD in man coupled to the clear toxicity of fibrils and protofibrils of α-synuclein presents an opportunity for interfering in the final pathogenic process. Based on a range of experimental models of PD, metformin acts to counter the toxicity of the protein. In MPTP-treated mice, metformin reduced α-synuclein expression and the number of α-synuclein positive cells (82). In *C. elegans*, metformin reduced the loss of dopaminergic neurons and decreased α-synuclein aggregation induced by 6-hydroxydopamine (94). Recently, metformin treatment was shown to attenuate dopaminergic cell loss and α-synuclein accumulation in the SN of rotenone-treated mice (95). How metformin alters α-synuclein toxicity is not clear but the drug is able reduce the phosphorylation of the protein that is key to the mediation of its toxicity (96). This may relate to the ability of metformin to increase the activity of phosphatases involved in α-synuclein dephosphorylation as shown in the SN of MPTP-treated mice (97).

Autophagy

Autophagy is a key cellular mechanism in protein homeostasis on which many of the pathogenic pathways involved in PD eventually converge (98). Autophagy plays a role in α-synuclein handling, mitochondrial function and oxidative stress, emphasizing its potential as a target for neuroprotection and disease modification (99, 100). Metformin has actions in experimental models of PD related to alterations in autophagy. For example, in MPTP-treated mice, metformin treatment prevented dopaminergic cell death and reduced motor impairment while decreasing α-synuclein aggregation, autophagic impairment, and ROS (82). The possible mechanism of this effect is not clear but may involve activation of AMP-activated protein kinase (AMPK) through the mitochondrial effects of metformin which in turn leads to an induction of autophagy involving in part, autophagosome formation and lysosomal biogenesis (101–104). Support for a role of AMPK comes from a study in MPTP-treated mice where downstream effectors of AMPK prevented dopaminergic cell death and motor impairment (105). This is supported by data from other areas, for example ischemia, where metformin exerts neuroprotective actions through manipulation of autophagy (106–108).

Neurogenesis

Dopamine modulates ontogenetic neurogenesis (109). Post-mortem studies suggest that dopamine depletion may impair neuronal precursor cell proliferation in PD (109, 110) and thus negatively impact neurogenesis. While the relationships are not fully understood, impaired neurogenesis in the subgranular zone of the hippocampus and olfactory bulb of PD patients (109) likely contributes to memory deficits (111), depression (112), and olfactory dysfunction (113), commonly present in PD. Wang et al. (114) showed that metformin treatment could stimulate neurogenesis via an atypical PKC-CBP (Protein kinase C-CREB-binding protein) pathway. In particular, the transcription factor CREB (c-AMP response element-binding protein), was found to be a key component for neurodevelopment, cell survival, plasticity, memory and learning (115). Additionally, CREB was shown to regulate TH gene expression (116). At the molecular level, metformin may also upregulate the expression of the brain-derived neurotrophic factor (BDNF) by activating AMPK/CREB-mediated histone acetylation improving the ability of mice to resist stress (117). Therefore, activation of CREB by metformin could boost compensatory and regenerative mechanisms in the brain.

STUDIES ON METFORMIN TARGETING AGE-RELATED DISEASES

Beyond the positive effects on multiple PD underlying mechanisms, metformin has also been shown to delay aging and extend lifespan in nematodes and rodents models (118–121). Metformin has, additionally, been considered to be effective in multiple human studies targeting age-related diseases. It was shown to delay cardiovascular disease, providing the rationale for metformin's designation as first-line therapy for most patients with T2DM UK Prospective Diabetes Study (UKPDS) Group (122). A recent study has shown lower mortality in patients with T2DM on metformin compared with non-diabetics despite the fact that the diabetic patients were more obese and had greater co-morbidities at baseline (123). Preliminary data support the concept that metformin may reduce the risk of cognitive impairment and dementia in both T2DM and non-T2DM (69, 72, 124). Long-term metformin therapy was also associated with lower incidence of neurodegenerative disorders among elderly veterans with T2DM (125). An ongoing trial called "Targeting Aging with Metformin" is validating metformin's ability to delay the onset of comorbidities related to aging (126). In relation to PD, clinical studies have mainly investigated the effects of metformin in comparison to, or in combination with other anti-hyperglycaemic agents and taken together all the studies look at different medications and are hardly comparable (40, 127). There is thus a lack of studies specifically evaluating the neuroprotective effects of metformin on PD development and/or progression.

THE SELECTION OF AN "ENRICHED" COHORT OF PRODROMAL PD FOR A METFORMIN TRIAL

To confer neuroprotection against PD, in addition to understanding the cellular mechanisms involved in PD pathogenesis, it is crucial to perform studies in a group of patients that reside in the prodromal stage of the disease and who will most certainly phenoconvert to clinical PD within a reasonable time. The identification of such group would allow the testing of a putative neuroprotective or disease-modifying agent, such as metformin, in the ideal time frame to maximize the possible beneficial impacts (18).

The definition of this stage requires working with the probability of conversion to overt PD in large populations of "at-risk" subjects. These include patients with idiopathic rapid eye movement behavior disorder (iRBD), olfactory dysfunction, autonomic dysfunction, depression, excessive daytime sleepiness, constipation, or carriers of a known PD mutation such as LRRK2 or GBA. iRBD is defined as apparent acting out of dreams during REM sleep, associated with a loss of normal REM sleep atonia (128). iRBD has a strong evidence on being a predictor of synucleinopathies as multiple single-center prospective cohort studies have documented that iRBD phenoconverts to PD, dementia with Lewy bodies or multiple system atrophy in 80% of cases over a 6–10 years and possibly to a higher rate over 12 years (129–133). This risk is higher if a person with iRBD also displays other prodromal features of PD (olfactory dysfunction or constipation), shows a reduced uptake on presynaptic dopamine transporters (DaTSCAN) (134–137) or has mild bradykinesia. It is impossible to give a definitive conversion rate in iRBD patients as data are only available from a few highly selected cohort studies (136, 138). However, a likelihood ratio (LR) of several motor and non-motor features of phenoconversion to clinical PD is available (132, 139, 140). The LR is the highest for RBD, followed by a positive DaTSCAN and hyposmia (140). Thus, "enriched"

iRBD cases (with hyposmia, or positive DaTSCAN) may provide an earlier window of phenoconversion and an optimal group to study neuroprotection (141). In addition, if such patients have mild bradykinesia, the time of phenoconversion is likely to be within 4 years as shown by a controlled study by Schrag et al. (142). We therefore would propose a randomized double blind placebo-controlled trial with metformin in an enriched iRBD cohort where other risk factors are also comorbid (hyposmia, abnormal DaTSCAN and/or bradykinesia) with a follow up period of 4–6 years to allow for the maximal possibility of phenoconversion to PD.

CONCLUSION

To date, all clinical disease-modifying trials in PD have been performed in individuals in the manifested "in-life" motor stage, targeting either early "*de novo*" PD or treated PD and have all failed to show any convincing effects on neuroprotection. Investigating the prodromal phase of the disease could offer greater promise of success, assuming the less-advanced pathology and the greater potential to intervene at key points of the molecular pathogenesis. There are currently no studies using metformin to evaluate a possible protective effect on motor and non-motor functions on PD, although the idea seems compelling. Given its properties and the fact that mitochondrial dysfunction, autophagy, α-synuclein, aging, have all been proposed to be involved in PD pathophysiological processes, and the potential benefits of metformin to counteract age-related disorders (cancer, cardiovascular and neurodegenerative diseases), metformin seems a reasonable pluripotent agent to try given its safety, ease of use and wide availability. The above evidence encourages us to pilot a study investigating the role of metformin as a potentially neuroprotective agent in prodromal PD. iRBD subjects have a high likelihood to convert to PD or a related synucleinopathy and may therefore represent an ideal group for neuroprotective trials, enabling the field to push into investigating the prodromal stage of the disease and hopefully prevent or slow the development of PD.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

CS conceptualized the paper, drafted, and revised the manuscript. DU drafted and revised the manuscript. PJ revised the manuscript. KC conceptualized the paper and revised the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00556/full#supplementary-material

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REVIEW
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A Neurologist's Guide to REM Sleep Behavior Disorder

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REM Sleep Behavior Disorder (RBD) is a chronic sleep condition characterized by dream enactment and loss of REM atonia. Individuals often present to clinic with complaints of injury to themselves or their bed-partner due to violent movements during sleep. RBD patients have a high risk of developing one of the neurodegenerative α-synucleinopathy diseases: over 70% will develop parkinsonism or dementia within 12 years of their diagnosis. RBD patients also exhibit accelerated disease progression and a more severe phenotype than α-synucleinopathy sufferers without RBD. The disease's low prevalence and the relatively limited awareness of the condition amongst medical professionals makes the diagnosis and treatment of RBD challenging. Uncertainty in patient management is further exacerbated by a lack of clinical guidelines for RBD patient care. There are no binary prognostic markers for RBD disease course and there are no clinical guidelines for neurodegeneration scaling or tracking in these patients. Both clinicians and patients are therefore forced to deal with uncertain outcomes. In this review, we summarize RBD pathology and differential diagnoses, diagnostic, and treatment guidelines as well as prognostic recommendations with a look to current research in the scientific field. We aim to raise awareness and develop a framework for best practice for RBD patient management.

Keywords: REMsleep behavior disorder (RBD), Parkinson's disease, prodromal Parkinson's disease, sleep disorders, neurology, neuroscience, sleep

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SYMPTOMS AND DIAGNOSTIC CONSIDERATIONS

Rapid Eye Movement (REM) Sleep Behavior Disorder (RBD) is a non-familial sleep disorder, characterized by the loss of the inherent muscle atonia observed during normal REM sleep. This phenomenon is often referred to as REM Sleep without Atonia (RSWA). Whilst isolated RSWA is frequently an incidental finding in sleep studies, it forms the substrate of the dream enactment behavior which defines RBD. Here, individuals experience vivid dreams which they act out during sleep.

It is important to remember that dream enactment and limb movements during sleep can occur in the healthy population, often in the context of heightened emotional states (1–3). The same symptoms may also be experienced during withdrawal from sedatives or alcohol. In non-pathological dream enactment, individuals typically respond to dream content during the transition from REM sleep to the awake state and while maintaining REM atonia during much of the REM period. In contrast, RBD individuals will maintain REM sleep during and immediately after most of their dream enactments. As acute dream enactment is generally self-limiting, the

chronicity of symptoms (>6 months) is a key distinguishing factor, and forms part of the diagnostic criteria for RBD (4).

Anecdotally, dreams are often reported by patients with RBD as violent or aggressive, resulting in violent motor behaviors which may pose a threat to them or their bedpartner (5). Whilst accounts of individuals kicking, punching, biting, or even strangling their bedpartners during sleep paint an emotive image of the condition and often capture public interest, they are prone to recall bias. More systematic studies have revealed that violent dreams and behaviors only make up a small percentage of all events (6–8). When a dream enactment is occurring, the individual's eyes will remain closed as they engage with the dream environment and their movements are generally contained to their immediate surroundings, thus differentiating these episodes from NREM parasomnias such as sleepwalking (4). Upon awakening from a large motor event, the RBD individual will be alert and orientated to their surroundings (4).

The frequency of motor events may vary greatly between RBD individuals; ranging from multiple episodes per night, to one episode per month (9). In any one patient, the severity and frequency of the behaviors may also vary from night to night, and over the course of their condition (10). The mechanisms behind this fluctuation remains unknown.

Etiology

The behavioral states of wake and sleep are initiated and maintained by complex interplay between multiple brainstem and diencephalic nuclei. Dysregulation, disease or degeneration of these nuclei can result in sleep disorders, such as narcolepsy, and subtle changes to sleep-wake patterns. In the case of RBD, the primary pathology appears to be an excitation/inhibition imbalance in the brainstem nuclei controlling REM muscle tone.

Movement during REM sleep is controlled by two systems: one controls the input to spinal cord motoneurons to generate muscle atonia (extrapyramidal), and the other controls motor cortex activation to suppress locomotor activity (pyramidal). The main generator of REM-sleep is the predominantly-glutamatergic Subcoeruleus/Pre-Locus Coeruleus complex [SubC/PC- analogous to the rat/mouse sublaterodorsal nucleus (SLD)], which is anatomically situated just below the noradrenergic locus coeruleus in the pons (11). As well as projecting to many subcortical brain regions to promote and maintain REM sleep, the SubC/PC projects caudally to control the REM atonia neural network (12). Preceding and during REM sleep, the REM-active SubC/PC excites the inhibitory ventromedial medulla (VMM) and glycinergic neurons of the spinal ventral horn, which in turn tonically hyperpolarize spinal motor neurons (12, 13). This results in a temporary paralysis of skeletal muscles and thus significantly reduced REM muscle tone.

Disruption to this process results in abnormal motor behaviors during REM sleep (**Figure 1**).

It is not definitively known whether RBD is caused by an imbalance originating in the glutamatergic SubC/PC or downstream in the GABA/Glycinergic VMM, though evidence from animal studies suggest the latter is more likely (14). This brainstem pathology does not exist in isolation. Given that RBD is characterized not just by an increase in small sleep twitches but also complex movements and dream enactment, it is likely that abnormal disinhibition occurs in the pyramidal motor tract during REM sleep, leading to execution of the complex movements "imagined" by the motor cortex. Imaging studies have shown that RBD can also be accompanied by changes in multiple neurotransmitter systems, including the cholinergic, noradrenergic, and dopaminergic circuits (15). Thus, one of the key challenges in treating RBD derives from the uncertainty surrounding its causative pathology and the extent of dysfunction throughout the brain.

RBD may present on its own, often referred to as idiopathic RBD (iRBD), or may exist as a secondary entity in the context another condition. Regardless of cause, all RBD subtypes are likely to reflect dysfunction at some point in the complex, interconnected REM atonia circuits.

"Idiopathic" RBD

These patients usually present to sleep clinics with a history of dream enacting behaviors and a present complaint of recent sleep-related injury to themselves or their bedpartner, despite no other health complaints or recent medication changes. Whilst previously considered an idiopathic phenomenon, the unquestionable link with alpha-synucleinopathies has challenged this view.

Approximately 10 years after the first description of RBD in the scientific literature, Schenck et al. reported the development of parkinsonism in ∼40% iRBD individuals (16). Since then, RBD has emerged as one of the most specific predictors of the synuclein-mediated neurodegenerative diseases: Parkinson's disease (PD), Multiple System Atrophy (MSA) and Dementia with Lewy Bodies (DLB). It is now estimated that up to 90% of patients with "iRBD" will eventually develop one of the α-synucleinopathies (17).

Given that RBD is found to occur, on average, 8 years before the presentation of the core motor or cognitive symptoms required for the clinically diagnosis of PD or DLB (18), there is increasing evidence to suggest that in most cases RBD is the early manifestation, or prodrome, of a clinically-defined neurodegenerative disease. Indeed, detailed assessments often reveal subtle features of these conditions, such as hyposmia, constipation, or a slight tremor (19), that can often be missed during a clinical consultation.

Whether any truly idiopathic RBD cases exist, or whether all cases of iRBD will eventually convert to an α-synucleinopathy given sufficiently follow-up time, is currently unknown. However, given currently available evidence many in the field have moved away from the idiopathic label (3).

Secondary RBD

The onset of RBD symptoms may coincide with the initiation of certain drugs (20), the most common being the anti-depressant SSRIs (21) which cause RBD behaviors in up to 6% of users (22). Treatment using SSRIs results in increased serotonergic tone during both wakefulness and sleep, which in turn may interfere with mechanisms of REM atonia (21).

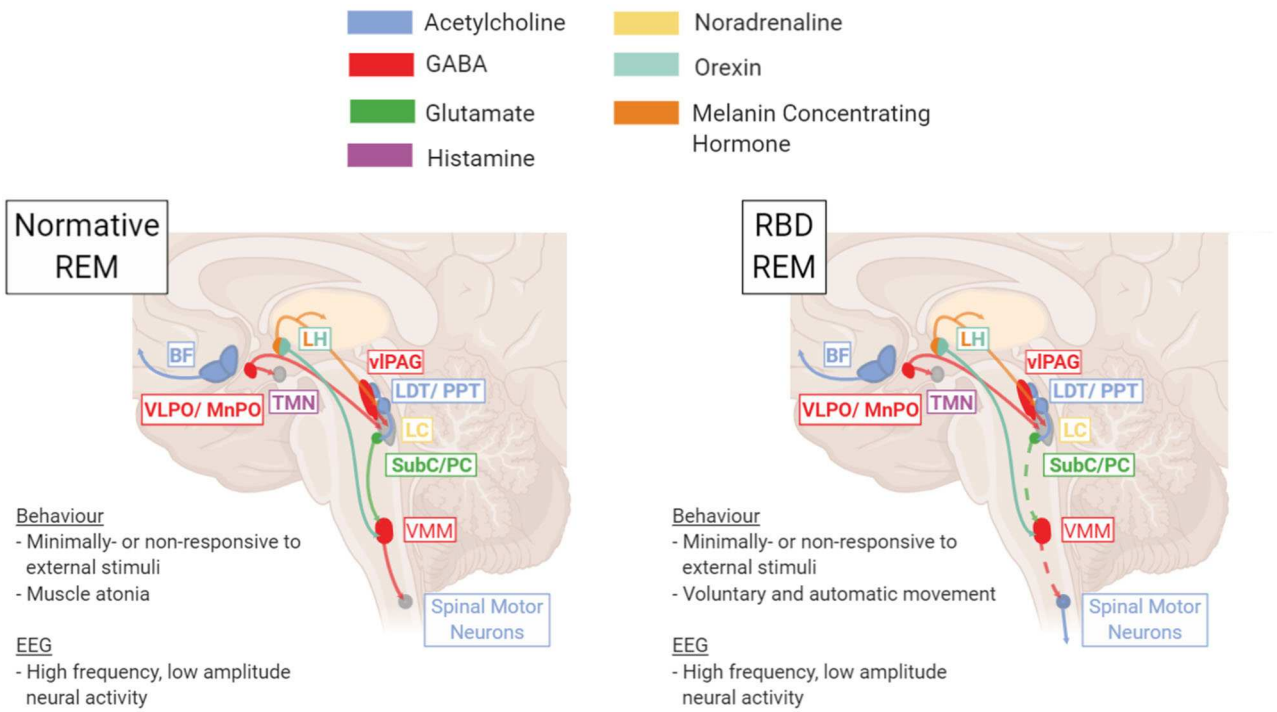

FIGURE 1 | Key brain regions and neurotransmitters involved in regulation and maintenance of the REM sleep stage under healthy normative or pathological RBD conditions. In RBD, dysfunction within the SubC→VMM→Spinal Motor Neuron pathway results in a lack of REM atonia (depicted with dotted line). BF, basal forebrain; LC, locus coeruleus; LDT/PPT, laterodorsal tegmentum/pedunculopontine tegmentum; LH, lateral hypothalamus; Subc/PC, subcoeruleus/pre-locus coeruleus; TMN, tuberomammillary nucleus; vlPAG, ventrolateral periaqueductal gray; VLPO/MnPO, ventrolateral preoptic nucleus/median preoptic nucleus; VMM, ventromedial medulla. Figure created using BioRender.com.

Other examples of secondary RBD include those caused by neurological lesion affecting sleep/wake regulatory brain regions, most commonly within the brainstem. RBD due to lesions is rare and most commonly associated with meningiomas and subsequent disruption of pontine REM-atonia structures (23), although cases of narcolepsy (24), pontine cavernoma (25), pontine lymphoma (26), multiple sclerosis (27), and acute inflammatory rhombencephalitis (28) all give further examples of RBD incidence secondary to lesion.

Though RBD may precede the diagnosis of a clinically-defined neurodegenerative disease, as seen in the majority of "idiopathic" RBD patients, it often emerges concomitantly around the same time or subsequent to a synucleinopathy diagnosis. The focus on RBD as a prodrome often leaves concomitant RBD, or RBD secondary to neurodegeneration, to fall by the wayside, with the distinction and prevalence of such cases in the α-synucleinopathy populations seldom reported in the literature.

RBD occurs concomitantly in up to 40% of PD patients (29), with studies suggesting the majority of PD patients develop RBD alongside or after their first parkinsonian symptoms (30, 31). These individuals also exhibit a more advanced disease profile (30), with greater cognitive impairment (31) compared to those whose RBD preceded their PD, warranting further investigation into the temporal spread of neurodegeneration in α-synucleinopathies. For DLB and MSA, prevalence of concomitant RBD may be as high as 76% (32) and 88% (33), respectively, though no research has investigated the timing of RBD occurrence in these populations.

The temporal variation in RBD occurrence highlights the importance of differentiating between RBD as a prodrome and as a concomitant symptom of α-synucleinopathies, especially when conducting research to phenotype and stratify RBD in α-synucleinopathic populations.

EPIDEMIOLOGY

RBD typically presents from the 6th decade of life onwards, with cases of medication- or lesion-induced disease more commonly seen in those under 50 years (34).

The true prevalence of RBD in the general population is very difficult to gauge. Due to its REM state-specific occurrence, individuals are often unaware of their behaviors. Therefore, clinic-based estimates typically only capture those prompted to seek medical advice by their bedpartner or, less frequently, those that have injured themselves during violent dream enactment.

Several studies have attempted to assess the prevalence in the general population using screening questionnaires, finding the rate of probable RBD to be 0.4–5% (35–37). These investigations are not without their limitations—several conditions have similar symptomology to RBD, including the increasingly common condition obstructive sleep apnoea (38), and the aforementioned prevalence rates are likely to include individuals without RBD. Polysomnography screening of the general population gives a more accurate RBD prevalence of ∼1–2% (39–41).

Divergence between clinical and general population representations of RBD are further demonstrated by reported RBD sex differences. RBD is commonly regarded as a strongly male-predominant disease, largely based on clinical cohorts reporting a male to female ratio of 9:1 (42). This does not reflect population-based studies, where an equal sex split is reported (39). While sex differentials in disease can be due to true pathological mechanisms, or persist due to gender-biased underreporting, it is unknown which accounts for the sex difference seen in RBD. It has been speculated that men are naturally more aggressive than women and therefore are more likely to experience violent dreams and RBD behaviors. However, studies have shown that RBD dream content does not differ between sexes (43, 44) and violent dreams are not associated with higher testosterone levels (45). If and why women are susceptible to underreporting RBD, as in the case of snoring and obstructive sleep apnoea (46, 47), therefore needs further investigation. Interestingly, male sex is an identified risk factor for all of the α-synucleinopathies (48–51), though the reason for this remains unknown.

As well as male sex and antidepressant use, large cross-sectional cohort studies have consistently identified low socioeconomic status and jobs with toxic environmental exposures (e.g., farming or mining) as environmental risk factors for RBD development (35, 36, 52). Additional measures associated with compromised health—including smoking, drinking, low physical activity, cardiovascular risk factors (such as diabetes), and psychological distress—have also been implicated (35, 36, 52). The complex interplay between most of these factors is not specific to RBD and rather reflects the influence of social structures upon population health. Interestingly, the link between occupational and environmental exposures (most notably pesticides) and RBD is one that mirrors the PD population. The link between pesticides and MSA or DLB is less clear (50, 53, 54), suggesting alternative triggers for the specific strain pathology of these conditions.

RBD is non-familial and therefore susceptibility to disease development is likely to be a combination of multiple environmental and genetic determinants. Studies of RBD genetics tend to center upon single nucleotide polymorphisms (SNPs) and genetic mutations known to be associated with the α-synucleinopathies. Genetic changes in RBD populations include underrepresentation of a PD-protective MAPT (Microtubule Associated Protein Tau) SNP (55), glucocerebrosidase missense variant overrepresentation (56), altered clock gene expression (57), and SNCA (Synuclein Alpha) gene variants (58). In each instance, however, the mutations are present in only a small minority of the RBD population thus limiting the conclusions that can be drawn regarding genetic determinants of RBD.

Patient Presentation and Diagnosis

Of those that seek medical advice, patients will usually present to their GP in the instance of self- or bedpartner injury due to their dream-enacting behaviors. In the authors' experience, this is often a significant barrier to accessing help, with many patients, and GPs, not recognizing RBD as a medical problem. This is reflected by the diagnostic delay seen in RBD, cited between 7 and 9 years on average (59, 60).

When RBD is suspected outside of the setting of a designated sleep clinic, simple screening questionnaires, such as the single-question RBD1Q (61), or the more detailed RBDSQ (62), may be used to prompt further assessment or onward referral.

Whilst these questionnaires hold some value when screening for the disorder, they do not inherently encompass the diagnostic criteria, and the cut-off points used are somewhat contentious (63). Thus, these scales are yet to be used as a standardized clinical resource.

Individuals suspected of having RBD should be referred to a specialist Sleep Medicine or Neurology service for a diagnostic assessment (see **Figure 2** for an outline of the RBD diagnostic process). Although there is likely significant variability between the individual clinicians (as there are no ICSD3 or other guidelines for RBD diagnostic assessment), this initial outpatient clinical assessment broadly consists of a general examination and neurological examination to rule out differential diagnoses, coupled with several tests, and rating scales designed to assess more specific sleep and neurodegenerative aspects of the condition. Whenever possible, clinicians should make use of any information from bed-partners, as they often provide a more accurate history of sleep-related behaviors. These are also the most reliable means to assess the severity of the RBD, which, in turn, can aid management options. Clinicians should additionally take detailed note of history indicative of secondary RBD, as well as screen for α-synucleinopathic prodromal symptoms such as hyposmia, constipation, and cognitive changes.

Where indicated from the history and clinical examination, further investigations e.g., brain MRI, may be required to diagnose secondary RBD and inform treatment. The utility of DaTSCAN for diagnosing RBD secondary to α-synucleinopathy is often limited at this stage and is not recommended (see "Current Research: RBD and the α-Synucleinopathies" section for further discussion).

The gold-standard protocol for RBD diagnosis is a clinical assessment coupled with a subsequent overnight video polysomnography (v-PSG) study. The collective measures from these form the basis of the most commonly used diagnostic criteria, the International Classification of Sleep Disorders (3rd Edition) (ICSD-3) (see **Table 1**).

While the diagnosis of probable RBD can be solely made based on a detailed history, meeting points 1, 2, and 4 on the ICSD-3 RBD Diagnostic Criteria, a confirmatory v-PSG study is required for a formal diagnosis to be made. The v-PSG is an inpatient sleep study, and therefore may require the patient to be further referred to a specialist sleep center. The study itself consists of electroencephalography (EEG) to assess brain activity, electromyography (EMG) for muscle activity, respiratory, oximetry, and heart rate monitoring and

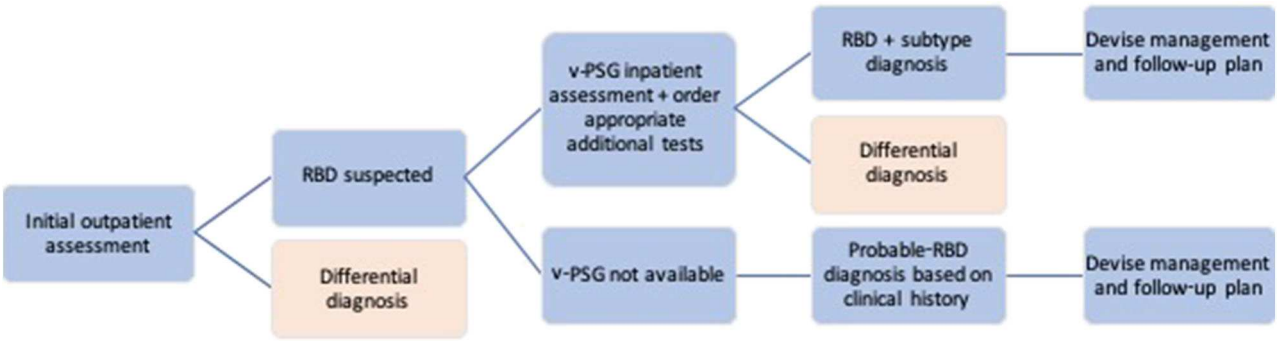

FIGURE 2 | Flow chart summarizing the process of RBD diagnosis.

TABLE 1 | Diagnostic criteria for REM sleep behavior disorder (4).

Criteria	Description
1	Repeated episodes of sleep-related vocalization and/or complex motor behaviors
2	These behaviors are documented by polysomnography to occur during REM sleep or, based on clinical history of dream enactment, are presumed to occur during REM sleep
3	Polysomnographic recording demonstrates REM sleep without atonia (RSWA)
4	The disturbance is not better explained by another sleep disorder, mental disorder, medication or substance abuse

All 4 criteria must be met for a definite RBD diagnosis. If v-PSG is not available, or RSWA is not captured during v-PSG then a provisional diagnosis of RBD can be given if all other criteria are met.

video recording of the patient while they sleep. It is also advisable for the patient to undergo a next-day Multiple Sleep Latency Test (MSLT) to control for excessive daytime sleepiness and narcolepsy.

The minimum aim of the v-PSG is to capture RSWA, which the ICSD-3 states is indicated by "excessive augmentation of chin EMG" or "excessive chin or limb phasic EMG twitching" during REM sleep (4). Scoring guidelines from the American Academy of Sleep Science (AASM) quantitatively define these as "a chin EMG amplitude greater than the minimum amplitude demonstrated in NREM sleep" and "transient muscle activity 0.1–5.0 s in duration at least 4 times as high amplitude as background EMG," respectively (64). Such twitches occur relatively frequently in RBD individuals so the likelihood of capturing RSWA during a diagnostic v-PSG is high. It is the large dream enactments and complex motor behaviors which are less common (for instance, which an individual may only experience once a month) and therefore rarer to capture during the sleep study. Therefore, the diagnostic criteria allow for RBD to be diagnosed based on v-PSG-confirmed RSWA with a *history* of dream enactment or sleep behaviors, acknowledging the limitations of a one-night sleep study for full phenotype capture.

The v-PSG requires not only a sleep center to physically host the study, but also technicians to score and interpret the data. The resources available to a health service will therefore determine access to v-PSG.

Though seemingly an attractive option given the limited access to sleep studies in most centers, the practice of solely relying on clinical assessment or screening questionnaires is likely to result in a significant number of false-positive diagnoses (65). This is particularly pertinent in suspected idiopathic cases, where a diagnosis of RBD should prompt discussion regarding future risk of developing a neurodegenerative disorder (see "RBD Prognosis and Communicating the Risks" section).

RBD PROGNOSIS AND COMMUNICATING THE RISKS

The prognosis for RBD depends largely upon the subtype. Patients diagnosed with RBD secondary to medication have the most promising prognosis of RBD resolution once the causative medication is withdrawn. However, it has been shown that RBD may persist following cessation of SSRIs (66, 67), and it is therefore possible that in some cases the medication simply "unmasked" an already underlying pathology, triggering early clinical presentation (22). For RBD secondary to defined lesion e.g., inflammatory plaques, the main symptoms of RBD can be controlled relatively reliably using a combination of pharmacological and behavioral treatments. As these patient's present with chronic but stable neural tissue damage, their RBD symptoms are unlikely to change over time.

For patients with RBD presenting as part of a clinically-defined neurodegenerative condition, such as PD, MSA, or DLB, the management of their sleep disorder should form part of their holistic care. Generally, the presence of RBD marks a less-favorable disease phenotype. In PD, for example, the presence of concomitant RBD is associated with a greater non-motor burden and a more adverse prognosis (68–71). There have been no studies of whether the symptomatic treatment of RBD impacts long-term outcomes in these patients.

Finally, for patients diagnosed with apparently idiopathic disease, the prognosis remains uncertain. There are currently no biomarkers or investigations to determine the personal risk of developing an α-synucleinopathy. A recent metanalysis

of the existing international prospective cohort studies found that after an average follow up of 4.6 years, 352 (28%) of 1,280 RBD patients were diagnosed with a clinically defined neurodegenerative disorder. Of those, 52% developed PD, 43.5% developed DLB, and 4.5% developed MSA (18). Color vision deficits, hyposmia, erectile dysfunction and constipation accompany the loss of REM sleep atonia in the "prodromal" disease stages, before a cluster of additional symptoms including cognitive deficits, urinary dysfunction and motor symptoms arise in the "preclinical" stage, defined as <5 years before diagnosable phenoconversion (72). Importantly, severity of all prodromal and preclinical symptoms increases over time (72), reinforcing the onus on clinicians to undertake vigilant symptom tracking of RBD patients to inform patient management and scientific research.

It is the responsibility of the clinician making the diagnosis to sensitively and clearly communicate the risks of neurodegeneration associated with RBD to their patients. Although there are no official UK guidelines for the care of RBD patients, it is in the patient's best interest to be fully informed of their condition (73). Not only does this respect and maintain the autonomy of the patient, it also facilitates the conversation of current RBD research and their potential involvement (74). It is highly beneficial for the RBD and α-synucleinopathy research fields if all patients diagnosed with RBD, regardless of subtype but especially those with iRBD, are encouraged to participate in experimental research. Further to any clinician-patient conversations, patient counseling, and advice services should be made available and recommended to the individual.

RBD MANAGEMENT

Given the general uncertainty of causative pathology and prognosis for RBD, the two greatest challenges for clinicians remain the successful management of the condition, and in the case of idiopathic or suspected prodromal RBD, symptom-tracking for neurodegeneration indicators. An overview of RBD patient management is provided in **Table 2**.

TREATMENT

The treatment of RBD falls into two categories: pharmacological and behavioral. Unfortunately, as no cure for the disorder exists, management remains symptomatic, with highest priority placed on controlling the extreme and potentially injurious motor behaviors. Many patients will, therefore, elect not to pursue any treatment, especially when the impact of the condition on their quality of life is low.

BEHAVIORAL

As there are no reported associations between daytime events (e.g., stress, alcohol intake) and subsequent night-time RBD behaviors (9), behavioral recommendations focus on the creation

TABLE 2 | Summary of RBD patient management recommendations according to condition subtype.

RBD subtype		Recommendation			Patient follow-up
		First-line	Second-line	Third-line	
Idiopathic	iRBD	Behavioral	Clonazepam 0.25–0.5 mg or melatonin modified-release 2 mg according to symptom severity	Clonazepam 0.25–0.5 mg or melatonin modified-release 2 mg	Regular 6 month-1 year follow-up. Review of current treatments, dosage titration if appropriate and monitoring of any motor, physiological, or cognitive changes
Secondary	Medication	Cessation and replacement of causative medication or dosage titration to stop RBD symptoms	Behavioral	Check medication contraindication and proceed with prescription of clonazepam 0.25–0.5 mg or melatonin modified-release 2 mg	Initial short-term follow up for review of treatment and dosage titration if appropriate. Further review at patient's request
	Lesion	Behavioral	Clonazepam 0.5 mg or melatonin modified-release 2 mg according to symptom severity	Clonazepam 0.25–0.5 mg or melatonin modified-release 2 mg	Initial short-term follow up for review of treatment and dosage titration if appropriate. Further review at patient's request
	α-synucleinopathy	Behavioral	Clonazepam 0.25–0.5 mg or melatonin modified-release 2 mg according to symptom severity	Clonazepam 0.25–0.5 mg or melatonin modified-release 2 mg	Initial short-term follow up for review of treatment and dosage titration if appropriate. Further review at patient's request

Titration of clonazepam dosage should be within the ranges of 0.25 mg−2.0 mg/night. Doses of modified-release melatonin are in the range of 2–6 mg, with higher doses up to 12 mg occasionally used.

of a safe sleep-environment. This can include removing or padding bedside furniture, or lowering the mattress and placing pillows on the floor beside the bed in case of falls (75). In some cases, it may be recommended for the patient and bedpartner to sleep in separate beds to minimize injury. Behavioral recommendations are applicable to both idiopathic and secondary RBD patients, and the extent of these measures should be appropriate to the severity and nature of the patient's RBD symptoms.

Besides changing the immediate sleep environment, physicians should as always encourage patients to observe good sleep hygiene and a healthy lifestyle. The lack of unique risk factors for RBD will however limit the specificity, and likely the efficacy, of this advice.

PHARMACOLOGICAL

Clonazepam is the generally the first-line agent used for the treatment of RBD symptoms. It is long-acting benzodiazepine with a half-life of 30–40 h, and typically commenced at a starting dose of 0.25–1 mg, taken nightly at bedtime. Clonazepam acts to non-specifically enhance inhibitory processes within the brain by binding to $GABA_A$ receptors, and thus temporarily quells overactive or disinhibited brain regions which control REM atonia, ultimately reducing the number of motor behaviors during subsequent sleep. It was first explored as a treatment for RBD given its efficacy in treating periodic leg movement disorder (76), another sleep condition characterized by excessive motor behaviors.

There have been no randomized, double-blind, controlled trials of clonazepam in an iRBD population, and only a handful of studies have looked at the effects of the drug on sleep and RBD symptoms. Clonazepam somewhat restores the EEG spectral profile of iRBD individuals to that of controls when compared to their drug-naïve counterparts, as well as improving both NREM and REM sleep stage stability (77, 78). However, long-term clonazepam use did not affect subjective measures such as daytime sleepiness (77) nor affect the REM sleep atonia index (77, 78). Neither study captured complex, violent REM movements in either the drug-naïve or clonazepam-treated iRBD groups and thus no conclusion can be drawn on the efficacy of clonazepam in reducing the severity of complex movements. However, if judged on RSWA index alone, clonazepam use does not sufficiently control muscle tone during RBD sleep.

Naturalistic follow-up studies have found similar results to the above cross-sectional studies. Li et al.'s v-PSG follow-up study found long-term clonazepam use increased the amount of stage 2 NREM sleep but failed to reduce the amount of REM sleep atonia in iRBD individuals; in fact, the amount of total and tonic RSWA significantly increased over time despite clonazepam use (79), demonstrating the progressive nature of the condition. In a follow-up survey, the majority of iRBD patients receiving clonazepam continued to experience sleep behaviors despite treatment, though frequency, severity and number of dream enactment behaviors were all reduced (80).

Over time, a number of patients will stop taking clonazepam due to side effects, while many of those who remain on clonazepam tend to experience the emergence of residual RBD symptoms (79).

The above follow-up study outcomes are relatively standard for long-term benzodiazepine use which, while a clinical common practice (81), should in and of itself be carefully considered case-by-case. Long-term benzodiazepine use is generally defined as $\geq$6 months (81) and holds the greatest risk for adverse effects such as cognitive impairment (82), dementia development (83), and risk of falling (84), in aged populations. While long-term benzodiazepine use in the elderly is generally maintained at a stable, albeit higher than average, dosage (81) it is common for clonazepam doses progressively over time in the RBD population (79, 85). Underlying this are three potential causes—dosage titration, the development of a tolerance to clonazepam or a progressive worsening of RBD symptoms over time. Long-term assessment of RBD symptoms does indeed show the latter (79, 86), and in light of conflicting evidence for long-term clonazepam tolerance in chronic conditions (87–89) it should generally be assumed that clonazepam dosage should be monitored closely to ensure sufficient control of RBD symptoms.

Clonazepam must be used with caution in the elderly, individuals with a history of depression and those with airways obstruction (90), such as obstructive sleep apnoea which is commonly concomitant with RBD (91). Additionally, the long half-life of clonazepam (92) can lead to "hangover" side effects of excessive daytime sleepiness the next morning (79). Efforts to replace or complement clonazepam therapy in unresponsive or non-tolerant patients has led to the exploratory prescription of alternative drugs with some success, namely in the prescription of zopiclone (93), sodium oxybate (93), or pramipexole (94) [for further review, see (95)]. As these cases are limited in size and are not extensive case-controlled studies, clonazepam remains the chosen pharmacological treatment for RBD, if only for the sole reason of upholding the status quo.

Over recent years melatonin and associated melatonergic agents have established their place in the management of RBD. Melatonin is indicated for the treatment of chronobiological disorders and insomnia, though is often prescribed off-label for all other sleep disorders. One of melatonin's main functions is to synchronize circadian rhythms by binding to its receptors at the hypothalamic suprachiasmatic nucleus (SCN) (96). Melatonin has been shown to improve sleep quality and duration in both healthy and diseased individuals (97, 98), and as sleep disorders are often multi-factorial, prescribing melatonin or melatonin-related compounds is often done to try to non-specifically stabilize any underlying circadian desynchronizations. In the case of RBD, where changes in REM circadian rhythmicity have been shown (57, 99), blanket-prescription of melatonin may be beneficial for patients. However, as no blinded case-controlled trials have confirmed the stabilization of REM circadian rhythmicity by melatonin in RBD, the clinical- and cost-effectiveness of blanket prescription policy for healthcare systems should be taken into consideration by the clinician (100).

Enhancement of melatonin signaling within the brain can be achieved directly with an exogenous, modified release

form of melatonin or indirectly with the melatonin receptor agonist Ramelteon. Melatonin is usually prescribed to treat RBD behavioral symptoms in the context of clonazepam shortcomings—either as a replacement monotherapy (if the patient cannot tolerate clonazepam due to side effects) or as a polytherapy in combination with clonazepam (if clonazepam is insufficient in controlling RBD symptoms and residual sleep behaviors persist).

The evidence for melatonin's efficacy is variable: several studies have found it to reduce RBD motor behavior occurrence (101–103), with long-term use ameliorating RBD symptoms in the majority of patients (104). However, a recent placebo-controlled trial found melatonin use at either 2 or 6 mg/night improved self-reported measures such as daytime sleepiness, sleep quality or dream enactment behaviors (105). A recent randomized, controlled trial of melatonin in a cohort of PD patients with RBD also found no effect of melatonin on RBD symptom frequency or severity (106), suggesting melatonin may be less effective at controlling RBD symptoms in the context of advanced neurodegeneration. The efficacy of melatonergic compounds such as the melatonin receptor agonist Ramelteon also do not significantly improve RBD symptom severity (107).

As melatonin causes fewer side effects has low tolerance risk and few drug interactions, it may be more suitable for RBD patients than clonazepam (108). Despite this, the uncertainty around melatonin's mechanism of action and overall efficacy means the popularity of clonazepam prevails.

At the time of writing, the UK RAG drug classification lists melatonin as a RED drug and clonazepam as a GREEN drug. The prescription of melatonin therefore requires secondary or tertiary care initiation and management whereas clonazepam prescription can be managed by GPs. This may be a choice-limiting factor for some patients. Ultimately, it is a combination of the clinician's personal preference and best judgement of which drug is prescribed.

SYMPTOM MONITORING

Follow-up studies and projected conversion rates estimate that the majority of idiopathic RBD patients will develop a clinically-defined α-synucleinopathy within 8 years of their initial diagnosis (18). Despite this, there are no guidelines for the routine monitoring of RBD patient symptomology (95). What further limits any attempts at symptom tracking is the lack of standardized clinical rating scales for RBD severity progression and conversion. The discrepancy between a lack of clinician-instigated patient follow up vs. the high risk of α-synucleinopathy development is largely explained by the fact that there are no medical interventions to stop or slow RBD conversion. The benefits of tracking neurodegenerative symptoms are therefore greatly outweighed by the economic cost to the healthcare system and the emotional burden to the patient.

The immediacy of such a situation, wherein both clinician and patient are powerless, can make symptom monitoring unattractive. Therefore, to date, the majority such follow up tends to be confined to research studies. Below, we detail how current practice in RBD management could be feasibly improved without changes to existing healthcare practices or clinic frameworks and explore possibilities for the development of a prodromal rating scale to track idiopathic RBD symptomology.

Ideally, once RBD has been confirmed on v-PSG, the newly diagnosed patient should undergo a series of standardized functional assessments to determine their current or "baseline" symptoms. These assessments should address the range of deficits which have been associated with RBD (and subsequent α-synucleinopathy development) in the literature and should be sensitive enough to capture subtle dysfunctions. Ultimately, the development of a unified assessment scale for clinical-practice deployment to RBD patients is recommended. This should be reflective of the Movement Disorders Society's recently published research criteria for prodromal PD (109), which demonstrates relatively high sensitivity for prediction of PD development (110). RBD patients should then be seen annually to discuss their disease phenotype with their clinician, and to repeat the functional assessments. This would generate an in-depth profile of each patient and their symptoms and ensure early signs of neurodegeneration can be addressed using available clinical tools.

CURRENT RESEARCH: RBD AND THE α-SYNUCLEINOPATHIES

The tests described in this section are used solely in experimental settings and therefore clinicians are not able to use them in the clinical RBD diagnostic or prognostic process. The majority of RBD research focuses upon the relationship between RBD and subsequent α-synucleinopathy development. In particular, the search for biomarkers which identify underlying α-synuclein pathology and predict RBD phenoconversion is perhaps the most relevant for clinical practice. As discussed previously there are no genetic markers with predictive power for RBD phenoconversion, and while rating scales may identify individuals with a high risk of phenoconversion, they do not have a binary outcome measure- a feature which is essential in prognostic testing.

Biomarkers for RBD conversion are essentially testing whether the RBD features of an individual are due to underlying α-synuclein pathology or not. This can be done by testing for misfolded, pathological α-synuclein (typically identified by serine-129 phosphorylation) in peripheral tissues and fluid samples. Dermal nerve fibers (111, 112), cerebrospinal fluid (113), submandibular glands (114), colonic submucosal nerve fibers (115), salivary glands (116), and parotid glands (117) have all been found to contain pathological α-synuclein proteins in idiopathic RBD patient populations and confirmatory PD populations. Such investigations are in their infancy but show promise as a basis for relatively low-cost clinical diagnostic biopsy tests for alpha-synucleinopathy in RBD patients.

Bioimaging techniques, such as MRI and PET scanning, are a less invasive alternative to biopsy-based diagnostic tests. Such methods are capable of identifying and quantifying dysfunction in deep structures which may otherwise be inaccessible. In general, the greatest focus has been upon

tissues and neurotransmitters whose dysfunction corresponds to the symptoms of α-synucleinopathies as there are no tracer molecules for direct pathological α-synuclein visualization. The degeneration of dopaminergic neurons seen in PD, and to some extent DLB, has led to an emphasis on imaging dopamine-associated molecules, such as dopamine and non-specific monoamine transporters, in RBD patients (118). DaTSCANs, which are a way to visualize presynaptic dopamine transporter density, are already used clinically in the diagnosis of PD and may also be enlisted in confirming whether an RBD individual has degeneration of dopaminergic terminals projecting to the putamen. However, RBD onset likely precedes gross dopamine dysfunction, and this is reflected in the literature that not all RBD patients present with abnormal DaTSCAN (18). Efforts to image neurotransmitter systems which may be affected earlier in the prodromal period accompanying RBD symptoms are therefore underway. Perhaps the most thorough and ambitious of these efforts demonstrated multimodal characterization of the sympathetic, parasympathetic, noradrenergic, and dopaminergic systems in RBD patients, using a combination of MRI, PET, CT, and scintigraphy (15). The authors found that the RBD patients had abnormal peripheral autonomic nervous system and brainstem results, but few demonstrated cerebral pathology (15). These results are in line with both clinical DaTSCAN findings and the Braak staging model of pathology and argue against the use of DaTSCAN for RBD diagnosis or prognosis prediction.

Though providing a useful insight into the progression and extent of neurodegeneration in an individual, there are several caveats to the use of imaging tests in regular diagnostic or prognostic practice. As seen above, DaTSCANs are unlikely to yield valuable results in an RBD patient, making the tests highly uneconomical. Multiple imaging tests are also inherently accompanied by radiation exposure and its associated risks. Therefore, while there is potential for assessment of alternative neurotransmitter systems in RBD patients, the clinical value of such tests must be assessed further. As with the majority of tests, neuroimaging currently adds to the overall picture rather than produces a binary diagnostic or prognostic outcome.

α-SYNUCLEINOPATHY TREATMENTS

Advances in preventative, slowing or curative α-synucleinopathy treatments would add credence to RBD patient monitoring, as the potential for therapeutic deployment during the RBD-characterized prodromal period could significantly disrupt the disease trajectory. Development of antibodies against pathological α-synuclein (119, 120) and neurorestorative/neuroprotective compounds (121) hold promise for treatments but as yet there are no clinically-approved α-synucleinopathy therapies.

CONCLUSIONS

RBD presents a multitude of considerations relevant for neurologists and non-specialized clinicians alike. From a public health perspective, RBD highlights the importance of sleep for good health and the need for greater awareness of sleep disorders and their detrimental effects. For scientists and researchers, RBD represents a window of opportunity for deployment of new interventions against neurodegenerative processes, as well as an opportunity to gain insight into the complex neural mechanisms of sleep and wake. Finally, the diagnosis, treatment, and ethical considerations of RBD require the clinician to demonstrate cross-speciality knowledge, emphasizing the importance and challenges of sleep medicine training in an over-stretched education system which provides inadequate training on sleep and it's disorders (122–124).

The dream enactment characteristic of RBD is associated with a variety of root causes- from acute emotional states to progressive neurodegeneration. The biological mechanisms underpinning the intrusion of waking behaviors into REM sleep remain to be fully characterized, plus it remains to be seen whether the same mechanisms underlie different disorders which share the symptom of dream enactment. For the majority of RBD patients, dream enactment behaviors will be the first symptom of impending α-synucleinopathic disease. Thus, the onus lies with the clinician to recognize these risks, communicate them effectively, and diagnose accordingly. While the prognosis of idiopathic RBD remains uncertain, an increase in basic and clinical research into the condition is already leading to greater understanding and endpoint prediction. The final barrier to RBD patient care remains effective treatments to slow, reverse, or stop the effects of α-synuclein mediated disease.

AUTHOR CONTRIBUTIONS

MR and AR conceived of the presented idea. AR drafted the manuscript with support from DR, AW, MJ, and MR. All authors contributed to the final version of the manuscript.

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MINI REVIEW
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Biofluid Markers for Prodromal Parkinson's Disease: Evidence From a Catecholaminergic Perspective

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Parkinson's disease (PD) is the most frequent of all Lewy body diseases, a family of progressive neurodegenerative disorders characterized by intra-neuronal cytoplasmic inclusions of α-synuclein. Its most defining features are bradykinesia, tremor, rigidity and postural instability. By the time PD manifests with motor signs, 70% of dopaminergic midbrain neurons are lost, and the disease is already in the middle or late stage. However, there are various non-motor symptoms occurring up to 20 years before the actual parkinsonism that are closely associated with profound deficiency of myocardial noradrenaline content and peripheral sympathetic denervation, as evidenced by neuroimaging experiments in recent years. Additionally, there is an inherent autotoxicity of catecholamines in the neuronal cells in which they are produced, forming toxic catecholaldehyde intermediates that make α-synuclein prone to aggregation, initiating a cascade of events that ultimately leads to neuronal death. The etiopathogenesis of PD and related synucleinopathies thus may well be a prototypical example of a catecholamine-regulated neurodegeneration, given that the synucleinopathy in PD spreads in synergy with central and peripheral catecholaminergic dysfunction from the earliest phases onward. That is why catecholamines and their metabolites, precursors, or derivatives in cerebrospinal fluid or plasma could be of particular interest as biomarkers for prodromal and *de novo* PD. Because there is great demand for such markers, this mini-review summarizes all catecholamine-related studies to date, in addition to providing profound neurochemical evidence on a systemic and cellular level to further emphasize this hypothesis and with emphasis on extracellular vesicles as a novel diagnostic and therapeutic incentive.

Keywords: biomarker, catecholamines, cerebrospinal fluid, DHPG/MHPG, DOPAC, extracellular vesicles, Parkinson's disease, plasma

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INTRODUCTION

Parkinson's disease (PD) is recognized as the second most common neurodegenerative disorder following Alzheimer's disease (AD), with an approximate incidence rate of 10–18 per 100,000 person-years. PD is present in about 1% of the population over 65 years of age and more than 4–5% in that over 80. Age and gender are established risk factors, followed by ethnicity. An

increase by more than 50% is expected by 2030, given the rising life expectancy worldwide (1, 2). Generally, PD is diagnosed when bradykinesia occurs alongside rigidity or tremor, so its clinical diagnosis mostly depends on motor findings. On the pathological level, this is when about 50–80% of the dopaminergic neurons of the substantia nigra pars compacta (SNpc) are lost due to α-synuclein deposits, known as Lewy bodies. PD is, therefore, often diagnosed clinically when the synucleinopathy is already advanced. On the other hand, patients frequently report having non-motor symptoms for 10–20 years before the diagnosis (3). These prodromes, defined as "early (non-specific) symptoms or signs which often indicate the onset of a disease before more diagnostically specific signs and symptoms develop," provide a potential temporal window during which disease-modifying therapy, once it becomes available, could be administered to prevent or delay the development and progression of disease. Similarly, researchers and clinicians recognize the need for a clinical diagnosis based on quantifiable measures (i.e., biomarkers) to refine qualitative assessments. Characteristic prodromal symptoms of PD are impaired olfaction (anosmia/hyposmia), constipation, depression, excessive daytime sleepiness, rapid eye movement (REM) sleep behavior disorder (RBD), impaired color vision, mild cognitive impairment, and autonomic dysfunction (e.g., orthostatic hypotension (OH), erectile dysfunction, bladder disturbances) (1, 3). From a neurochemical point of view, PD was the first neurodegenerative disease of which the underlying neurochemical abnormality was identified, i.e., striatal depletion of the catecholamine dopamine (DA) (4). This pivotal discovery led to the introduction of the first successful symptomatic treatment with levodopa/carbidopa therapy (5). Almost half a century later, this theory of "central catecholamine deficiency" in PD has expanded considerably, entailing both dopaminergic and noradrenergic neurotransmission deficits, not only in the central but also in the peripheral nervous system.

PERIPHERAL CATECHOLAMINERGIC DEFICIENCY AS NEUROCHEMICAL SUBSTRATE OF PRODROMES IN PD

A catecholamine is a monoamine neurotransmitter, an organic compound that has a catechol (benzene ring with adjacent hydroxyl groups) and one ("mono") side-chain amine group. Included among catecholamines are DA and (nor)adrenaline [(N)A]. All are derived from the amino acid tyrosine, which is retrieved from dietary sources, as well as synthesis from phenylalanine. Principal metabolites of DA and (N)A, are 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and, 3,4-dihydroxyphenylglycol (DHPG) and 3-methoxy-4-hydroxyphenylglycol (MHPG). Furthermore, NA is synthesized from DA (6). Catecholamine neurons are relatively rare in the central nervous system, but with abundant afferent and efferent projections. Toxic intraneuronal α-synuclein depositions in the brainstem nuclei that produce DA and NA, i.e., SNpc and locus coeruleus (LC), therefore, leads to widespread alterations on both central and peripheral levels. Interestingly, upregulated NA reuptake in the LC area of early-stage PD patients, compatible with enhanced NA release, has previously been suggested as a compensatory, protective mechanism against degeneration of nigrostriatal dopaminergic projections (7).

Apart from the central depletion of DA in the brain's nigrostriatal system, giving rise to the well-known motor phenomenology, PD is equally characterized by a severe deficiency of NA in the heart (8). Other Lewy body diseases such as pure autonomic failure or dementia with Lewy bodies (DLB) similarly involve decreased myocardial NA content (9). According to Braak's staging concept, nigrostriatal neuropathological lesioning in PD occurs in stage three out of six (10) and imputes early autonomic involvement (11). The LC, the brain's main source of NA, becomes affected by the synucleinopathy in stage two. During stage one, the dorsal motor nucleus of the vagal nerve becomes affected, which has strong connections with the parasympathetic nervous system of the gastrointestinal system and lungs and with the nucleus ambiguous, which partially involves the innervation of the heart via preganglionic parasympathetic neurons (12). Intriguingly, peripheral cardiac sympathetic neurodegeneration occurs even earlier in the disease process and has a significant clinical importance. The cardiac noradrenergic sympathetic deficiency has been associated with cognitive impairment (13), fatigue/exercise intolerance (14, 15), anosmia (16), RBD (17), visual hallucinations (18), falls from neurogenic OH (19), and decreased survival (20). Gastrointestinal symptoms due to local α-synuclein accumulation and as part of enteric neuronal dysfunction also appear well before the onset of motor symptoms (21).

In PD, OH is a common prodrome that occurs some years before or concurrent with the clinical motor phase and is associated with sympathetic neurocirculatory failure (22). The loss of sympathetic noradrenergic neurons thus might be assumed, but studies using immunoreactive tyrosine hydroxylase as a marker of myocardial catecholaminergic innervation have noted a 75% decrease (23), whereas the loss of NA levels is about 95–99% (8, 9). This points to a proportion of inactive/dysfunctional but not completely eradicated residual nerves and may involve abnormalities in vesicular storage of NA, altered enzymes, decreased vesicular uptake via the vesicular monoamine transporter (VMAT) type 2, and, increased vesicular permeability (24). The cardiac sympathetic denervation does not affect cardiac structure or function under resting conditions but creates a failure to increase the myocardial contractility following stimuli that depend on NA release, for instance during exercise, and is linked with generalized fatigue (25). In AD, myocardial sympathetic innervation is unaltered, so cardiac sympathetic neuroimaging can provide a means for improvement of the differential diagnosis between AD and DLB, as was evidenced previously using ^{123}I-metaiodobenzylguanidine scintigraphy (26).

Remarkably, neither the severity of noradrenergic sympathetic denervation nor values for measures of other non-motor manifestations seem to be 100% related to the severity of

loss of central nigrostriatal dopaminergic neurons (16, 27). If compatible with Braak's concept of ascending pathology, such patients with decreased striatal dopaminergic innervation should also have cardiac noradrenergic denervation. In contrast, autonomic dysfunction seems to occur independently of the dopaminergic cell loss that causes the parkinsonian triad of motor symptoms (9).

LOW AND HIGH FUNCTIONAL THRESHOLD SYSTEMS

The hypothesis of Braak et al. of the ascending and trans-synaptically prion-like spreading of α-synuclein from peripheral nerves, such as via a nasal or gastric route (28), to the brainstem and midbrain up to higher cortical structures is questioned due to contradictory neurochemical, neuroimaging, and clinical evidence [(9), for review: (29)]. In part, the evidence against the idea of the trans-synaptic spread comes from Tysnes et al. (30), who concluded that the idea of performing full truncal vagotomy in reducing the risk of PD (31) may be too premature. Recently, Engelender and Isacson (29) challenged the theory of Braak et al. and proposed a model based on evidence of parallel degeneration and pathology of the central and peripheral nervous system in PD. Under this alternative, systems reach their individual thresholds for symptoms at different rates. Even though the brainstem, peripheral and autonomic neurons seem more resilient to insults as opposed to dopaminergic midbrain neurons, the threshold for the brainstem and peripheral motor symptoms to become apparent is lower than that for motor symptoms. The key factor in understanding this theory is the greater functional reserve of the dopaminergic midbrain neurons, which are more sensitive but have vast interconnections throughout the midbrain, striatal, pallidal, thalamic, and cortical nuclei, providing extensive compensatory mechanisms and redundancy to allow initiation of movement. This is in contrast to catecholaminergic neurons of the autonomic, peripheral, and enteric nervous systems which interconnect with the brainstem nuclei (29). Various types of inputs to the striatal medium spiny neurons derived from, e.g., cortex (glutamatergic), thalamus (glutamatergic), and even the dorsal raphe nuclei (serotonergic), could compensate for a progressively reduced input of dopaminergic SNpc neurons. Direct and indirect pathways of the striatal system have strong bidirectional interactions. On the other hand, the functional network of the enteric nervous system (cholinergic, noradrenergic) is much less developed, suggesting that enteric neurons would have less functional reserve and thus may elicit constipation as a prodromal symptom. Cardiac autonomic dysfunction (noradrenergic) and RBD due to lesioning of the brainstem–reticular activating system (cholinergic, noradrenergic) may develop likewise.

In essence, 70% loss of sensitive dopaminergic midbrain neurons causes motor symptoms (high threshold), whereas only a 20–30% reduction in mainly noradrenergic neurons of the brainstem/peripheral/enteric/autonomic nervous system (low threshold) already seems sufficient to elicit non-motor symptoms. In agreement, the difference between the low and high functional threshold system may explain the appearance of prodromes up to 20 years before the onset of the motor symptomatology and supports the notion of an ideal biofluid marker for prodromal PD to potentially be of catecholaminergic origin (**Figure 1**).

THE CATECHOLAMINE AUTOTOXICITY THEORY: IMPLICATIONS FOR BIOMARKER RESEARCH

Dopamine spontaneously auto-oxidizes to form neuromelanin, the black pigment that pinpoints the SNpc, and the final product of the DA oxidative pathway. Nigral depigmentation, therefore, likely has a neurochemical basis. In a nutshell, the "catecholaldehyde hypothesis" in PD theorizes that long-term increased buildup of 3,4-dihydroxyphenylacetaldehyde (DOPAL)—the catecholaldehyde of DA—significantly contributes to the death of dopaminergic neurons (33, 34). This concept builds on the notion that there is an inherent cytotoxicity of catecholamines and metabolites in the cells in which they are produced [Figure 11 of Goldstein and Sharabi (9)]. The vesicular uptake of cytosolic DA is regulated via VMAT2. Next, DA leaks from the synaptic vesicle into the cytosol, undergoing exocytotic release. DA transporter takes most of the released DA back into the cytosol. However, within the neuron, monoamine oxidase (MAO)-A enzymatically oxidizes DA into an intermittent form, i.e., DOPAL. This reaction yields hydrogen peroxide, which reacts with metal cations to produce extremely harmful hydroxyl radicals. DOPAL can also auto-oxidize, forming DOPAL-quinone, which can be transformed into cysteinyl-DOPAL. Simultaneously, this reaction produces deleterious reactive oxygen species (ROS). Under physiological conditions, DOPAL is metabolized into DOPAC by aldehyde dehydrogenase (ALDH). In glial cells, DOPAC is further metabolized into HVA by catechol-O-methyltransferase (COMT).

Apart from the enzymatic deamination of cytoplasmic DA into DOPAL, DA itself can also auto-oxidize into DA-O-quinone, producing cysteinyl-DA (cys-DA), (amino)chrome, 5,6-indolequinone, polydopamine, and condensation products (e.g., salsolinol), most of which are toxic for the cellular environment. Finally, from 5,6-indolequinone, neuromelanin is formed and stored.

It seems that enzymatic or spontaneous DA oxidation creates aldehydes (DOPAL), ROS, hydrogen peroxide, and thio-catecholamines (cys-DA/cys-DOPAL) within the neuron. The peculiar tendency of α-synuclein to precipitate in dopaminergic neurons can be explained by the fact that DOPAL-quinone can induce oligomerization of α-synuclein in the cytoplasm, forming Lewy bodies. Moreover, ROS inhibit ALDH and thus build up cytoplasmic DOPAL, which leads to an imbalanced system.

The same goes for the production of the noradrenergic aldehyde dihydroxyphenylglycolaldehyde (DOPEGAL). DOPEGAL is formed in the sympathoneural cytosol upon

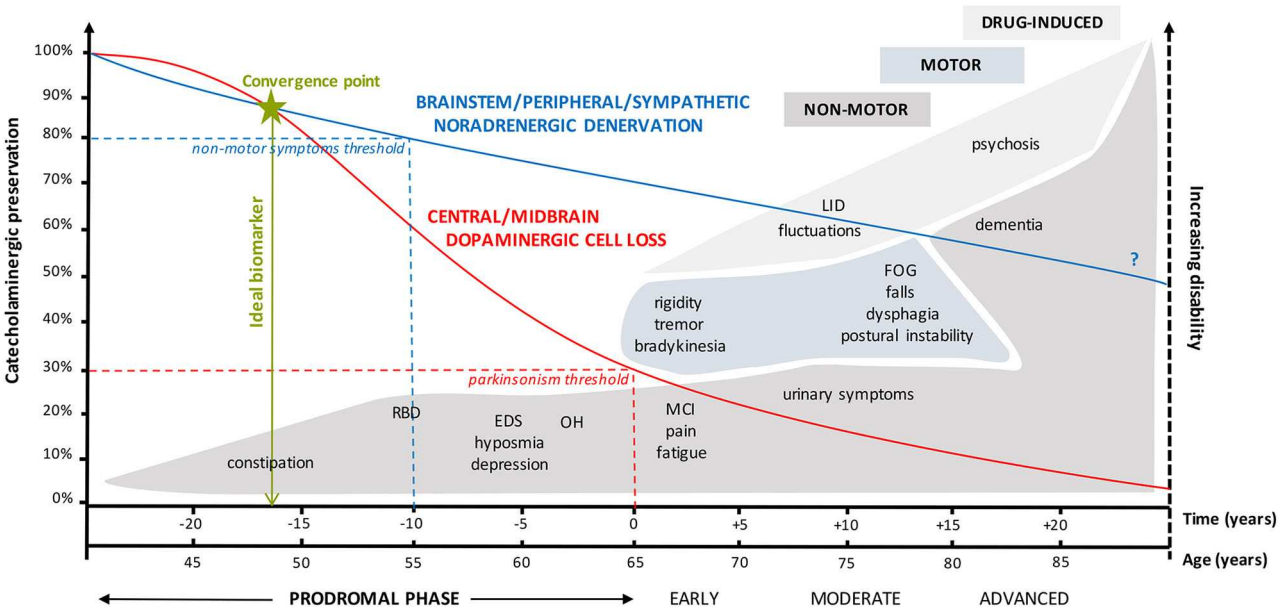

FIGURE 1 | Graphical representation of the complex interplay between catecholaminergic neuronal preservation, the high and low functional threshold theory, and the time course of (non-)motor symptoms in prodromal, early, moderate, and advanced Parkinson's disease. The high threshold for clinical appearance of parkinsonian motor symptoms is only reached when there is at least 70% reduction in dopaminergic midbrain neurons (32). On the other hand, a 20 to 30% loss of mainly noradrenergic brainstem-RAS/peripheral/autonomic/enteric neurons of the low threshold system is already sufficient to elicit a variety of non-motor symptoms such as constipation, RBD, EDS/fatigue, hyposmia, and neurogenic OH. These can be regarded as prodromes. Both catecholaminergic systems progressively degenerate over a period of almost 50 years, albeit with an initial less steeper decline in the noradrenergic system (29). The question mark relates to the uncertainty regarding the extent of noradrenergic decline from early to advanced PD. Figures vary between 40 and 90%, depending on whether NA loss was measured in the myocardium (e.g., 90–95%) or sympathetic ganglia (e.g., 40%). These results are consistent with the concept of centripetal, retrograde "die-back" degeneration of cardiac sympathetic nerves in Lewy body diseases (9). Preferentially, the ideal biofluid marker or combination of markers should be sensitive enough to detect the first subtle catecholaminergic alterations at the convergence point from where both neurotransmitter systems tend to deteriorate in an accelerated fashion. EDS, excessive daytime sleepiness; FOG, freezing of gait; LID, levodopa-induced dyskinesia; MCI, mild cognitive impairment; OH, orthostatic hypotension; PD, Parkinson's disease; RAS, reticular activating system; RBD, REM sleep behavior disorder.

oxidative deamination of NA and undergoes metabolization to form DHPG, mainly via aldehyde/aldose reductase (6). Similar as with DOPAL-induced synucleinopathy in the SN in PD, the DOPEGAL-promoted formation of tau aggregates in the LC in AD has recently been demonstrated (35).

Various early alterations within the dopaminergic neuron, such as decreased vesicular sequestration of DA via VMAT2 and decreased DOPAL metabolism by reduced activity of ALDH, are the multifactorial result of genetic predispositions, exposure to environmental toxins, stress, and aging. Ultimately, this cascade of events could trigger PD pathophysiology. The fungicide benomyl, for instance, increases PD risk by inhibiting ALDH, causing subsequent DOPAL accumulation (36). Furthermore, neuromelanin has the potential to bind environmental redox-active metal ions *in situ*. These are released upon neuronal death, augmenting DOPAL-induced oligomerization of α-synuclein (37, 38).

Altogether, the autotoxicity theory clarifies the selective vulnerability of central and peripheral catecholaminergic neurons, converting a stabile negative feedback-regulated in-cell system to a fragile, unstoppable positive feedback loop (33). This context naturally provides rationale for the development of biofluid markers of dopaminergic/noradrenergic origin.

BIOFLUID CATECHOLAMINE MARKERS FOR PRODROMAL PD: THE EVIDENCE

So far, only a handful of researchers investigated the biomarker potential of circulating catecholamines or derivatives in prodromal or *de novo* PD (dnPD), evidently with no record of present or past therapy with anti-parkinsonian drugs (**Table 1**). For matters of comparison, neurochemical investigations in early-stage PD patients have also been enlisted in the table.

Kienzl et al. (39) included 16 dnPD patients with Hoehn and Yahr stage scores I or II. The study of D'Andrea and colleagues included 16 dnPD patients diagnosed within 1 year or less following the onset of parkinsonism (40). In 2019, the same group analyzed a larger variety of catecholamines and trace amines, with inclusion of 21 dnPD patients with a disease duration of less than 2 years (47). As for Goldstein et al. (41), 14 out of 34 PD patients were dnPD, with cerebrospinal fluid (CSF) obtained within 2 years or even before the onset of parkinsonism. The authors also excluded DA data from 17 PD patients to eliminate potential treatment effects, even after levodopa washout. Next, the same group performed similar research in early-stage PD patients, complying with three out of four clinical PD criteria, that were off levodopa or MAO inhibitor treatments, as confirmed by

TABLE 1 | Enlistment of studies evaluating biofluid catecholamines and their derivatives or precursors as potential markers for prodromal, *de novo*, or early-stage Parkinson's disease.

Study	Subjects (N)	CA marker	Main findings	Remarks
Kienzl et al. (39)	15 HC 16 dnPD 21 PD 11 OTHER	Urinary DA-3-o-sulfate and DA-4-o-sulfate	DA-4-o-sulfate is reduced in dnPD compared to HC and OTHER	DA-4-o-sulfate levels remained unaltered during levodopa therapy
D'Andrea et al. (40)	28 HC 16 dnPD 47 nfPD 21 fPD	Plasma octopamine and NA	Octopamine levels were lower in dn/nf/fPD vs. HC; NA levels were only lower in nf/fPD vs. HC	Trace amines are hard to detect; very low circulating levels
Goldstein et al. (41)	38 HC 34 PD (14 dn) 54 MSA 20 PAF	Plasma and CSF l-DOPA, DA, DOPAC, NE and DHPG	CSF DOPAC and DHPG strongly decreased in all groups vs. HC; CSF DOPAC lower, and CSF DHPG higher, in PD than PAF; CSF DOPAC 100% sensitive and 89% specific in dnPD vs. HC	Plasma DHPG levels were lower in PAF than in HC; in PD, CSF and plasma DHPG were positively correlated
Goldstein et al. (42)	26 HC 12 PD+OH 11 PD-OH 21 MSA-p 5 MSA-c 11 PAF	Plasma F-DOPAC and DHPG	F-DOPAC levels were higher, and, DHPG levels lower, in PD+OH vs. MSA-p and HC	F-DOPAC:DHPG ratio differentiated PD+OH from MSA-p
Goldstein et al. (43)	32 HC 24 PD 32 MSA-p 18 PAF	CSF (cys-)l-DOPA, (cys-) DA, DOPAC, (N)A, DHPG	DOPAC was decreased in PD and MSA-p vs. HC	cys-DA:DOPAC two times as high in PD and MSA-p than HC or PAF
Figura et al. (44)	22 early PD 28 aPD+LID 23 aPD-LID	Serum phenylalanine and tyrosine	Phenylalanine levels were higher in early PD than aPD+LID	No differences in tyrosine; no inclusion of HC
Goldstein et al. (45)	26 subjects at risk for PD with 3.7 years FU	CSF l-DOPA and DOPAC	4 out of 26 with low baseline l-DOPA and DOPAC developed PD	At least three risk factors: genetic, olfactory, RBD, and/or OH
Kim et al. (46)	26 untreated dnPD with ±2.5 years of disease duration	Tear fluid DA, NA and A levels	NA and DA were increased, A decreased, in PD vs. HC; increases were pronounced on the ipsilateral motor side	Results were confirmed in (pre)clinical stages of a neurotoxic PD mouse model
D'Andrea et al. (47)	10 HC 21 dnPD 27 PD-treat	Plasma tyrosine, tyramine, tryptamine, octopamine, TRP, β-PEA, 5-HT, NA, MNE	Tyramine differed between all three groups; tyramine, tyrosine and NA combined acted as biomarkers of disease progression	Trace amines are hard to detect; very low circulating levels

β-PEA, beta phenylethylamine; 5-HT, serotonin (5-hydroxytryptamine); A, adrenaline; aPD+/-LID, advanced PD patients with(out) levodopa-induced dyskinesia; CA, catecholamine; CSF, cerebrospinal fluid; cys-DA, cysteinyl-dopamine; cys-l-DOPA, cysteinyl-levodopa; DA, dopamine; DHPG, 3,4-dihydroxyphenylglycol; dnPD, de novo PD subjects; DOPAC, 3,4-dihydroxyphenylacetic acid; F-DOPAC, fluoro-DOPAC; fPD, fluctuating PD patients; FU, follow-up; HC, healthy controls; l-DOPA, levodopa (3,4-dihydroxyphenylalanine); MNE, metanephrine; MSA, multiple system atrophy; MSA-c, cerebellar variant of MSA; MSA-p, parkinsonian variant of MSA; NA, noradrenaline; nfPD, non-fluctuating PD patients; OH, orthostatic hypotension; OTHER, other neurological disorders, such as polyneuropathy, multiple sclerosis, cerebral infarction, myasthenia gravis, arthritis, cerebral atrophy, and Wilson's disease; PAF, pure autonomic failure; PD, Parkinson's disease; PD-treat, PD patients on levodopa or other dopaminergic drugs; RBD, REM sleep behavior disorder; TRP, tryptophan.

additionally measured CSF DOPA levels (42, 43). Figura et al. examined serum amino acids in four dnPD and 18 early PD subjects. The latter group was defined as having a score of I or II on the Hoehn and Yahr staging scale, less than 3 years of disease duration, and stable levodopa response (44). Goldstein et al. (45) also clinically evaluated 26 prodromal subjects who had at least three risk factors for PD, i.e., olfactory dysfunction, RBD, OH, and/or genetic predisposition, and, no motor symptoms. Finally, Kim et al. (46) assessed tear fluid catecholamines in 26 untreated dnPD patients having Hoehn and Yahr stages I–II.

At first glance, (i) three CSF and plasma metabolites [DOPAC (41, 43, 45), DHPG (41, 42), L-DOPA (41, 43, 45)], (ii) one plasma and three tear fluid catecholamines [N(A) (40, 41, 46), DA (46)], and (iii) two derivative plasma trace amines [tyramine (47) and octopamine (40), whether or not combined with plasma NA (47)] seem promising as markers for prodromal or dnPD (**Table 1**). Regarding CSF DOPAC, results are quite unequivocal, with very low to low levels across PD stages, from prodromal (45) to *de novo* (41) and early-stage (43). For instance, four out of 26 subjects at risk for PD with low CSF levels of DOPAC (<1.22 pmol/ml) and L-DOPA (<2.63 pmol/ml) at baseline inclusion eventually developed PD (45).

Just like DHPG, MHPG—the final metabolite of NA metabolism—has also been indicated as a potential biofluid marker for Lewy body diseases, albeit in the context of cognitive dysfunction in PD (48), or to differentiate DLB from AD (49). Because MHPG crosses both blood–brain (BBB) and CSF–blood barriers (50), plasma alterations may well be indicative of central noradrenergic dysfunction.

EXTRACELLULAR VESICLES AS A NOVEL DIAGNOSTIC AND THERAPEUTIC APPROACH

It is still challenging to efficiently treat PD patients, due to a BBB impeding passage of most drugs. The development of various drug delivery systems encapsulating, e.g., DA, is, therefore, desirable (51). The focus herein lies upon extracellular vesicles (EVs), nanoparticles including exosomes (<100 nm), microvesicles (100–1,000 nm), and apoptotic bodies (up to 4,000 nm) (52). Their cargo mostly contains lipids, proteins, mRNA, and microRNA (miRNA). EVs show low immunogenicity and can easily cross the BBB. As such, small-molecule therapeutics like paclitaxel, doxorubicin, and curcumin have been encapsulated into exosomes to treat cancer and inflammatory diseases (53–55).

One great advantage over levodopa of an exosomal DA delivery system to the brain via a peripheral route would be that exosomes can be engineered to strategically target specific neurons or neuronal populations (56). This would prevent the non-targeted levodopa-derived DA exocytosis in serotonergic rather than residual dopaminergic nerve terminals. These serotonergic projections innervate entirely different systems, such as the prefrontal cortex, nucleus accumbens, subthalamic nucleus, and hippocampus (57). Since VMAT2 is contained in serotonergic neurons too for the uptake of *in situ* synthesized DA, increased extracellular DA levels in these extrastriatal regions have been linked to various (non-)motor symptoms, such as psychosis and LID (58).

On the other hand, exosomes may intercellularly transfer and sequester misfolded, pathogenic α-synuclein from neuron to neuron and from neuron to glia, in turn leading to the activation of an inflammatory response, and thus contributing to neuronal dysfunction and overall disease progression (59). However, few studies also reported functional evidence of a neuroprotective functionality via exosomal externalization of α-synuclein, which may be beneficial for the surviving dopaminergic neurons of the SNpc (60, 61).

Despite the potential role of EVs in contributing to the onset or progression of PD, exosomes could represent a valuable drug delivery tool (62). Qu et al. (63) administered DA-loaded blood exosomes to PD mice, which successfully entered the nigrostriatal system, induced nigral dopaminergic neurogenesis, and improved the symptomatic performance compared to exogenous DA treatment (64). More recently, Narbute et al. (65) developed EVs derived from stem cells from the dental pulp of human exfoliated deciduous teeth, which are highly proliferative and capable of differentiating into, e.g., neural cells (66–68). These derived EVs were administered intranasally and improved gait parameters in a PD rat model (65).

Furthermore, EVs contain miRNA-124a as part of their cargo (69), which is a potent regulator of MAO-A expression in dopaminergic neurons (70). Regulating EV-containing miRNA-124a expression levels in the brain might be an inventive therapeutic approach, especially because it avoids adverse effects caused by conventional MAO-A inhibitors. Meanwhile, Gui et al. (71) found 16 downregulated miRNAs in CSF exosomes of PD patients compared to controls. Cao et al. (72) concluded that miRNA-19b was downregulated and miRNA-195 and miRNA-24 upregulated in PD. This raises the possibility that serum exosomal miRNA profiling may be a novel strategy for diagnosing prodromal PD.

CONCLUSIONS AND FUTURE PERSPECTIVES

By and large, in PD, α-synuclein pathologically spreads in synergy with central and peripheral catecholaminergic dysfunctioning, so the quest for biofluid catecholamine markers seems a rational choice. Above, we provided neurochemical, clinical, and pathophysiolocial evidence. On the systemic level, the threshold theory acknowledges that the earliest symptoms are caused by catecholaminergic deficiency of the peripheral/autonomic nervous system, followed by a central dopaminergic depletion. On the cellular level, the hypothesis of DOPAL- and DOPEGAL-induced synucleinopathy and a consequentially altered metabolic route of DA and NA are in agreement with a handful of studies so far that indicated CSF DOPAC and plasma DHPG/MHPG to be of potential interest as biomarkers for prodromal PD in particular. Moreover, the concept of EVs in CSF/plasma needs further refinement, since these nanoparticles may contain a vast amount of information about disease progression, for instance as encoded by exosomal miRNA profiles.

Preferentially, future studies should include more PD patients in prodromal and *de novo* stages based on strict inclusion criteria [e.g., risk factors (45)] and with prolonged follow-up, since current studies only comprised a mere 10–30 study subjects per group. Baseline and intermittent sampling with preceding levodopa washouts would be necessary if early-stage PD subjects are to be included or if treatment was initiated in the meanwhile. Additionally, optimal cutoff CSF/plasma values of DOPAC, L-DOPA, and DHPG/MHPG need to be determined and independently verified on an international scale. In this regard, "very low, low, normal, or high" levels can be attributed to numerical meanings. Metabolomic approaches, such as liquid chromatography with sensitive electrochemical detection, whether or not coupled to mass spectrometry, are routinely implemented in most research hospitals and are feasible techniques for catecholamine biomarker analyses in daily clinical practice (73). Finally, one should also reckon with important methodological issues that are inherent in monoaminergic research, such as sampling conditions (e.g., CSF rostrocaudal concentration gradient, circadian rhythm (74), and dietary effects) and pre-analytical stability of CSF catecholamine metabolites (75).

AUTHOR CONTRIBUTIONS

Conceptualization, design, figure, and table: YV. Drafting of original manuscript: YV and YH. Critical manuscript revision and editing: IM and PD. Approval of final version: YV, YH, IM,

and PD. Funding acquisition: YV, IM, and PD. All authors agree to be accountable for all aspects with regard to this work.

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REVIEW
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Future Perspectives on the Relevance of Auditory Markers in Prodromal Parkinson's Disease

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Research on auditory processing in Parkinson's disease (PD) has recently made substantial progress. At present, evidence has been found for altered auditory processing in the clinical stage of PD. The auditory alterations in PD have been demonstrated with low-cost and non-invasive assessments that are already used in routine clinical practice. Since auditory alterations have been reported early in disease progression, it would be highly relevant to investigate whether auditory markers could be provided in the prodromal stage of PD. In addition, auditory alterations in early stage PD might be modulated by dopaminergic medication. Therefore, the aim of this review is (1) to summarize the literature on auditory processing in PD with a specific focus on the early disease stages, (2) to give future perspectives on which audiological and electrophysiological measurements could be useful in the prodromal stage of PD and (3) to assess the effect of dopaminergic medication on potential auditory markers in the prodromal stage of PD.

Keywords: Parkinson's disease, auditory processing, prodromal markers, audiometry, otoacoustic emissions, dichotic listening, auditory reflexes, auditory evoked potentials

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INTRODUCTION

Biomarker research in Parkinson's disease (PD) covers the development and validation of diverse clinical, biochemical, neuroimaging and genetic markers of pathological alterations, preferably in the early preclinical and prodromal stages of PD when a clinical diagnosis is not yet possible (1, 2). Valid and useful biomarkers potentially target a window of therapeutic opportunity in the early stages of the pathological process before clinical signs and symptoms emerge. Currently, evidence has been found for 16 prodromal markers in PD (3). Among these, non-motor symptoms are specifically interesting since they often precede the characteristic motor deficits in PD (4, 5). The non-motor symptoms in PD include, among others, autonomic dysfunctions, cognitive deficits, depression, sleep disorders, and sensory-perceptual alterations (6–8).

Biomarkers are specifically beneficial when they are non-invasive, easy and relatively inexpensive to administer, sufficiently available, and reliable (9, 10). These features seem explicitly present in assessments related to auditory processing, ranging from audiological toward electrophysiological measurements. At present, evidence has been found for altered auditory processing in the clinical stage of PD. The alterations range from disturbances in the processing of basic acoustic features toward the perception of affective and linguistic prosody (11, 12) and can be demonstrated with

low-cost and non-invasive assessments that are already used in routine clinical practice. Since auditory alterations have also been reported in the early stages of PD, it would be highly relevant to investigate whether auditory markers can be found in the prodromal stage of PD. However, to date, no studies have assessed the potential of auditory markers in the prodromal stage of PD. In addition, auditory alterations might be modulated by dopaminergic medication. As such, assessing the effect of dopaminergic medication on auditory processing in the prodromal and early stages of PD will be important to identify pharmacological strategies to optimize auditory processing in PD. Therefore, the aim of this review is (1) to summarize the literature on auditory processing in PD with a specific focus on the early disease stages, (2) to give future perspectives on which audiological and electrophysiological measurements could be useful in the prodromal stage of PD and (3) to assess the effect of dopaminergic medication on potential auditory markers in the prodromal stage of PD.

METHODS

A stepwise approach was followed to summarize the literature on auditory processing in PD and to achieve the objectives of the current review. First, a comprehensive literature search was performed in four databases: MEDLINE (PubMed interface), Embase (Embase.com interface), Web of Science and The Cochrane Central Register of Controlled Trials (CENTRAL Cochrane Library). The search strategy was based on two separate search strategies for a systematic review covering central auditory processing in parkinsonian disorders and a systematic review covering the auditory P3 in PD. Finally, to be able to provide a comprehensive review on all auditory processing stages, elements related to peripheral auditory processing were added that were not covered by any of the two existing search strategies. The search strategy included key terms related to PD on the one hand (e.g., "Parkinson Disease," parkinson*), and key terms related to auditory processing and its audiological and electrophysiological measurements on the other hand (e.g., "Hearing Threshold," "Auditory Perception," "Evoked Potentials Auditory, Brain Stem"). Only peer-reviewed articles in English were retained.

Second, titles and abstracts were screened to identify relevant studies. Subsequently, they were listed according to the type of audiological or electrophysiological measurement. Of these studies, Hoehn and Yahr (H&Y) staging (mean, SD, and range) and disease duration (mean, SD, and range) of the patients with PD who participated in the study, were determined. Articles were considered as "early stage PD" when the patient characteristics (of subgroups) met the following criteria: (1) all individual participants were at H&Y stage $<$ III, (2) mean group H&Y stage was $\leq$ II, and (3) mean group disease duration was $\leq$ 6.0 years (13–15). The literature on auditory processing in all clinical disease stages of PD was synthesized for each type of audiological or electrophysiological measurement. Detailed outcomes and results of the systematic review are published elsewhere (16). Finally, a specific focus was given on the results of "early stage PD" studies.

AUDIOLOGICAL MEASUREMENTS

Peripheral Auditory Tests

Pure-Tone Audiometry

Pure-tone audiometry has been considered a gold standard in the audiological examination to evaluate hearing sensitivity. Based on the participant's response to pure-tone stimuli, hearing thresholds are measured at a frequency range of 0.250–8 kHz. The clinical aim of pure-tone audiometry is to determine the type, degree and configuration of the patient's hearing loss. In study groups with older participants, presbycusis or progressive bilateral hearing loss related to aging can be expected. Indeed, a high-frequency age-related hearing impairment has been shown in studies that used pure-tone audiometry to evaluate the hearing sensitivity of patients with PD and age-matched control participants (HCs). On the one hand, multiple studies have demonstrated the same sloping audiometric pattern in patients with PD compared to HCs, consistent with the presence of an age-related hearing loss (17–20). These studies did not find significant differences between patients with PD and HCs regarding pure-tone hearing thresholds. On the other hand, higher pure-tone hearing thresholds in patients with PD have been reported by other research groups in the middle to high frequency range (1.5–8 kHz) (21–28). The differences between study results may be influenced by the approach chosen to analyze the data (e.g., categorical vs. continuous data-analysis, consideration of confounding variables, correction for multiple comparisons). So, although a sensorineural hearing loss has been shown in patients with PD, it remains unclear whether hearing thresholds as measured with pure-tone audiometry are indeed higher in patients with PD compared to age-matched HCs.

Regarding early stage PD, Pisani et al. (23) carried out pure-tone audiometry to investigate the effect of dopaminergic treatment on hearing sensitivity in 11 previously untreated *de-novo* patients with PD. In the drug-naïve condition, a significantly higher hearing threshold was found at 2 kHz in the PD group compared to age-matched HCs. Following the initiation of dopaminergic treatment for a period between 1 and 3 months, pure-tone hearing thresholds remained unchanged in *de-novo* patients with PD. In the study of Yylmaz et al. (28), higher hearing thresholds at 4 and 8 kHz were demonstrated in patients with PD at H&Y stage II. However, in their study, patients with PD were on average 6.1 years older compared to the HC group. In a recent study, Scarpa et al. (29) also reported higher hearing thresholds at 4 to 8 kHz in patients with PD with an average disease duration of 4.8 year compared to HCs. However, the authors suggested that increased hearing thresholds in the high frequency range may not be a distinct feature of PD, as patients with multiple system atrophy demonstrated a similar sloping audiometric pattern in the same study.

Speech Audiometry

In addition to pure-tone audiometry, speech audiometry is also routinely administered in audiological practice. Speech

audiometry compromises many tests, varying according to the type of speech material, task demands, response format and the presence of background noise. Speech recognition scores are calculated as the percentage of correct responses for each stimulus presentation level (relative to the noise). The resulting performance-intensity function is characterized in terms of its configuration and the level at which speech can be correctly identified half of the time [Speech Recognition Threshold, SRT (30)]. The clinical aim of speech audiometry is differentiating cochlear from retrocochlear lesions. Regarding speech audiometry in silence, two studies have found normal speech recognition scores in patients with PD compared to age-matched HCs (17, 31). In contrast, Vitale et al. (26) found a significantly higher SRT in patients with PD compared to age-matched HCs. In their study, significantly more patients with PD demonstrated a performance-intensity function that was suggestive of a cochlear dysfunction. The differences between study results are likely due to differences regarding hearing sensitivity of patients with PD and HCs, as measured with pure-tone audiometry. More specifically, if pure-tone hearing thresholds are increased compared to HCs, as was the case for patients with PD in Vitale et al. (26), abnormal speech recognition scores can be expected. Regarding speech audiometry in noise, no significant differences regarding speech recognition scores were reported between medicated patients with PD and HCs (17, 22). Interestingly, a modulatory effect of dopaminergic medication state was found on both speech audiometry in silence and in noise (17). The same patients with PD performed slightly better when they were tested without medication compared to when they were tested with medication.

No study has specifically focused on speech audiometry in early stage PD. As speech audiometry generally demonstrates normal results in patients with PD at more advanced disease stages, no differences are expected regarding speech perception in early stage patients with PD, provided that pure-tone hearing thresholds are within the normal range accounting for age and gender.

Otoacoustic Emissions

Otoacoustic emissions (OAEs) provide a non-invasive and objective measurement of outer hair cell (OHC) functioning. OAEs are sounds of cochlear origin, which are recorded with a microphone probe fitted in the ear canal. The sounds originate from vibrations of the eardrum that are transmitted backwards from the cochlea through the middle ear. These vibrations are caused by the motion of OHCs as they respond to auditory stimulation (32) (**Figure 1**). There are two widely used OAE measurements: transient evoked OAEs (TEOAEs) and distortion product OAEs (DPOAEs). Robust TEOAE responses are evoked by click stimuli around 80 dB SPL. Although clicks are broadband signals, TEOAEs give a frequency specific indication of OHC functioning between 1 and 4 kHz. On the other hand, DPOAEs are evoked using pairs of pure tones at a lower stimulus level, usually 65/55 dB SPL. DPOAEs offer a wider frequency range than TEOAE measurements with less sensitivity to minor and subclinical alterations in adults. Similar to hearing thresholds, age-related changes in the OAE response can be expected (34). In routine audiological practice, it is the presence of a detectable OAE response to a particular stimulus that is important. Frequencies at which hearing thresholds exceed 35 dB HL typically show an absent OAE response. Hence, the presence of OAE decreases with increasing age, especially at higher frequencies. In patients with PD, the presence of TEOAE and DPOAE responses did not differ significantly from HCs (17, 20). Additionally, for research purposes, the response amplitudes of OAEs can be used for group comparisons. Regarding TEOAEs, two studies reported lower response amplitudes in medicated patients with PD compared to HCs (18, 23). In contrast, De Keyser et al. (17) found no significant differences regarding TEOAE response amplitudes between medicated patients with PD and age-matched HCs. Regarding DPOAEs, similar response amplitudes were found between medicated patients with PD compared to HCs (17, 23). Nevertheless, a modulatory effect of dopaminergic medication on the OAE response amplitudes has been found. OAE response amplitudes were higher when the same patients with PD were tested without their medication (17). This result corroborates with the study of Lopes et al. (19), who found higher DPOAE response amplitudes in patients with PD receiving low daily doses of dopaminergic medication compared to patients receiving higher doses.

Moreover, OAEs can be used to examine the integrity of the efferent auditory pathways by applying contralateral acoustic stimulation (CAS). In normally hearing individuals, OAE response amplitudes decrease during CAS, because acoustic stimulation activates the medial olivocochlear (MOC) efferent system, which in turn has an inhibitory effect on OHC motility. This inhibitory effect is termed efferent suppression (ES). Generally, if CAS fails to reduce the OAE response amplitudes by 1 dB or more, ES is described as abnormal (35). In PD, abnormal ES of TEOAEs has been reported by Di Mauro et al. (18). In this study, "increased TEOAE response amplitudes during CAS" were described in patients with PD. It is, however, unclear whether response amplitudes were increased compared to HCs or whether they were increased compared to response amplitudes of the same patients, but in the absence of CAS. In any case, this result indicates decreased to absent ES of TEOAEs in patients with PD, which may support the involvement of the efferent auditory pathway in the pathology of PD. Regarding ES of DPOAEs, both patients and HCs generally demonstrated adequate reduction during CAS and no significant difference could be demonstrated regarding the amount of ES between patients with PD and HCs (19). Nevertheless, a modulatory effect of dopaminergic medication dosage has been found on ES in patients with PD. ES of DPOAEs was higher in patients receiving higher daily doses of dopaminergic medication compared to those receiving lower doses, albeit only statistically significant at 2 and 3 kHz.

Regarding early stage PD, only Pisani et al. (23) evaluated OAEs in 11 *de-novo* patients with PD. In their study, OAEs were administered to investigate possible alterations of cochlear functioning after initiating dopaminergic treatment in previously untreated patients with PD. In the drug-naïve condition, lower TEOAE and DPOAE response amplitudes were found in patients with PD compared to HCs. Following the initiation of dopaminergic treatment for a period between 1 and 3

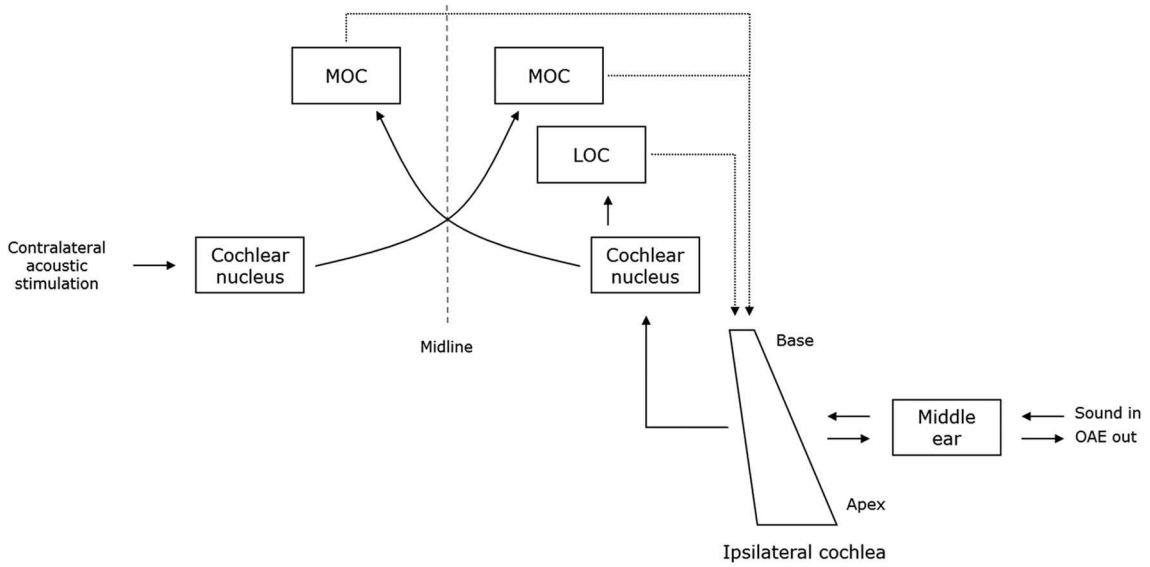

FIGURE 1 | A schematic representation of the structures that are involved in the generation and suppression of OAEs. The dashed and full lines represent the afferent and efferent auditory pathways, respectively. Sound enters the ipsilateral cochlea through the middle ear and is analyzed along the length of the cochlea. Outer hair cells amplify the motion of the basilar membrane. Vibratory energy is transmitted backwards through the middle ear and produces the OAEs. The medial (MOC) and the lateral olivocochlear (LOC) system provide efferent innervation to the cochlea. The MOC system is stimulated by crossed afferents and projects to the ipsilateral and contralateral cochlea. MOC efferents have an inhibitory effect on OHC activity. Therefore, acoustic stimulation of the contralateral ear leads to a reduction of the response amplitudes of OAEs (i.e., efferent suppression). Based on Guinan (33).

months, TEOAEs remained unchanged, while DPOAE response amplitudes significantly increased compared to the drug-naïve condition, resulting in an outcome more similar to HCs.

Auditory Reflexes

Stapedial reflex testing is usually administered as part of acoustic immittance measurements (tympanometry). When presented with a sufficiently high-intensity stimulus, the stapedial muscles contract bilaterally and stiffen the ossicular chain, thus decreasing middle-ear admittance (36). The stapedial reflex depends on the intact function of the entire reflex arc, including the middle and inner ear (sensory receptors), the cochlear nerve (afferent neurons), the lower brainstem (interneurons) and the facial nerve (efferent neurons). The stapedial reflex can be measured both ispilaterally and contralaterally. The specific site of lesion is determined by comparing the stapedial reflexes in response to ipsilateral vs. contralateral acoustic stimulation. The clinically most relevant outcome of stapedial reflex testing is the acoustic reflex threshold (ART) or the lowest intensity at which a minimal change of admittance is measurable. The ART generally ranges between 70 and 100 dB HL for pure tones. In the study of Murofushi et al. (37), patients with PD and HCs demonstrated an ART that was well within normal limits, although the ART of patients with PD was significantly lower compared to HCs. For research purposes, a series of latency measures can be extracted from the stapedial reflex. In patients with PD, the latency between stimulus onset and the time at which the stapedial reflex reaches 50% of its maximal amplitude was significantly prolonged compared to HCs (37). In addition, following cessation of the stimulus, patients with PD demonstrated a significantly longer latency for the amplitude to decrease to 50% of its maximal amplitude compared to HCs. These latency measures did not differ significantly between patients with PD taking dopaminergic medication and those who were not taking dopaminergic medication.

An auditory reflex that is also elicited by a high-intensity stimulus, is the auditory startle response (ASR). The ASR is a generalized motor response produced reflexively in response to a sudden, loud sound that results in a quick, usually observable movement. It is generally believed to be a brainstem reflex that originates in the pontine reticular formation, which is innervated by auditory afferents through the cochlear nucleus and connects with efferents that supply the target muscles, including the bulbar reticular tract, the reticulospinal tract, spinal interneurons and cranial and spinal motor neurons (38). The muscular activity of interest, such as the ocular, facial, neck, upper, and lower extremity muscles, can be objectively measured with electromyography (EMG). A variety of response parameters can be extracted from the EMG response, including the amplitude, latency, duration and habituation of the ASR. In the auditory field, the ASR is known as a cursory test for hearing sensitivity (39). In addition, an increased ASR has been associated with reduced sound tolerance (40), hyperacusis (41) and tinnitus (42). In PD, the ASR has primarily been studied as a potential marker for the differentiation from patients with progressive supranuclear palsy. The neural loss in this type of parkinsonism specifically involves the cholinergic neurons of the pontine reticular formation, including those involved in the ASR (43). In patients with PD, studies have generally found present ASRs demonstrating a normal amplitude, duration and

habituation compared to HCs (43–46). Regarding the onset latency of the ASR, both prolonged (45, 46), as well as shortened latencies (44) have been reported in patients with PD, depending on the muscular activity of interest. Prolonged latency measures have been related to withdrawal of facilitatory input to brainstem centers from the basal ganglia (45, 46). No modulatory effect of dopaminergic medication has been found on the ASR in patients with PD (45, 46).

No study has specifically focused on auditory reflexes in early stage PD. While auditory reflexes may be able to differentiate between patients with PD and progressive supranuclear palsy in the clinical stages of PD, it appears unlikely that abnormalities regarding the latency of auditory reflexes will be able to reliably detect early stage PD. Furthermore, latency abnormalities have also been reported in other parkinsonian disorders, such as multiple system atrophy (44, 47) and dementia with Lewy bodies (44). These findings suggest that auditory reflex testing has limited potential to discriminate PD from atypical parkinsonian disorders. In addition, auditory reflexes are mediated by multiple neural circuits and abnormal or absent responses do not necessarily comprise a problem with auditory function.

Central Auditory Tests

Psychoacoustic Experiments Assessing, Pitch, Loudness, Temporal Perception

Psychoacoustic experiments are concerned with the relationship between the physical characteristics of an auditory stimulus and their perceptual attributes (48). In PD, psychoacoustic research has been used to examine the perception of basic acoustic features (frequency, intensity, duration), i.e., pitch, loudness and duration. The methods used in these studies vary greatly according to stimulus type (verbal and non-verbal stimuli) and task demands (detection, discrimination, categorization and estimation). Regarding psychoacoustic experiments using pure-tone stimuli, patients with PD demonstrated increased discrimination thresholds for duration (49–52) and frequency [albeit not statistically significant (51, 53)] compared to HCs. Likewise, patients with PD had more difficulty at discriminating perceptually small acoustic differences compared to HCs (12, 54). On the other hand, when perceptually larger differences were used to assess auditory discrimination, no significant differences could be found between patients with PD and HCs (12, 53, 55). Auditory categorization and estimation tasks of intensity and duration have generally not found significant differences between patients with PD and HCs (53, 56–60). Regarding psychoacoustic experiments using speech stimuli, multiple studies have found abnormal perception of speech intensity (61–64). More specifically, patients with PD appear to overestimate the intensity of less intense speech and to underestimate the intensity of more intense speech.

Only a few studies have administered psychoacoustic experiments in patients with early stage PD. Breitenstein et al. (54) evaluated duration and frequency discrimination to assess the contribution of altered auditory perception to the disturbed perception of prosody. In this study, a subgroup of six recently diagnosed (on average 16.2 months) patients with PD were included who had not yet received dopaminergic treatment. No significant differences were found between early stage patients with PD and age-matched HCs for the discrimination of pure tones differing either with respect to frequency or duration. In the study of Lopes et al. (20), patients with PD were also divided into two subgroups regarding disease stage. As such, 34 patients with PD at H&Y stage I to II were included in the early stage PD group. The Duration Pattern Test demonstrated that early stage patients with PD had more difficulty to identify the order of a sequence of three pure tones (e.g., long—short—short) compared to HCs. This difference was, however, only significant in a subgroup of patients with PD aged 42–64 years, but not in older patients with PD. In the same study, the Gap-in-Noise test did not reveal significant differences between early stage patients with PD and HCs, as the duration detection threshold for silent intervals embedded in ongoing noise was similar in both groups. Likewise, patients in the more advanced PD group did not differ significantly from HCs. Lastly, Graber et al. (65) examined whether altered duration perception in PD affects their categorical perception of phonemes. They included nine early stage patients with PD, who were all at H&Y stage I to II and had a maximum disease duration of 6.0 years. The word-medial occlusion length and voice onset time were varied in order to create a continuum between two unambiguous endpoints, the minimal word pairs boden/boten and dick/tick, respectively. For both manipulations, early stage patients with PD made, on average, similar categorical decisions compared to age-matched HCs. Three patients, however, perceived "boden" throughout the whole continuum and needed a considerably longer occlusion interval to perceive the minimal pair cognate "boten." This result could not be readily explained by differences regarding disease staging (as all patients were in an early disease stage), age, dopaminergic medication state, nor verbal intellectual abilities. Altogether, current research has found no evidence for alterations regarding the perception of basic acoustic features in early stage PD. In more advanced PD, psychoacoustic studies suggest that (ab)normal auditory perception is highly dependent on the paradigm that is used (16). More specific, tasks that probe the lower limits of the ability to detect and discriminate auditory changes have demonstrated abnormal results in PD. Further research might indicate whether more complex psychoacoustic experiments could be suitable to differentiate early stage patients with PD from HCs.

Dichotic Listening Tests

Dichotic listening tests are among the most widely used behavioral tests to assess central auditory processing. Dichotic listening refers to listening to different speech stimuli presented to each ear simultaneously or in an overlapping manner (66). A great variation of dichotic listening tests exists, varying with regards to the speech stimuli (i.e., consonant-vowels, digits, words or sentences), as well as the response condition. Two types of response conditions can be used that engage the integration or segregation of binaural auditory input, respectively (30). Namely one condition that requires divided attention (free recall condition) and one condition that requires selective attention (right/left ear recall condition). Multiple studies have addressed dichotic listening in a divided attention paradigm in patients

with PD. Overall, these studies did not find significant group differences between patients with PD and HCs (20, 22, 67, 68). Only Richardson et al. (69) reported that a substantial part of patients with PD demonstrated abnormal dichotic listening compared to normative values.

In agreement with research in more advanced patients with PD, Lopes et al. (20) found no alterations regarding dichotic listening in a divided attention paradigm in early stage PD. In their study, a subgroup of 34 patients with PD at H&Y stage I to II did not differ significantly from HCs regarding the percentage of correctly repeated digits. On the other hand, Sharpe (70, 71) has evaluated dichotic listening in a more complex task to examine (auditory) attentional deficits in patients with early stage PD. The task consisted of dichotically presented word pairs containing the target word or a phonemic distractor paired with a phonetically unrelated word. Two studies used this task in either a divided attention paradigm (71) or a selective attention paradigm (70). In both studies, 14 patients with PD were included that were at H&Y stage I to II and had a mean disease duration of 4.2 years. In the divided attention paradigm, patients with PD discriminated significantly less target words compared to HCs. In the selective attention paradigm, patients with PD and HCs did not differ significantly regarding the discrimination of target words in the to-be attended ear, considering the percentage of ipsilateral responses to targets and distractors was similar in both groups. Nevertheless, the author suggested that "patients with PD were more prone to the interference of phonemic distractors," as patients with PD made "more contralateral responses to false positive errors in the unattended ear." In sum, most studies point to a preserved performance in conventional dichotic listening tests that probe divided auditory attention in all clinical stages of PD, including the earliest disease stage. Nevertheless, more complex dichotic listening tests may be able to differentiate early stage patients with PD from HCs.

Binaural Interaction Procedures

Binaural interaction tests depend upon intact binaural processing in the central auditory nervous system. Binaural processing enables sound localization and lateralization, and improves speech perception in adverse listening conditions. These tests require the combination of complementary input presented to both ears simultaneously, synthesizing intensity, temporal, and spectral differences of otherwise identical stimuli (66). Binaural interaction is presumed to occur in the brainstem (72). Hence, these tests are thought to be sensitive to brainstem lesions. A great variation of procedures can be used to evaluate binaural interaction. Some of these tests may be useful to demonstrate alterations regarding binaural processing in patients with PD compared to HCs, including auditory lateralization (73) and spatial listening tasks (22). In contrast, binaural masking level difference tasks, that assess the ability to detect a tonal stimulus in noise when the binaural signal-to-noise phase relationships are altered, show no significant differences in patients with PD compared to HCs (22).

No study has specifically focused on binaural auditory processing in early stage PD.

Summary: Audiological Measurements in PD

A summary of study findings regarding audiological measurements in early stage patients with PD can be found in **Table 1**. Altogether, auditory research in PD has found alterations at all clinical disease stages using audiological measurements associated with both peripheral and central auditory processing. Unfortunately, to date, few studies have specifically examined auditory processing in early stage PD. Nevertheless, these studies suggest an involvement of the auditory system early in disease progression. At the peripheral level, three studies using subjective pure-tone audiometry provided evidence that hearing thresholds in the middle to high frequency range may be increased in early stage patients with PD. In one of these studies, hearing thresholds did not change following initiation with dopaminergic medication. Likewise, objective OAE measurements demonstrated altered auditory processing in early stage patients with PD, based on decreased response amplitudes compared to HCs. Moreover, OAE response amplitudes increased following initiation with dopaminergic medication. Although OAEs are generally associated with cochlear dysfunction, alterations may also indicate a dysfunction of the efferent control of the OHCs by the MOC system. In this regard, decreased ES of OAEs has been reported in patients with more advanced PD. However, further research is warranted into early alterations of the MOC system in PD, assessed using OAE and ES measurements. Especially since an involvement of the MOC system can be hypothesized during the early stages of PD pathology involving the lower brainstem (75). Behavioral measurements associated with central auditory processing in PD demonstrated that results are generally highly dependent on the paradigm that is being used. Therefore, experiments may have to be tailored toward specific hypotheses regarding early stage PD. For example, more complex tasks that stress the auditory system to a higher level, such as adaptive measurements of auditory discrimination and adapted tasks of dichotic listening, may be more suitable to discriminate patients with early stage PD from HCs.

ELECTROPHYSIOLOGICAL MEASUREMENTS

Short Latency Auditory Evoked Potentials

Short latency auditory evoked potentials (AEPs), better known as the auditory brainstem response (ABR), are routinely evaluated in audiological practice. In adults, the peak latency and interpeak latency (IPL) of the ABR are typically used in the diagnosis of retrocochlear pathologies, such as vestibular schwannoma and lesions of the brainstem. The ABR consists of seven distinct waves that occur within 10 ms and is recorded with a high-intensity transient acoustic stimulus, most commonly a click. The auditory nerve and auditory nuclei located in the brainstem are the major structures involved in the generation of the ABR. Each wave, labeled using Roman numerals, reflects the synchronous firing of different auditory cell populations. As such, waves I and II are derived from the distal and proximal regions of the

TABLE 1 | Summary of study findings regarding audiological measurements in early stage patients with Parkinson's disease.

Audiological measurements	Description	Early stage PD studies	Study findings	Effect of dopaminergic medication	Strengths/limitations
Peripheral auditory tests					
i. Pure-tone audiometry	Measurement of hearing sensitivity to pure-tone stimuli	(23*, 28, 74)	Higher hearing thresholds at 2, 4, 6, and 8 kHz	No effect was found	+ Gold standard in audiology, standardized clinical method – Low specificity (high prevalence of high-frequency hearing impairment in the general elderly population), subjectivity
ii. Speech audiometry	Measurement of speech recognition	No early stage PD studies	–	–	+ Information on functional auditory status – Time consuming, influence of cognitive factors, low sensitivity (most patients in the clinical PD stage demonstrate no abnormalities), subjectivity
iii. Otoacoustic emissions	Measurement of outer hair cell cochlear functioning	(23)*	Lower TEOAE and DPOAE response amplitudes in *de-novo* patients with PD	DPOAE response amplitudes increased	+ Objectivity, direct link to neurotransmission, time efficiency, standardized clinical method, ability to detect subclinical auditory alterations – Low specificity (reduction of OAE response amplitude in the general elderly population), absent response at hearing thresholds >35 dB HL
iv. Auditory reflexes	Measurement of stapedial reflex or auditory startle response	No early stage PD studies	–	–	+ Objectivity – Low specificity (atypical parkinsonian disorders demonstrate abnormal auditory reflexes as well)
Central auditory tests					
i. Psychoacoustic tests	Measurement of acoustic feature perception	(20, 54, 65)	No difference in gap-in-noise detection, frequency and duration discrimination, phoneme categorization, altered duration pattern recognition	–	– Time consuming, subjectivity, no standardized clinical method, low sensitivity (most patients with early stage PD demonstrate no abnormalities)
ii. Dichotic listening tests	Measurement of the integration or segregation of binaural auditory input	(20, 70, 71)	Divided attention paradigm: no difference in digit repetition, altered target word discrimination in a complex task Selective attention paradigm: no difference	–	+ Sensitized test (high level of difficulty) – Influence of cognitive factors, no standardized clinical method, subjectivity
iii. Binaural hearing tests	Measurement of the integration of intensity, temporal, or spectral differences of otherwise identical stimuli	No early stage PD studies	–	–	– No standardized clinical method, subjectivity

Study findings indicate the results of audiological measurements in early stage patients with Parkinson's disease (PD) compared to healthy control participants. Studies investigating the effect of dopaminergic medication on auditory processing in early stage PD are indicated with an asterisk (*). The main strengths and/or limitations whether or not to incorporate the audiological measurements into a prodromal auditory marker protocol for PD are given. PD, Parkinson's disease.

auditory nerve as it enters the brainstem, while waves III to VII are generated from successively higher brainstem structures (76). The components following wave V show large intersubject and within-subject variability and are, therefore, less useful for research purposes. Studies on age-related changes of the ABR have reported only slight changes regarding wave latencies and IPLs. Generally, prolongations appear to be limited to the early waves I to III, suggesting that age primarily affects peripheral auditory nerve transmission (77, 78). As increasing evidence suggests that PD initially affects the brainstem and follows a

predominant upwards course (75), considerable attention has been devoted to measures of brainstem functioning in PD, such as the ABR. On the one hand, various studies have reported normal latency measures in patients with PD compared HCs (25, 27, 79, 80). On the other hand, multiple other studies have reported prolonged latencies for the centrally generated waves III to V in patients with PD compared to age-matched HCs (21, 24, 28, 81–84). In addition, prolonged IPLs have also frequently been found in patients with PD compared to HCs for waves III-V, and I-V. The differences between study results cannot be readily explained by methodological differences or patient variables. At present, although there is some evidence that suggests abnormal ABR results, experimental support for altered auditory brainstem processing in patients with PD is inconsistent.

Regarding early stage PD, two studies have investigated the ABR in early stage patients with PD. Karayanidis et al. (79) administered ABR testing in 16 patients with PD (to ensure that the main outcome of their study, which was centered around the long latency event-related potentials (ERPs), was not confounded by differences in auditory brainstem processing). All patients had a relatively recent diagnosis (on average 3.0 years) and "most of them were at H&Y stages I or II". Compared to age-matched HCs, patients with PD exhibited a non-significant prolongation of the IPL between wave I and III. No significant group differences were found for the IPL between wave I and V and wave V latency. In contrast, Yylmaz et al. (28) found significantly prolonged IPLs between wave I and V and wave V latencies in 20 patients with PD at H&Y stage II compared to HCs. However, in their study, patients with PD were on average 6.1 years older compared to the HC group. The result of Yylmaz et al. (28) may indicate a similar pattern of centrally located ABR abnormalities found in more advanced patients with PD. Nonetheless, further research is warranted.

Middle Latency Auditory Evoked Potentials

Following the ABR, middle latency AEPs are generated between 10 and 50 ms after the onset of a transient auditory stimulus, such as a tone burst or a click. Middle latency AEPs are not routinely administered in audiological practice, as they are highly sensitive to the participant's attention and state of arousal, as well as to several recording parameters, especially stimulus presentation rate (85–87). However, when these variables are adequately controlled for, middle latency AEPs can be useful to assess the functional integrity of the auditory pathways and to localize lesions at the thalamocortical and primary auditory cortex levels (87). Traditionally, four positive and three negative peaks are included in the middle latency AEPs, namely V, N0, P0, Na, Pa, Nb, and P50. In patients with PD, P50 is the most frequently studied middle latency AEP. When using a sufficiently low stimulus presentation rate, no abnormalities regarding P50 amplitude or latency appear evident in patients with PD compared to HCs (88–91). On the other hand, higher presentation rates may lead to P50 being absent or having a prolonged latency in a substantial part of patients with PD (92). In addition to stimulus trains, the P50 can be evoked by pairs of stimuli. The paired-stimulus paradigm is useful for investigating auditory gating. Auditory gating represents the central auditory nervous system's ability to suppress irrelevant auditory stimuli (93). When two identical stimuli are presented in a paired-stimulus paradigm, P50 to the second stimulus is inhibited relatively to the first stimulus in HCs. Patients with PD exhibited significantly less inhibition of P50 to the second stimulus compared to age-matched HCs, suggesting diminished auditory gating in PD (90, 91). All patients were in rather advanced disease stages (H&Y ranging between III and V). Interestingly, when patients were divided according to disease stage, both studies found that patients at H&Y stage III did not differ significantly from HCs regarding P50 inhibition, and that abnormalities were limited to patients with PD at H&Y stages IV and V.

No study has specifically focused on the middle latency AEPs in early stage PD. It may be interesting to investigate middle latency AEPs in early stage patients with PD in response to high stimulus presentation rates, as abnormalities were found in a large sample of patients with PD ($n = 46$) that varied greatly regarding H&Y disease stage (range I to IV) and disease duration (range 1–24 years) (92). Regarding auditory gating, based on the studies of Teo et al. (90, 91), it appears unlikely that P50 inhibition would be able to discriminate early stage patients with PD from HCs, given the finding that only patients in the most advanced disease stages differed significantly from HCs in terms of P50 inhibition.

Long Latency Auditory Evoked Potentials

P1-N1-P2 Complex

The auditory P1-N1-P2 complex represents an exogenous stimulus-related response associated with sound detection (94, 95). The ERP-waveform consists of three deflections, namely P1, N1, and P2 that reach their maximal amplitude at around 50, 100, and 160 ms respectively. The first component, P1, has been considered to overlap -to some extent- with the P50 studied as a middle latency AEP. The auditory P1-N1-P2 complex can be evoked in both passive or active listening conditions based on different stimulus paradigms, such as stimulus trains, paired-stimulus paradigms or oddball paradigms. In audiological practice, the P1-N1-P2 complex has been most commonly investigated as an objective counterpart of behavioral audiometry to estimate a participant's hearing thresholds. On average, the P1-N1-P2 complex can be found at 10 dB above the behavioral hearing threshold (96). From a broader point of view, the outcome of the P1-N1-P2 waveform highly depends on the acoustic characteristics of the stimulus, such as frequency, intensity, duration, and location (94). Based on the majority of studies that used an auditory oddball paradigm, no altered auditory P1-N1-P2 complex in patients with PD compared to HCs could be found (16). Significant differences in latency or amplitude values of the different subcomponents have been reported between patients with PD and HCs [e.g., (97–101)], however, no conclusive pattern of auditory alterations emerged. It is unclear which participant, clinical or ERP related variables could explain the heterogeneous study results.

Regarding early stage PD, the same inconclusive pattern was found (79, 99, 100, 102–107). Therefore, no clear alterations in stimulus-related sound detection in the early stage of PD can

be assumed based on the current results from auditory oddball paradigms. However, in the study of Beucke et al. (108), altered intensity dependence of AEPs (IDAEP) was demonstrated in early stage PD. A significantly increased IDAEP of the N1/P2 amplitude, indicating low serotonergic activity, was found in unmedicated patients with PD compared to HCs. This difference was no longer evident after 12 weeks of dopaminergic treatment in patients with PD (108). In addition, based on a paired-stimulus paradigm, Lukhanina et al. (109) and Lukhanina et al. (110) demonstrated significantly reduced post-excitatory inhibition of the auditory N1/P2 complex following the second stimulus in patients with PD evaluated without dopaminergic medication state compared to HCs. Subgroup analyses based on disease stage, revealed that diminished post-excitatory auditory cortical inhibition may be evident at the early stage of the disease (110). Furthermore, auditory inhibition of the second stimulus seems to improve after dopaminergic intake in patients with PD (110).

Mismatch Negativity

The auditory mismatch negativity (MMN) is an ERP associated with the automatic pre-attentive detection of a deviant auditory stimulus in a sensory memory trace (85, 111, 112). As such, the MMN may be considered as a signature of auditory discrimination abilities (94, 113). The component is classically obtained with an auditory oddball paradigm in which a deviant stimulus infrequently occurs in a sequence of standard stimuli. The deviant stimulus may comprise a change in frequency, duration, intensity, location, inter-stimulus interval, or the omission of the stimulus compared to the standard stimulus (85, 113, 114). The component is very useful in clinical conditions that require no cooperation of the patient since the MMN can be elicited in the absence of the participant's attention (113). Accordingly, the participant can be asked to perform a visual task, such as watching a silent-video or reading a book, whilst ignoring the auditory stimuli, or to focus on stimulus characteristics other than that of the deviant stimulus during an attentive oddball paradigm (113, 115, 116). The MMN response is detectable with a deviant-minus-standard wave and peaks between 100 and 250 ms at the frontocentral and central scalp electrodes. Based on the review by Seer et al. (117) no evidence was found for altered MMN latency and amplitude values in non-demented patients with PD compared to HCs. In addition, no effect of dopaminergic medication on the auditory MMN could be demonstrated (115, 116).

In most of the studies, the auditory MMN was evaluated in (subgroups of) patients with early PD (79, 99, 115, 116, 118). Thus, it can be assumed that no deficiencies in automatic auditory discrimination are present in the early stage of PD as investigated with a MMN paradigm, irrespective of the dopaminergic medication status (115). Furthermore, no significant staging effect on the MMN was found in non-demented patients with PD in a later study of the same research group (116). Decreased auditory MMN amplitudes may be evident when PD is associated with PD dementia and hence, neurodegeneration is in an advanced stage (116, 118).

Processing Negativity or Nd

In selective attention tasks, attended and unattended auditory stimuli differ, for example, in location, frequency (i.e., pitch) or both on which basis the participant may select the task-relevant stimuli (96, 119, 120). More specifically, the participant attends one channel in which a deviant stimulus must be detected in a sequence of standard stimuli whilst ignoring the other channel (oddball paradigm in a dichotic listening condition) (119). Generally, the ERPs related to the standard stimuli in the attended channel are compared to those of the unattended channel. The negative shift of the ERPs to the attended stimuli relative to the unattended stimuli has been related to selective auditory attention and has been identified as the Nd or processing negativity (PN) (119, 121–123). Based on decreased amplitude values of the Nd in patients with PD, alterations in selective auditory attention in PD have been suggested (79, 106, 124). However, it remains unclear which aspects of selective auditory attention might be altered in patients with PD, as different results have been reported regarding the early central subcomponent (Nd1) and the late frontal subcomponent (Nd2) (16). The Nd1 may reflect the matching process of incoming auditory stimuli with the internal template, whereas Nd2 is thought to represent the updating of the internal template (125). Nonetheless, further research is needed since selective auditory attention has not yet been investigated sufficiently based on electrophysiological dichotic listening paradigms. Yet, investigating this aspect of auditory processing might be highly relevant even more because alterations of the Nd have been demonstrated in studies in which early stage PD patients were included (79, 106, 124). Vieregge et al. (124) evaluated the effect of dopaminergic medication on the Nd in patients with PD with an average disease duration of 5.0 years and H&Y stages ranging between I and III. Compared to HCs, a decreased Nd amplitude was reported in patients with PD after a 12-hour withdrawal from dopaminergic medication. Following dopaminergic medication, the Nd remained unchanged in patients with PD.

N200

The auditory N200 or N2 is a negative wave between 200 and 350 ms post stimulus onset that is endogenous in nature (96, 126). Different components of the N2 wave have been described based on the design of the ERP paradigm and its modality, namely N2a, anterior N2 (N2b) and posterior N2 (N2c) (85, 126). An auditory N2a can be elicited by an inattentive auditory mismatch effect in which case the component is commonly known as the MMN as described above (85, 126). Regarding the N2b component, latency and amplitude values have been defined based on the deviant target stimuli in attentive auditory oddball paradigms. In this regard, various studies have reported an increased N2 latency in non-demented patients with PD compared to HCs [e.g., (97, 101, 127)] although the result was not always statistically significant and non-differences have also been demonstrated [e.g., (105, 128)]. Most studies found no differences in N2 amplitude between patients with PD and HCs [e.g., (101, 105, 127)]. Yet, a decreased N2 amplitude has been reported in the studies of Lagopoulos et al. (129), Lagopoulos et al. (98), and Pekkonen et al. (99) using an auditory oddball

paradigm. Regarding the N2c component, PD related results are beyond the scope of the current review since this component has been specifically considered for the visual modality.

Regarding early stage PD, Broussolle et al. (130) evaluated N2 latency in 8 *de-novo* patients with PD with an average disease duration of 1.3 years. Six of these patients had not yet received dopaminergic medication. N2 latency did not significantly differ between patients with PD compared to age-matched HCs. Likewise, the majority of studies reported no abnormalities regarding N2 latency in patients with early stage PD, neither without dopaminergic medication (102), nor with dopaminergic medication (79, 99, 105, 107). Only Philipova et al. (100) found a prolonged N2 latency in patients with PD compared to HCs, but only when participants were instructed to provide a motor response to the presented stimuli and not when they were required to count the target stimuli. Overall, however, no clear N2 alterations can be assumed early in disease progression.

P300

The auditory P300 or P3 is a high-level endogenous cognitive ERP generated by an attentive response to an infrequent deviant stimulus (131). Usually, the distinction is made between a parietally maximal P3b component and a frontally maximal P3a component. The P3b component is elicited after the presentation of a task-relevant deviant stimulus and may be considered a signature of auditory categorization based on top-down voluntary goal-driven attention and working memory processing (132, 133). In addition, the P3a is elicited by a non-target task-related or novel distractor stimulus and can be regarded as a neurophysiological marker of bottom-up involuntary stimulus-driven attention and the orienting response (134–137). The auditory P3 component has been classically obtained with a two (standard, deviant) or three-stimulus (standard, target deviant, non-target deviant/novel distractor) auditory oddball paradigm in which the participants are instructed to count the task-relevant deviant stimuli or to respond by a button-press. The P3 can be described by the average of the deviant stimuli or a deviant-minus-standard wave and starts from 250 ms at the parietal and frontal scalp electrodes for the P3b and P3a respectively. Regarding P3b, the review of Seer et al. (117) concluded that a prolonged P3b latency may be evident in demented patients with PD. The authors found no conclusive pattern of P3b differences in non-demented patients with PD, although P3b latency was significantly increased in 38% of the related studies. In addition, it was stated that P3b amplitude was generally found to be unaltered in demented and non-demented patients with PD (117). However, it should be noted that regarding the P3b specifically, both auditory and a smaller amount of visual oddball paradigms were considered. Regarding P3a, latency and amplitude findings were rather heterogeneous (117), although a decreased P3a amplitude seems to be related to disease duration (116, 117).

Regarding early stage PD, significant differences in latency or amplitude values of the P3b component have been reported in patients with PD compared to HCs (100, 103–105, 107, 138). Overall, however, no clear pattern of P3b alterations emerged in the early stage of cognitively non-impaired patients with PD (79, 100, 102–105, 107, 130, 138–141). In addition, no effect of dopaminergic medication could be shown in the study of Georgiev et al. (140). In contrast, evidence has been demonstrated for alterations of the P3a component in early stage patients with PD compared to HCs (15, 104, 115, 116, 138, 140, 142). In the study of Cavanagh et al. (142), a trend for an increased P3a amplitude was found in early stage patients with PD. The authors suggested that—when filter settings are considered—the altered P3a amplitude is in line with the study of Solis-Vivanco et al. (116) in which a decreased P3a amplitude could be demonstrated in patients with PD compared to HCs. Moreover, a diminished habituation to novel stimuli over time was found in the PD group (142). Likewise, alterations of the P3a amplitude were evident in the study of Pauletti et al. (138). In their study, an increased P3a latency and decreased P3a amplitude were shown in PD patients with central fatigue (138). Although no significant differences in the P3a component were found between patients with PD and HCs in the study of Solis-Vivanco et al. (15), the authors suggested that impaired novelty detection may be evident in the early stage of PD based on a reduced phase alignment for deviant stimuli using time-frequency based analyses (15). Finally, amplitude alterations of the P3a component seem most evident when patients with PD are evaluated with dopaminergic medication (115, 140, 142).

Summary: Electrophysiological Measurements in PD

A summary of study findings regarding electrophysiological measurements in early stage patients with PD can be found in **Table 2**. Based on various AEPs, neurophysiological alterations in auditory processing have been demonstrated in the clinical stage of PD. Considering early stage PD, a careful selection of electrophysiological paradigms may be suitable to discriminate patients with early stage PD from HCs. Study results particularly suggest a pattern of centrally located ABR abnormalities (wave III–V) in PD. However, as ABR studies in early stage PD are limited and rather inconsistent, further research is warranted. Especially because of the potential involvement of the lower brainstem during the early stages of PD pathology (75). Furthermore, altered long latency AEPs in early stage PD may be evident when ERP paradigms are tailored to evaluate specific and/or more complex auditory processes. Regarding paired-stimulus and intensity dependence paradigms, an increased N1/P2 amplitude in early stage patients with PD has been found. These differences disappeared following initiation with dopaminergic medication in patients with PD. Regarding selective attention and three-stimulus oddball paradigms, studies have shown a decreased Nd amplitude and a decreased or increased P3a amplitude. Regardless of the direction of the P3a amplitude alteration, shifts of the P3a component were most evident when patients with PD were evaluated with dopaminergic medication.

DISCUSSION

Prodromal markers are defined as indicators of an ongoing neurodegenerative process in the central or peripheral nervous

TABLE 2 | Summary of study findings regarding electrophysiological measurements in early stage patients with Parkinson's disease.

Electrophysiological measurements	Description	Early stage PD studies	Study findings	Effect of dopaminergic medication	Strengths/limitations
Short latency AEPs	Measurement of auditory brainstem responses	(28, 79)	Non-significant prolonged IPL I–III, no difference or prolonged IPL I–V and wave V latency	–	+ Objectivity, standardized clinical method, established neural generators, high specificity – Inconsistent evidence
Middle latency AEPs	Measurement of subcortical and primary auditory cortex responses	No early stage PD studies	–	–	+ Objectivity – No standardized clinical method, low sensitivity (most patients in the clinical PD stage demonstrate no abnormalities)
Long latency AEPs					
i. P1-N1-P2	Measurement of auditory signal detection	(79, 99, 100, 102–107, 108*, 109, 110*)	Increased IDAEP of the N1/P2 amplitude Decreased inhibition of the N1/P2 amplitude using a paired-stimulus paradigm No clear alterations using an auditory oddball paradigm	IDAEP differed no longer with HCs Inhibition of the second stimulus increased	+ Objectivity, information on distinct auditory (sub-)processes, direct link to neurotransmission, correlated with behavioral outcome measures – Influence of subject related variables, such as alertness and arousal, low sensitivity for specific ERP components (e.g., MMN, N2 and P3b)
ii. MMN	Measurement of automatic pre-attentive auditory discrimination	(79, 99, 115*, 116*, 118)	No clear alterations	No effect was found	
iii. Nd/PN	Measurement of auditory selective attention	(79, 106)	Decreased Nd amplitude	–	
iv. N2	Measurement of voluntary auditory discrimination and categorization	(79, 99, 100, 102, 104, 105, 130)	No clear alterations	–	
v. P3	Measurement of attentive auditory categorization related to (1) bottom-up involuntary stimulus-driven attention and the orienting response (P3a) or to (2) top-down voluntary goal-driven attention and working memory processing (P3b)	(15, 79, 100, 102–105, 107, 115, 116, 130, 138, 139, 140*, 141*, 142*)	Evidence for P3a alterations No clear P3b alterations	P3a: Increased P3a amplitude P3b: No effect was found	

Study findings indicate the results of electrophysiological measurements in early stage patients with Parkinson's disease (PD) compared to healthy control participants. Studies investigating the effect of dopaminergic medication on auditory processing in early stage PD are indicated with an asterisk (*). The main strengths and/or limitations to whether or not incorporate the electrophysiological measurements into a prodromal auditory marker protocol for PD are given. PD, Parkinson's disease; AEPs, auditory evoked potentials, IPL, interpeak latency; IDAEP, intensity dependence of auditory evoked potential; MMN, mismatch negativity; PN, processing negativity.

system prior to the typical symptoms allowing a clinical diagnosis (143). A prodromal marker can refer to any disease indicator (1), whether it be of clinical, neuro-imaging, biochemical or genetic origin. Of critical importance in the utility of prodromal markers is their sensitivity (the certainty with which a marker can identify prodromal PD) and specificity (the ability of a marker to identify disease-free individuals). In addition, the practicalities and difficulties involved in assessing a prodromal marker must be considered (10). More specifically, a prodromal marker should be non-invasive, easy and relatively inexpensive to administer, sufficiently available, and reliable (9, 10).

Ideally, studies that aim to define prodromal markers should be prospective and prodromal markers should be identified before the patients develop PD (10). Unfortunately, to date, no prospective studies have assessed the potential of auditory markers in the prodromal stage of PD. Nevertheless, indirect evidence for the relevance of auditory markers in prodromal PD comes from studies in which auditory assessment was carried out in the early clinical disease stage. At present, multiple studies have investigated clinically diagnosed patients with early stage PD (H&Y $\leq$ II). H&Y stage I and II represent early pathology in the olfactory bulb and the lower brainstem (75), possibly causing prodromal symptoms (e.g., olfactory dysfunction, constipation

and rapid eye-movement sleep behavior disorder) (144). As such, the earliest pathological disease stages are an important step in delineating which auditory measurements may be useful to detect prodromal PD. However, whether audiological or auditory electrophysiological measurements have the potential to detect prodromal PD depends on the necessary characteristics of a biomarker. Overall, auditory alterations in PD can be evaluated with relatively low-cost and non-invasive audiological and electrophysiological measurements that are already routinely available in clinical practice.

Regarding audiological measurements associated with peripheral auditory processing, pure-tone audiometry revealed significantly increased middle to high frequency hearing thresholds in early stage patients with PD compared to HCs. However, increased pure-tone hearing thresholds are highly prevalent in the elderly population, which seriously affects the specificity of hearing thresholds as a marker of prodromal PD. In addition, pure-tone audiometry is a behavioral test that relies on a subjective response and requires repeated responses to provide a reliable outcome. Hence, the potential of pure-tone audiometry as a marker of prodromal PD is rather limited. An objective measurement that is generally associated with peripheral auditory processing involves OAEs. OAEs are relatively easy to acquire, fast to administer and require limited cooperation of the participant. Research has found preliminary evidence for altered OAE response amplitudes in *de-novo* patients with PD. Moreover, OAEs have been reported to be sensitive to dopaminergic medication, which might make them relevant for monitoring the pharmacological response. However, older patients, as well as patients with a history of excessive noise exposure will potentially produce rather weak OAEs with no measurable responses in the high frequency range. Hence, OAE measurements in PD comprise both strengths and shortcomings regarding biomarker perspectives. Therefore, further research into the sensitivity and specificity of OAE alterations in prodromal PD is warranted.

The involvement of the peripheral auditory system in PD has generally been regarded as an age-dependent sensorineural dysfunction (i.e., presbycusis) (23, 27). Structural changes underlying presbycusis include loss of cochlear hair cells, which in turn, affects afferent transmission of auditory information to higher auditory structures (145). Cochlear hair cells are subject to feedback control from the olivocochlear efferent system, which has been proposed to play an important role in preventing noise-induced and age-related hearing impairment. The auditory efferent system originates in the brainstem and involves two major pathways. The LOC efferents provide innervation of the inner hair cells (IHCs), whereas the MOC efferents primarily project to the OHCs. Interestingly, dopamine is released from the LOC efferents and exerts a neuroprotective circuitry for the cochlea by preventing excitotoxic damage during glutamate overstimulation (146). In addition, alpha-synuclein has been located in the cholinergic MOC system (147). By directly controlling OHC motility and subsequently modulating basilar membrane motion, the MOC system is proposed to exert a second efferent pathway preventing hearing impairment. In sum, peripheral auditory alterations in PD most likely represent the combined effects of physiological aging processes and the neuropathological changes intrinsic to PD, which may leave patients with PD at a higher risk for developing noise-induced, as well as age-related, hearing impairment. However, the effect of dopaminergic medication on OAEs cannot be readily explained by dopaminergic expression at the level of IHCs, as OAEs mainly represent OHC function. Despite the apparent lack of dopaminergic terminals in the OHC region, Pisani et al. (23) suggested that dopamine may exert a modulatory effect on OHCs via LOC synapses on the MOC efferents. Alternately, De Keyser et al. (17) argued that abnormal OAEs might result from a dopamine deficiency at the level of the brainstem.

Audiological measurements associated with central auditory processing are inherently subjective in nature (66) and, therefore, have limited potential as a reliable marker for prodromal PD. In addition, in contrast to peripheral auditory tests, these measurements are no standardized audiological methods, using a variety of stimuli and procedures, and differing greatly regarding task demands. Generally, central auditory processing has been linked to behavioral phenomena such as sound localization, auditory discrimination and auditory performance with competing auditory signals (148). Interestingly, many of these central auditory processes can be objectively and possibly more sensitively assessed using electrophysiological measurements.

Auditory electrophysiological measurements are characterized by a high temporal resolution and provide a continuous measure of auditory processing, whereas behavioral measures of central auditory processing reflect the combined effect of many neural processes (85). Therefore, ERPs have the ability to demonstrate how auditory processing unfolds over time and to uncover which distinct neural processes may be altered in prodromal PD. For example, in contrast to behavioral measures of selective auditory attention, electrophysiological registration during dichotic listening tasks may reveal which specific stages in the process of selective auditory attention are altered in prodromal PD. Moreover, electrophysiological measurements meet the necessary practicalities of a biomarker. At present, electrophysiological studies of auditory processing in PD have primarily focused on the processing of non-verbal auditory stimuli using two-stimulus oddball paradigms. Based on early stage PD studies, auditory ERPs derived from this type of paradigm (e.g., MMN, N2, and P3b) seem unlikely to detect prodromal PD. Nevertheless, paradigms that assess specific and/or more complex auditory processes have demonstrated differences between early stage patients with PD and HCs. ABR measurements are well-known in clinical neurological practice and may be relevant to further investigate the potential early involvement of the lower brainstem in PD pathology (75). Nonetheless, bilateral central ABR prolongations have been reported in a wide variety of neurological conditions (e.g., demyelinating diseases, Alzheimer's disease, schizophrenia) (74, 149, 150) which may affect the specificity of ABR measurements as a marker of prodromal PD. Regarding intensity dependence paradigms, an increased IDAEP of the N1/P2 component has been found in early stage patients with PD compared to HCs suggesting lower serotonergic activity and related depression in PD (108, 151). Interestingly, symptoms of

depression may occur before the clinical onset of PD (152, 153) and have already been prospectively established as a prodromal marker of PD (3). As such, IDAEPs are highly relevant as an objective indicator for this prodromal marker in PD. Finally, ERP paradigms that assess the modulation of incoming auditory stimuli have shown alterations in early stage PD compared to HCs ranging from automatic pre-attentive to attentive auditory processing. More specific, pre-attentive auditory gating abnormalities in early stage PD have been suggested given a disinhibited N1/P2 component in paired-stimulus paradigms. At a higher level, Nd and P3a component abnormalities have been shown using dichotic oddball or attentive three-stimulus paradigms in early stage PD. Therefore, electrophysiological measurements focusing on these aspects of auditory processing may have a potential as a marker for prodromal PD. However, further research is needed to detect and validate the AEPs that show abnormalities in the prodromal stage of PD.

The current study results may suggest an involvement of the central auditory system in early stage PD when involuntary inhibitory or attentive selective auditory processing is required. Along the auditory pathway, nuclei of the brainstem auditory system have been found to exert an inhibitory function in auditory processing (154). Since PD pathology especially involves brainstem structures during the early disease stages (75), inhibitory dysfunction of auditory processing in early stage PD can be hypothesized. Regarding higher-order auditory processing, the involvement of a dysfunctional fronto-striatal circuitry in attentive auditory processing in PD has been considered. To date, the pathophysiological mechanisms underlying central auditory processing deficits in PD are poorly understood and thus, are subject for further research. Nonetheless, dopamine deficiencies may be related to both auditory dysfunctions at the brainstem and cortico-subcortical level. Dopamine has been suggested a neurotransmitter involved in sensory processing and therefore, may be hypothesized to have a modulatory role in auditory processing. In the current review, study results may imply a positive effect of dopaminergic medication on inhibitory function of repeated auditory stimuli. In contrast, increased resource allocation for the unattended channel or novel (distractor) stimuli processing was suggested in early stage PD. Moreover, alterations of the P3a component were most evident when patients with PD were evaluated with dopaminergic medication. Since the cortico-subcortical circuits are not equally affected by PD pathology, dopaminergic medication may differentially effect higher-order cognitive auditory processing (155). Taken together, dopaminergic medication could modulate both peripheral and central auditory processing in patients with PD. However, few studies have directly investigated the effect of dopaminergic medication on auditory processing in early stage PD.

Overall, the current review provided evidence for auditory alterations in the early stages of PD. At present, however, the relevance of auditory markers in prodromal PD is unclear. In order to truly gain insight into the value of prodromal auditory markers, prospective studies in large populations or in selected high-risk groups are needed. The assessment of auditory processing is already routinely carried out in the general population and compromises standardized and reliable measurements, making them highly suitable for prospective biomarker studies.

CONCLUSION

At present, research has demonstrated altered auditory processing in early stage PD using audiological and electrophysiological measurements. Future perspectives for auditory markers in the prodromal stage of PD can be found in the use of objective audiological and specific electrophysiological measurements. However, further research is warranted to assess the sensitivity and specificity of these auditory measurements as well as their relationship to other markers of prodromal PD. Since few studies have directly investigated the effect of dopaminergic medication on auditory processing in early stage PD, it is currently unclear whether patients with PD would benefit from early pharmacological intervention regarding auditory processing.

AUTHOR CONTRIBUTIONS

ED and KD wrote the manuscript under the supervision of MD. PS, DT, AB, DB, and MD provided critical feedback and helped shape the manuscript. All authors approved the final version for submission.

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A Study of the Relationship Between Uric Acid and Substantia Nigra Brain Connectivity in Patients With REM Sleep Behavior Disorder and Parkinson's Disease

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Low levels of the natural antioxidant uric acid (UA) and the presence of REM sleep behavior disorder (RBD) are both associated with an increased likelihood of developing Parkinson's disease (PD). RBD and PD are also accompanied by basal ganglia dysfunction including decreased nigrostriatal and nigrocortical resting state functional connectivity. Despite these independent findings, the relationship between UA and substantia nigra (SN) functional connectivity remains unknown. In the present study, voxelwise analysis of covariance was used in a cross-sectional design to explore the relationship between UA and whole-brain SN functional connectivity using the eyes-open resting state fMRI method in controls without RBD, patients with idiopathic RBD, and PD patients with and without RBD. The results showed that controls exhibited a positive relationship between UA and SN functional connectivity with left lingual gyrus. The positive relationship was reduced in patients with RBD and PD with RBD, and the relationship was found to be negative in PD patients. These results are the first to show differential relationships between UA and SN functional connectivity among controls, prodromal, and diagnosed PD patients in a ventral occipital region previously documented to be metabolically and structurally altered in RBD and PD. More investigation, including replication in longitudinal designs with larger samples, is needed to understand the pathophysiological significance of these changes.

Keywords: uric acid, REM sleep behavior disorder, parkinson's disease, resting state, functional connectivity

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INTRODUCTION

Parkinson's disease (PD) is a chronic, progressive neurologic disease characterized by motor deficits that include tremor at rest, rigidity, slowing of movement, and postural instability. PD pathology includes extensive loss of brain dopaminergic neurons, which occurs before the emergence of gross neurologic deficits, as well as the presence of Lewy body eosinophilic inclusion within neurons. Etiologic factors include a role for aging (1), a role for environmental factors (2, 3) including herbicide/pesticide exposure, and specific disease-causing genetic mutations, most notably in the α-synuclein (4), and the parkin (5) genes. Pathogenic mechanisms proposed to underlie the

neuronal degeneration in PD include free radicals, deficits in energy metabolism, specifically abnormalities of iron metabolism and mitochondrial complex I, programmed cell death, and protein aggregation. The free radical-mediated injury theory, which is also referred to as the oxidant stress hypothesis, is arguably the leading explanation for pathogenesis due most notably to the fact that the major degradative pathway for dopamine is its oxidative deamination by monoamine oxidase A and B resulting in highly reactive hydroxyl radicals (6). While the oxidant stress hypothesis receives indirect support from numerous lines of evidence which have been extensively reviewed (7, 8), it remains only a hypothesis with shortcomings that include that aspects of the hypothesis that are dependent on catecholamine metabolism are not relevant to degeneration of structures like the nucleus basalis, which is cholinergic, and which also degenerate in PD. Nevertheless, since oxidative stress appears to play a role in the pathogenesis of Parkinson's disease (PD) (9, 10), questions remain about the role of antioxidants in PD and especially during the prodromal stage.

Uric acid (UA) is the end product of purine metabolism, and it accounts for most of the antioxidant capacity in human blood (11). Higher serum UA levels are associated with lower risk of developing PD in the general population (12, 13) and slower disease progression in the PD population (14). The presumptive neuroprotective action of UA includes suppression of oxygen radical accumulation, stabilization of calcium homeostasis, preservation of mitochondrial function, chelation of iron and blocking of iron dependent oxidation reactions, and the slowing of the dopamine auto-oxidation rate in the caudate and SN of PD patients (15–19).

A notable characteristic of UA's influence in PD is sex differences (20). In men only, UA was the first biomarker shown to be consistently associated with lowering risk of developing PD (12, 21–23) by 33% (24) as well as changing disease prognosis in males with PD (25–29). A link between UA and the risk of development or progression of PD in women is weaker possibly due to biological differences in interactions between sex-specific hormones and UA. This occurs independently of factors including age, smoking, obesity, hypertension, thiazide use, and caffeine consumption, all of which have been associated with PD and uricemia (30, 31).

A major pathological signature of PD is cell death in the SN (32–37). Previous research also indicates reduced SN functional connectivity and basal ganglia dysfunction in rapid-eye-movement sleep behavior disorder (RBD) (38, 39). RBD is a parasomnia that is associated with an increased likelihood of developing either PD or another alpha-synuclein neurodegenerative disorder (40, 41). Identifying the neural changes accompanying RBD has become a research priority for developing objective markers of early diagnosis (42–44). Neuroimaging studies show that RBD is associated with altered striatal dopaminergic innervation (45, 46), striatal volumetric differences (47), and reduced nigrostriatal and nigrocortical resting state functional connectivity (38). More recent neuroimaging studies including structural MRI, [18F]fluorodeoxyglucose PET, and fMRI resting state connectivity implicate posterior cortical regions, including lingual gyrus, in PD and RBD (37, 48–50). A large proportion of patients develop Parkinson's disease years after the diagnosis of their RBD (51, 52). The presence of polysomnography-proven RBD is a prodromal marker, with a positive likelihood ratio (LR+) of 130 and a negative likelihood ratio (LR–) of 0.65. Meanwhile, low plasma urate levels are a risk marker, with an LR+ of 1.8 (in men) and an LR– of 0.88 (in men) (53).

To our knowledge, no previous study has investigated the relationship between SN functional connectivity and levels of UA in RBD or PD patients. UA levels are directly reduced within the SN of Parkinson's patients (15), which adds to the rationale for investigating how connectivity of this structure covaries with UA levels. Studies using the horseradish peroxidase retrograde transport technique motivate investigation of SN connectivity with cortex because they show SN cells give rise to highly collateralized axons and innervate different regions of cortex, including cingulate cortices, prefrontal and suprarhinal cortex, and entorhinal cortex, as well as subcortical sites (54). To date, only one cross-sectional study has been reported investigating the role of UA in RBD (55), but no studies have investigated the relationship between levels of UA and SN functional connectivity in either RBD or PD. One hypothesis is that SN connectivity decreases in the prodromal phase of idiopathic RBD and further decreases by the time the early diagnosis of Parkinson's disease is made. Understanding how the relationship between UA and SN functional connectivity differs during the prodromal and clinically-defined stages of PD may inform the ongoing debate about how UA levels are associated with changes in neural connectivity. Therefore, the objective of the present study is to explore the relationship between UA levels and SN functional connectivity in males using the eyes-open resting state fMRI method. Resting state fMRI allows for measurement of intrinsic neuronal fluctuations to identify markers of prodromal neurodegeneration (42). We used whole-brain voxelwise ANCOVA and a cross-sectional design to test the prediction that SN functional connectivity exhibits a positive relationship with UA in controls, and that this positive relationship decreases in RBD patients and decreases further in PD patients.

MATERIALS AND METHODS

Experimental Design

A total of 66 participants were recruited from the sleep clinic or movement disorders clinic or referred to our study from its entry at www.clinicaltrials.gov (NCT00817726). All participants provided written informed consent under a study protocol (HSC-MS-08-0147) that was approved by the UTHealth Institutional Review Board. The study was conducted in accordance to the International Conference on Harmonization Good Clinical Practice Guideline and the principles of the Declaration of Helsinki. Inclusion criteria were men aged 35–75 years old who did not have an unstable medical condition, and met criteria for one of the study groups including an early-to-moderate PD group, an idiopathic RBD group, and a control group. For all participants in the study, we asked about first- and second-degree family members with PD or any other neurodegenerative

TABLE 1 | Participant demographics and clinical measures.

	Control	RBD	PD
Number of subjects	11	32	23
Age (yrs.), mean (SD)	54.82 (12.77)	57.47 (8.20)	61.17 (10.91)
DoD, mean (SD)	NA	3.13 (2.23)	5.89 (5.48)
RBD by polysomnography (*n*), %	0 (0.0)	32 (100)	16 (69.56)
Handedness	9 R, 2 L	28 R, 3 L, 1 LR	21 R, 2 L
UA (mg/dl), mean (SD)	5.60 (1.02)	5.19 (1.24)	4.81 (0.92)
MOCA, mean (SD)	28.36 (2.01)	27.44 (4.35)	27.23 (2.89)
UPSIT, mean (SD)	34.36 (6.04)	29.31 (7.55)	19.48 (6.77)
UPDRS-M, mean (SD)	0.64 (1.21)	2.16 (4.04)	25.22 (14.96)
H&Y, mean (SD)	0.00 (0.00)	0.00 (0.00)	1.91 (0.95)
Tremor-dominant/ akinetic-rigid*	NA	NA	10 TD, 9 AR, 4 Mixed
Laterality of disease involvement	NA	NA	PD (RBD+): 7 R, 9 L PD (RBD-): 5 R, 2 L Total: 12 R, 11 L
Interfering medications	None	2, Thiazide	1, Thiazide

*DoD, duration of disease; MOCA, Montreal Cognitive Assessment; UPSIT, University of Pennsylvania Smell Identification Test; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr Staging. *Sub-type was determined using Schiess et al. criteria. NA,Not Applicable.*

disorder, but this information was not used as an exclusion criterion for RBD or PD. Each was assigned to one of these three groups according to the inclusion criteria detailed in the next paragraphs and listed in **Table 1**. Included are 11 control subjects (CON), 32 patients with REM Sleep Behavior Disorder (RBD), and 23 patients with Parkinson's disease (PD). A total of 16 of the PD patients were confirmed to have RBD, denoted in this paper as PD (RBD+) and seven of the PD patients were confirmed not to have RBD, denoted as PD (RBD–).

The diagnosis of PD was made based on the United Kingdom Brain Bank Diagnostic Criteria (56). Patients with parkinsonian symptoms due to atypical parkinsonism, vascular PD, or medicine/toxin-induced parkinsonism were excluded. No genetic tests were administered to identify genetic forms of PD. However, young-onset PD was excluded, which has a higher genetic prevalence. To exclude advanced disease, we used the Hoehn and Yahr disability scale (57) with a cutoff of $\leq$ 3.5 in the off-medicine state. Twelve of the 23 PD patients had right lateralized disease involvement, while 11 of the 23 patients had left lateralized disease involvement. Of the 16 patients with PD (RBD+), seven were right lateralized and nine were left lateralized. Of the seven patients with PD (RBD–), five were right lateralized and two were left lateralized.

The RBD group diagnostic criteria was based on the American Academy of Sleep Medicine (AASM) (58). Nocturnal video-polysomnography (NPSG) was performed in an AASM-accredited sleep disorders center, with a minimum of 10% REM sleep recorded and at least 10% of REM epochs documented to be without atonia. The criteria for the control group included individuals who have no personal history or primary family history of PD or neurodegenerative disease, and no history of dream enactment or REM sleep without atonia. All subjects met the above inclusion criteria and were matched to members of the PD or RBD groups in age ($\pm$ 3 years). All controls underwent a PSG to rule out the presence of RBD. The PD patients also underwent a PSG to determine whether they were PD (RBD+) or PD (RBD–).

All individuals underwent clinical, behavioral, and brain imaging in the off-medicine state defined as no PD medicines for at least 12 h before the assessment the night before. The clinical assessment included a Montreal Cognitive Assessment Test (MoCA), and Unified Parkinson's Disease Rating Scale (UPDRS) evaluation with I-IV subscales. Medications like thiazide diuretics, loop diuretics, allopurinol, colchicine, febuxostat among others could interfere with UA levels. None of the controls used interfering medications, and only two patients with RBD and one patient with PD used thiazide medication that could impact UA levels (**Table 1**).

Uric Acid

Serum UA concentration was measured in an early morning blood sample drawn from a peripheral vein by using a uricase colorimetric method on non-fasting blood. A one-way analysis of variance (ANOVA) with one factor, group, with four levels of CON, RBD, PD (RBD+), and PD (RBD–) was performed on the UA measurements using Prism 8 for macOS Version 8.3.

Magnetic Resonance Imaging

Each participant underwent magnetic resonance imaging (MRI) using a Philips 3T scanner (Philips Medical Systems, Bothell, WA). Since dopaminergic medications influence the functional MRI signal of the task and rest state (59, 60), patients with PD were scanned in the off-medication state. The structural images acquired included a T1-weighted magnetization-prepared rapid acquisition turbo field echo sequence (repetition time/echo time [TR/TE] = 8.4/3.9 ms; flip angle = 8 degrees; matrix size = 256 $\times$ 256; field of view = 240 mm; slice thickness = 1.0 mm, sagittal acquisition. The functional images acquired included a whole-brain echo-planar imaging (EPI) run sensitive to BOLD contrast (TE = 30 ms; flip angle = 90 degrees; 2 s TR; 150 dynamics; 2.75 $\times$ 2.75 $\times$ 3.5 mm voxel resolution) which was acquired while participants were instructed to rest while remaining still and fixating a white cross hair displayed on a black background during the functional acquisition.

Image Analysis

Image processing was performed with the Analysis of Functional Neuroimages (AFNI) (61). AFNI's python script afni_proc.py was used to process each participant's structural and functional MRI using the "example 11" resting state analysis procedure (https://afni.nimh.nih.gov/pub/dist/doc/program_help/afni_proc.py.html). The steps included in this analysis were (1) Despiking: the shrinking of any large spikes in the fMRI time series, (2) time shifting: the correction of slice timing differences, (3) Aligning: determining the alignment between the fMRI time series and anatomical T1, (4) Spatial normalization: determining the alignment between the anatomical T1 and a template brain, and (5) Censoring and regression: removing timepoints due to

excessive motion and regressing out from the censored time series the contributions to signal from the ventricles and local white matter. We used a relatively conservative threshold to censor TR pairs where the Euclidean Norm of the motion derivative exceeds 0.3. The proportion of volumes censored in each of the four groups was computed [CON = 0.199 (±0.162), RBD = 0.215 (±0.241), PD (RBD+) = 0.179 (±0.214), PD (RBD–) = 0.082 (±0.108)] and did not differ significantly among the groups [$F_{(3,64)} = 0.7449$, $p = 0.5297$]. For volumes that were not censored, the computed motion regressors were regressed out as covariates of no interest. We used the recommended default -KILL option where the censored timepoints were removed rather than filled with zeros or by values interpolated from neighboring non-censored timepoint values.

Resting state functional connectivity was estimated in each subject using an average fMRI time series accumulated in voxels representing left and right SN. A mask representing left and right SN was built using the multi-contrast PD25 atlas (62) using the following steps. First, the PD25 T1 MPRAGE volume with 1 mm resolution was co-registered to each subject's native-space skull-stripped T1 anatomical volume using a non-linear warping algorithm in AFNI. The segmented left and right SN volumes of the PD25 atlas were warped into the native-space of the T1 anatomical by applying the non-linear warp transformation. Then the transform from the native T1 anatomical space to the TT_N27 brain template that was computed in the *afni_proc.py* was applied to the segmented left and right SN volumes of the PD25 atlas, which resulted in segmented left and right SN masks for each subject in Talairach space. The SN masks in Talairach space were averaged and thresholded to create a single group mask representing the location of left and right SN. The thresholding level ensured that voxels in this mask represented complete overlap in the location of SN in at least 50% of the subjects so that the SN seed vectors for each subject were derived from the same voxel locations in standard space for the exploratory group analyses.

Statistical Analysis

Group resting state analysis was conducted in AFNI. First, the *3dSetupGroupInCorr* command was used to build files of whole-brain masked residual error time series (niml *.ertts) for the CON, RBD, PD (RBD+), and PD (RBD–) groups. Next, the *3dGroupInCorr* command was used to compute a standardized Z-score difference map of the slope of the inverse hyperbolic tangent of the correlation (i.e., Fisher transformation) of a seed vector with every voxel time series in the brain. The seed vector was made by averaging the time series of non-zero seed voxels in the bilateral SN mask. The output of the *3dGroupInCorr* command resulted in a standardized correlation coefficient (*zcorr*) volume for each subject in each of the four groups CON, RBD, PD (RBD+), and PD (RBD–). These volumes were next input to AFNI's *3dMVM* command (63), a group-analysis program that performs traditional analysis of covariance (ANCOVA). A between-subject factor *group* included four levels, CON, RBD, PD (RBD+), and PD (RBD–), and a quantitative covariate variable *uric acid* was included to produce F statistical maps representing the main effect of *group*, the main effect of *uric acid*, and the interaction between *group* and *uric acid*. An additional *post-hoc* analysis was performed using the same ANCOVA model but with the images for the 11 patients with left disease onset [2 PD (RBD–) and 9 PD (RBD+)] flipped about the x-axis using AFNI's *3dLRflip* command. The rationale for this analysis was that it would align for these 11 patients their diseased and non-diseased hemispheres with the 12 other patients [5 PD (RBD–) and 7 PD (RBD+)] with right disease onset whose images were not flipped.

Given the exploratory nature of these analyses, statistical tests with a height threshold of $p < 0.05$ and a cluster extent threshold (k) of 50 or greater were evaluated. A cluster-wise multiple comparison correction was computed using *3dClustSim* to estimate the probability of false positive (noise-only) clusters. *3dClustSim* is based on simulating the noise field that interferes with detection of the "true" signal in the dataset. To do this, *3dClustSim* needs statistics about the spatial smoothness of the noise. These estimates were computed for each subject using *3dFWHMx* with the -acf option, which computes the spatial autocorrelation of the data as a function of radius, then fits that to a model of the form $ACF(r) = a*exp(-r*r/(2*b*b))+(1-a)*exp(-r/c)$. *3dFWHMx* output the 3 model parameters (a,b,c). An average of these parameters across all subjects were input to *3dClustSim* (ACF 0.65, 3.70, 9.77) resulting in a FWHM of 9.51 mm with 3D grid dimensions of 64 × 76 × 60 (2.5 × 2.5 × 2.5 mm^3) and 91,631 voxels in the brain mask (31.40% of total voxels). *3dClustSim* determined given an uncorrected height threshold (pthr) of 0.05 that a cluster of size 358 voxels or greater would occur <5% by chance assuming first-nearest neighbor clustering (above threshold voxels cluster together if faces touch). In *3dClustSim* smoothing simulated data over a finite volume is known to introduce edge artifacts. To minimize this, extra-large padded simulated volumes were made before blurring (64 × 76 × 60 pads to 96 × 120 × 96), which were trimmed back down to the desired size before continuing with the thresholding and cluster-counting steps. Clusters of a size that survived the multiple comparisons correction are denoted in results tables. Given the exploratory nature of these analyses, clusters with a size that did not reach statistical significance after the multiple comparisons correction are also included in results tables as it has been pointed out that reporting of statistical results using arbitrary cluster-forming height thresholds and arbitrary minimum cluster size is not in itself problematic (64). These tables can be used to confirm replication of results in other exploratory analyses (65) and facilitate meta-analyses (66).

RESULTS

Analysis of Uric Acid Levels

The lab reference range for UA levels at Houston's Memorial Hermann Hospital is 3.80 to 8.00 mg/dl. All CON subjects had UA levels (mean = 5.62, SD = 1.15, min = 4.10, max = 7.50, range = 3.40) within the lab reference range. A total of 29 of 32 RBD subjects had UA levels (mean = 5.19, SD = 1.24, min = 3.10, max = 8.00, range = 4.90) within the lab reference range, with three having UA levels below the reference minimum of 3.80 mg/dl. A total of 6 of 7 PD (RBD–) subjects had UA levels

(mean = 5.16, SD = 1.22, min = 3.5, max = 7.20, range = 3.70) within the lab reference range with one having a UA level below the reference minimum of 3.80 mg/dl. A total of 13 of 16 PD (RBD+) subjects had UA levels (mean = 4.66, SD = 0.81, min = 2.80, max = 5.90, range = 3.10) within the lab reference range with three having UA levels below the reference minimum of 3.80 mg/dl.

An ANOVA showed no main effect of group on UA levels [$F_{(3, 64)} = 1.789$, $p = 0.158$, $R^2 = 0.077$]. The distribution of UA in each of the four groups is shown in **Figure 1**. *Post-hoc* analyses using Tukey's multiple comparisons test indicated no significant pairwise differences between the groups (**Table 2**). The largest difference was between CON and PD (RBD+), 5.623 vs. 4.662 mg/dl, but the mean difference of 0.961 was not significantly different (adjusted $p = 0.110$).

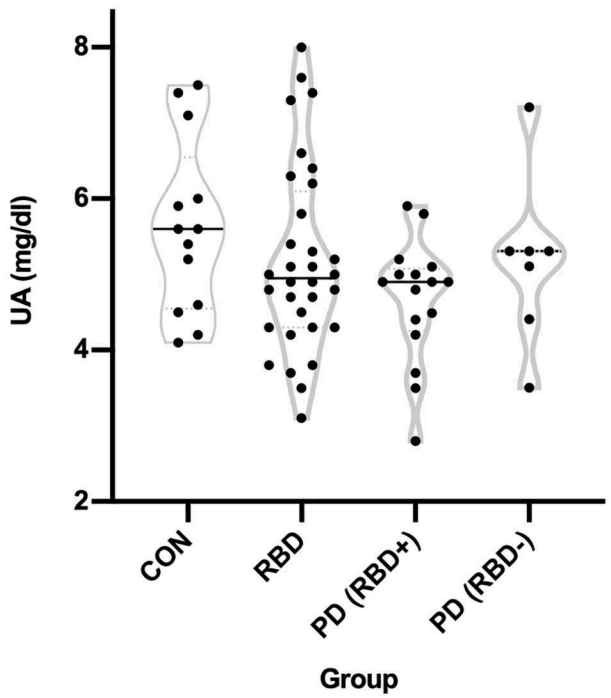

FIGURE 1 | Distribution of uric acid levels in each group. Measured UA levels in the CON, RBD, PD (RBD+), and PD (RBD–) groups are shown using violin plots where the width of the distribution of points is proportionate to the number of points at a given Y value.

Analysis of Substantia Nigra Functional Connectivity

Voxelwise ANCOVA identified eight brain clusters showing a main effect of group on SN functional connectivity (**Figure 2**). These clusters encompassed right nucleus accumbens, left superior temporal gyrus, right parahippocampal gyrus, left caudate, left cingulate gyrus, left superior frontal gyrus, right brainstem, and right cerebellum (**Table 3a**).

Voxelwise ANCOVA identified 15 clusters showing a main effect of UA on SN functional connectivity (**Figure 3**). These clusters included left lingual gyrus, right angular gyrus, right postcentral gyrus, left inferior frontal gyrus, left caudate, left insula, right hippocampus, right inferior gyrus, right cerebellum and right putamen (complete list in **Table 3b**). The largest cluster, encompassing 459 voxels [peak $F_{(1, 58)} = 14.13$, $p = 0.0004$], survived the multiple comparison correction and was centered at MNI $x = -17$, $y = -86$, $z = -9$ in left lingual gyrus (BA 18).

Voxelwise ANCOVA also identified 15 clusters exhibiting an interaction between group and UA on SN functional connectivity. These clusters encompassed left lingual gyrus, right middle frontal gyrus, right precentral gyrus, left cerebellum, left middle occipital gyrus, left thalamus, right lingual gyrus, left calcarine gyrus, right brainstem, and right Heschl's gyrus (**Table 3c**). The left lingual gyrus cluster of size 250 [peak $F_{(3,58)} = 8.33$, $p = 0.0001$] was centered at MNI $x = -23$, $y = -90$, $z = -20$ and encompassed a subset of the 459 voxel left lingual gyrus cluster identified in the main effect of UA F-map (cluster 1, **Table 3b**), which survived the multiple comparison correction. For the *post-hoc* ANCOVA in which the 11 patients with left disease onset were flipped, the previous largest cluster encompassing lingual gyrus (cluster 1, **Table 3c**) reflecting a Group-by-UA interaction increased in extent from 250 voxels to 416 voxels (cluster 1, **Supplemental Table 1**). A smaller cluster in the other hemisphere encompassing lingual gyrus (cluster 7, **Table 3c**) increased in extent from 79 voxels to 108 voxels (cluster 5, **Supplemental Table 1**).

TABLE 2 | Pairwise group comparisons of uric acid levels.

Pairwise Comparison (Group 1 vs. Group 2)	Group 1 UA (mg/dl)	Group 2 UA (mg/dl)	Mean diff	SE of diff	95% C.I. of diff	Adjusted *p*
CON vs. RBD	5.62	5.19	0.44	0.37	−0.54 to 1.41	0.64
CON vs. PD (RBD+)	5.62	4.66	0.96	0.42	−0.14 to 2.07	0.11
CON vs. PD (RBD–)	5.62	5.16	0.47	0.53	−0.92 to 1.85	0.81
RBD vs. PD (RBD+)	5.19	4.66	0.53	0.34	−0.38 to 1.43	0.43
RBD vs. PD (RBD–)	5.19	5.16	0.03	0.47	−1.20 to 1.27	>0.99
PD (RBD+) vs. PD (RBD–)	4.66	5.16	–0.49	0.51	−1.84 to 0.85	0.76

UA levels among the groups were compared using a 1-way ANOVA with group as factor and four levels of CON, RBD, PD (RBD+), and PD (RBD−). The main effect of group was not significant [$F_{(3, 64)} = 1.79$, $p = 0.15$]. Each pairwise comparison is shown with mean UA for each group, the group difference, the standard error of the group difference, the 95% confidence interval (C.I.) for the difference, and the adjusted p-value resulting from the Tukey's multiple comparisons test. The largest mean difference was between CON vs. PD (RBD+) with 0.96 higher UA for CON, but this difference did not reach significance ($p = 0.11$).

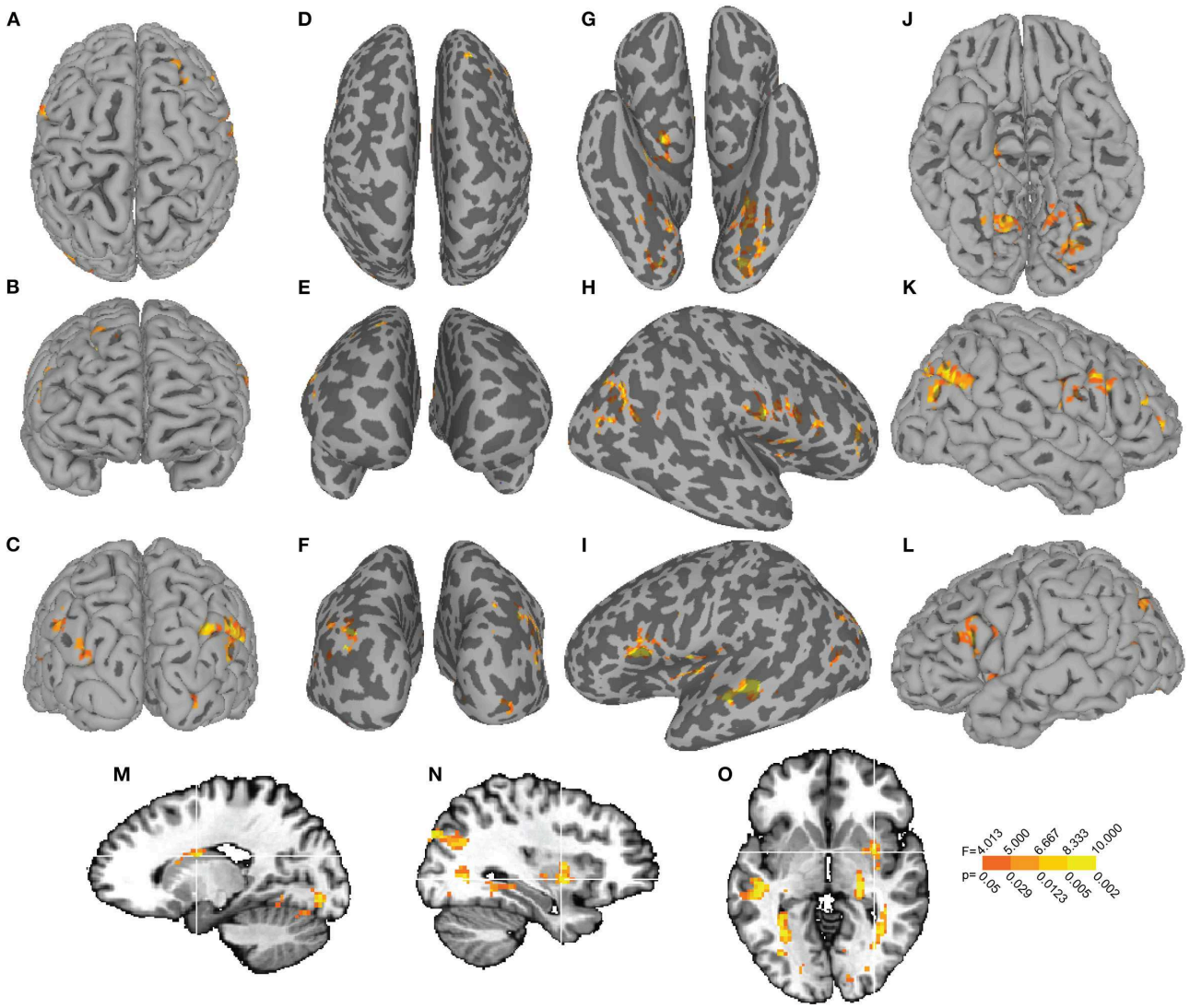

FIGURE 2 | The main effect of uric acid on SN functional connectivity. The main effect of UA F-map is displayed on dorsal **(A)**, anterior **(B)**, and posterior **(C)** views of the pial surface with corresponding inflated representations **(D–F)**. Inflated views of the ventral **(H)**, right hemisphere **(I)**, and left hemisphere **(J)** cortex are displayed next to their pial representations **(K–M)**. Sagittal slices through left **(N)** and right **(O)** hemisphere and an axial slice **(P)** show main effects in left caudate (cluster 5, **Table 3b**) and right putamen (cluster 10, **Table 3b**).

Of the 15 clusters identified in the ANCOVA interaction F-map, six clusters (**Figures 4A–F**) exhibited a relationship in which CON individuals had the highest positive slope, with RBD patients showing a less positive slope, and the PD (RBD+) and PD (RBD–) patients having slopes lower than the RBD subjects. This graded decrease in slopes across the groups was evident in the left lingual gyrus cluster 1 (**Figure 4A**) in which the CON slope was 0.04803 [$F_{(1, 9)} = 6.438$, $p = 0.0318$], the RBD slope was a less positive 0.01859 [$F_{(1, 30)} = 7.705$, $p = 0.0094$], the PD (RBD+) slope was near flat at 0.003069 [$F_{(1, 14)} = 0.04061$, $p = 0.8432$] and the PD (RBD–) slope was negative at −0.06649 [$F_{(1, 5)} = 9.517$, $p = 0.0273$]. For the *post-hoc* ANCOVA in which the 11 patients with left disease onset were flipped and for which the cluster encompassing lingual gyrus increased in extent, the slopes exhibited a similar pattern with CON = 0.03763 [$F_{(1, 9)} = 6.442$, $p = 0.0318$], RBD = 0.01592 [$F_{(1, 30)} = 7.856$, $p = 0.0088$], PD (RBD+) = 0.00797 [$F_{(1, 14)} = 0.5477$, $p = 0.4715$], but with the biggest difference being a more significantly negative slope for PD (RBD–) = −0.0688 [$F_{(1, 5)} = 16.10$, $p = 0.0102$] (**Supplemental Figure 1**).

Of the 15 clusters identified in the ANCOVA interaction F-map, four clusters exhibited a relationship in which RBD patients had a positive slope while the CON individuals had negative or flat slopes. This relationship was evident in the right middle frontal gyrus cluster two (**Supplemental Figure 2**). In this right middle frontal gyrus cluster RBD subjects had a positive slope of 0.03231 [$F_{(1, 30)} = 13.05$, $p = 0.0011$], CON subjects had negative slope of −0.07525 [$F_{(1, 9)} = 10.60$, $p = 0.0099$], PD

TABLE 3 | Brain regions identified by ANCOVA.

Cluster	Cluster Size	X	Y	Z	Brain Region	Peak (F, p)
a) Main effect: group						
1	200	6	1	−12	R. Nucleus Accumbens	6.30,0.0009
2	73	−42	−42	11	L. Superior Temporal Gyrus	6.15, 0.001
3	72	25	8	−23	R. Parahippocampal Gyrus	7.72, 0.0002
4	68	−10	17	−5	L. Caudate	7.19, 0.0003
5	61	−17	24	29	L. Cingulate Gyrus	5.11, 0.003
6	60	−10	26	56	L. Superior Frontal Gyrus (BA 6)	7.55, 0.002
7	55	4	−30	−35	R. Brainstem	5.77, 0.001
8	52	28	−65	−35	R. Cerebellum (Crus 1)	6.32, 0.0009
Cluster	**Cluster Size**	**X**	**Y**	**Z**	**Brain Region**	**Peak F(F, p)**
b) Main effect: UA						
1	459[†]	−17	−86	−9	L. Lingual Gyrus (BA 18)	14.13, 0.0004
2	209	52	−73	34	R. Angular Gyrus	12.80, 0.0007
3	171	62	−12	20	R. Postcentral Gyrus	14.39, 0.0003
4	118	−53	19	16	L. Inferior Frontal Gyrus (BA 45)	20.24, 3.33e-05
5	118	−17	4	19	L. Caudate	10.74, 0.002
6	90	−44	−10	3	L. Insula	13.70, 0.0005
7	88	22	−15	−16	R. Hippocampus	10.34, 0.002
8	86	38	−58	−2	R. Inferior Temporal Gyrus	12.71, 0.0007
9	86	12	−60	−11	R. Cerebellum (Culmen)	12.03, 0.001
10	74	33	2	−6	R. Putamen	11.91, 0.001
11	66	22	42	37	R. Superior Frontal Gyrus (BA 8)	11.72, 0.001
12	65	−49	−26	−4	L. Superior Temporal Gyrus	15.39, 0.0002
13	64	49	−1	2	R. Insula	15.45, 0.0002
14	57	43	28	12	R. Inferior Frontal Gyrus (BA 45)	12.91, 0.0007
15	54	17	−91	−3	R. Lingual Gyrus	12.33, 0.0009
16	51	−26	−33	4	L. Thalamus	15.07, 0.0003
Cluster	**Cluster Size**	**X**	**Y**	**Z**	**Brain Region**	**Peak F**
c) Interaction: group-by-UA						
1	250[‡]	−23	−90	−20	L. Lingual Gyrus (BA 18)	8.33, 0.0001
2	215	28	35	34	R. Middle Frontal Gyrus (BA 8)	7.66, 0.0002
3	134	31	−19	50	R. Precentral Gyrus	7.19, 0.0003
4	89	−23	−28	−35	L. Cerebellum (IV-V)	9.70, 2.78e-05
5	85	−36	−88	14	L. Middle Occipital Gyrus (BA 19)	4.99, 0.004
6	80	−17	−9	14	L. Thalamus	10.01, 2.06e-05
7	79	15	−63	−10	R. Lingual Gyrus	6.89, 0.0005
8	74	−6	−69	13	L. Calcarine Gyrus	5.17, 0.003
9	72	1	−23	−38	R. Brainstem	10.56, 1.22e05
10	72	36	−32	16	R. Heschl's Gyrus	7.14, 0.0004
11	60	22	52	−9	R. Superior Frontal Gyrus (BA 10)	6.23, 0.001
12	59	−36	−31	8	L. Heschl's Gyrus	7.27, 0.003
13	56	17	58	10	R. Superior Frontal Gyrus (BA 10)	5.58, 0.002
14	56	−6	55	24	L. Superior Frontal Gyrus (BA 9)	7.24, 0.0003
15	50	31	−26	−4	R. Lentiform Nucleus	6.99, 0.0004

The voxelwise ANCOVA analysis produced clusters for the main effect of group (a), the main effect of uric acid (b), and the group-by-uric acid interaction (c). For each of the three F maps, clusters exceeding a joint threshold (cluster height $p < 0.05$ & cluster size $k \geq 50$ voxels) are listed in descending order by k along with the corresponding Montreal Neurological Institute x,y,z coordinate, labeled brain region with Brodmann Area (BA) where applicable, peak F value of the cluster and associated probability value. [†]Cluster exceeds threshold corrected for multiple comparisons. [‡]Cluster is a subset of cluster [†] in b.

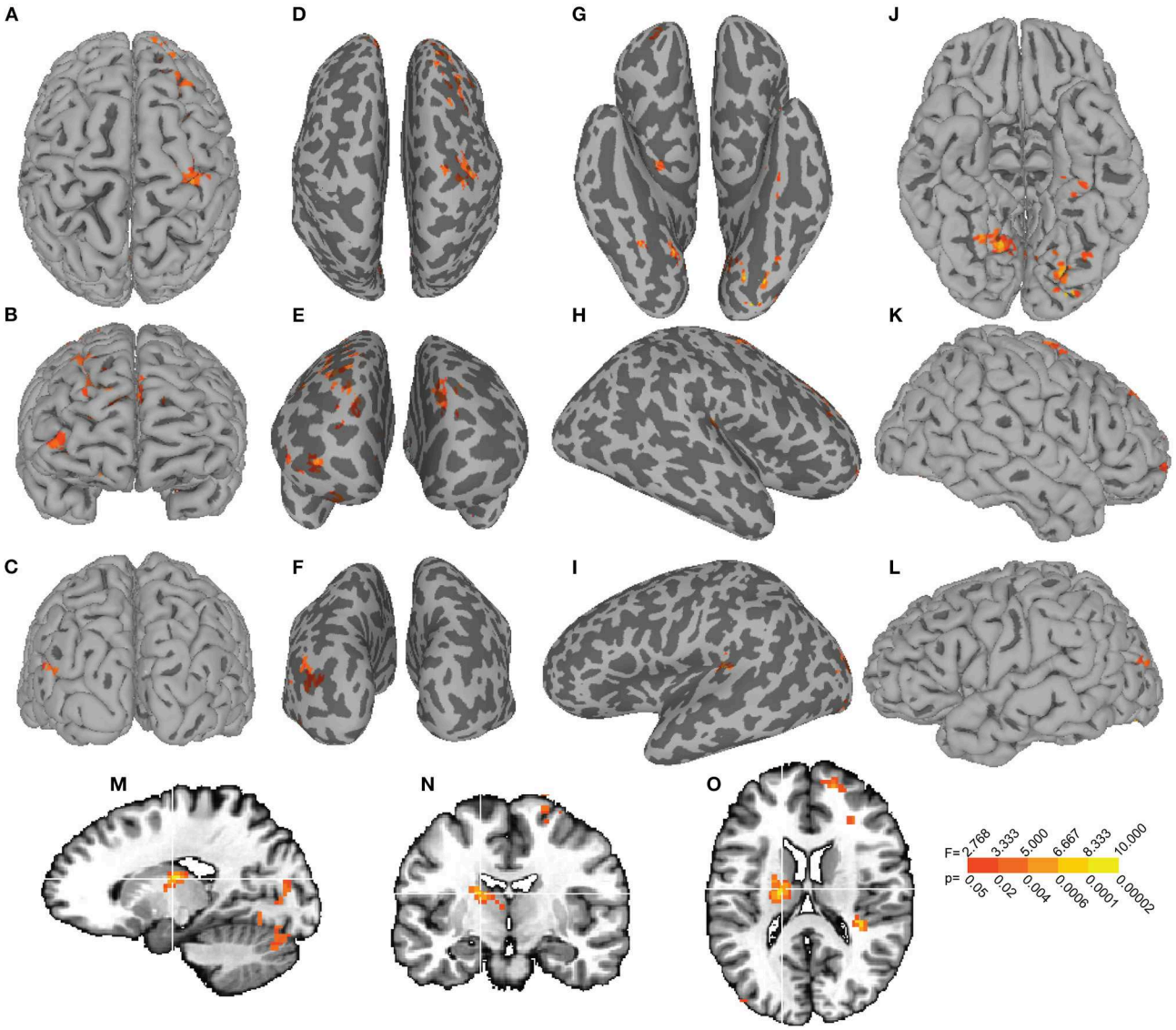

FIGURE 3 | The group-by-uric acid interaction effect on SN functional connectivity. The group-by-UA interaction F-map is displayed on dorsal **(A)**, anterior **(B)**, and posterior **(C)** views of the pial surface with corresponding inflated representations **(D–F)**. Inflated views of the ventral **(H)**, right hemisphere **(I)**, and left hemisphere **(J)** cortex are displayed next to their pial representations **(K–M)**. A sagittal slice through left hemisphere **(N)** and coronal **(O)** and axial **(P)** slices show the interaction between group and UA on SN functional connectivity with the left thalamus (cluster 6, **Table 3c**).

(RBD−) patients had a negative slope of −0.0446 [$F_{(1, 5)} = 12.86$, $p = 0.0158$], and PD (RBD+) patients had a nearly flat slope of −0.0215 [$F_{(1, 14)} = 1.565$, $p = 0.2315$].

Of the 15 clusters identified in the ANCOVA interaction F-map, four clusters exhibited a relationship in which the PD (RBD+) patients had a positive slope, while the CON subjects had a negative slope. This relationship was evident in the right precentral gyrus cluster 3 (**Supplemental Figure 3**). In this right precentral gyrus cluster PD (RBD+) patients had a positive slope of 0.09794 [$F_{(1, 14)} = 14.53$, $p = 0.0019$], CON subjects had a negative slope of −0.08411 [$F_{(1, 9)} = 9.915$, $p = 0.0118$], the PD (RBD−) subjects had a less negative slope of −0.03183 [$F_{(1, 5)} = 3.603$, $p = 0.1161$], and the RBD subjects had a flat slope of −3.496e-005 [$F_{(1, 30)} = 6.411e006$, $p = 0.9980$].

Of the 15 clusters identified in the ANCOVA interaction F-map, two exhibited a relationship in which the PD (RBD−) patients had a positive slope while the PD (RBD+) patients had a negative slope. This relationship was evident in the right lingual gyrus cluster 7 (**Supplemental Figure 4**). In this right lingual gyrus cluster the PD (RBD−) patients had a positive slope of 0.1741 [$F_{(1,5)} = 23.74$, $p = 0.0046$], the PD (RBD+) patients had a negative slope of −0.03982 [$F_{(1, 14)} = 4.775$, $p = 0.0464$], the RBD patients had a nearly flat slope of

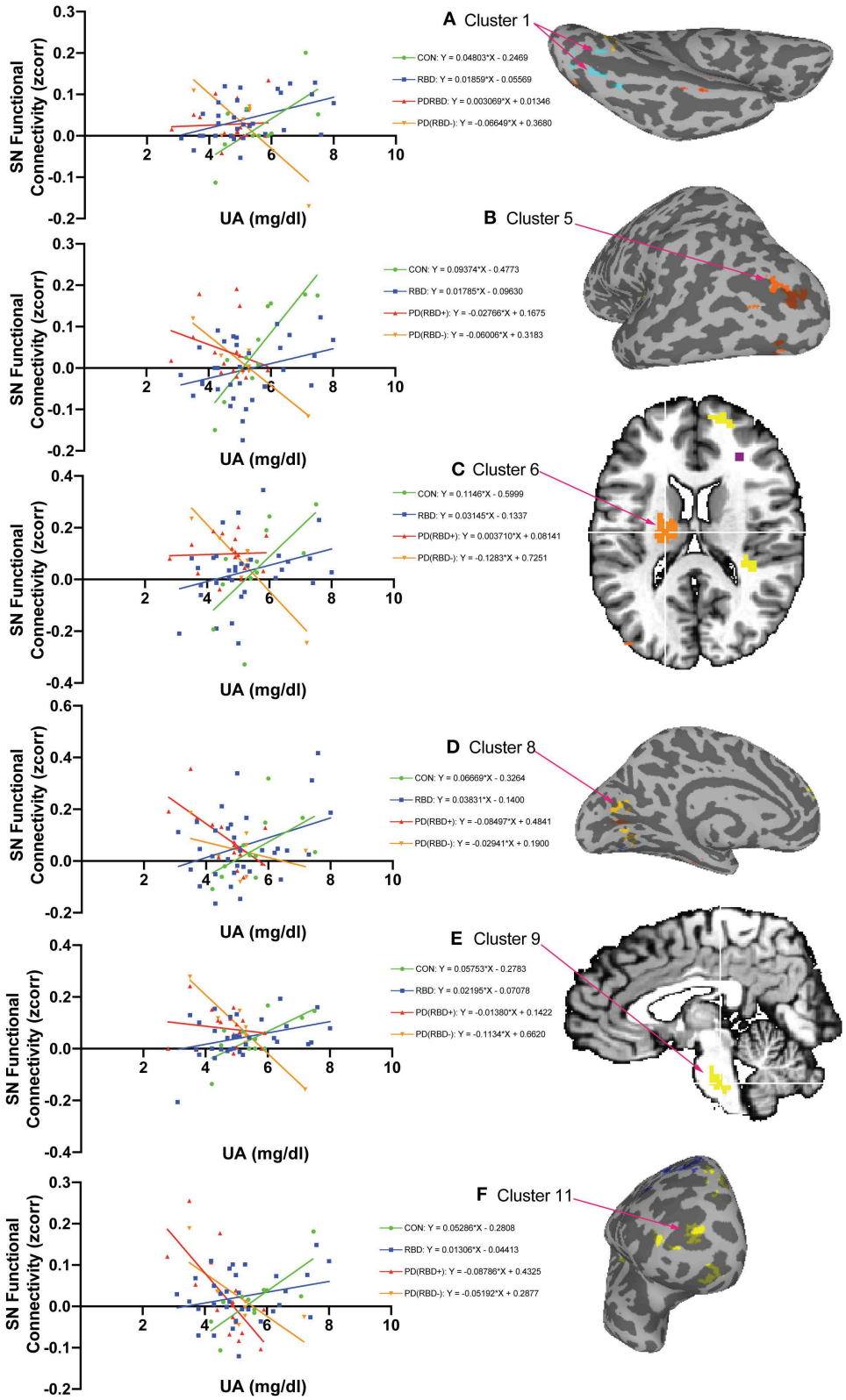

FIGURE 4 | The group-by-uric acid interaction reveals six brain regions with decreasing SN functional connectivity as a function of uric acid from controls to PD with RBD in between. Of the 15 clusters identified in the ANCOVA interaction F-map, six exhibited a relationship in which CON subjects showed the highest positive

(Continued)

FIGURE 4 | regional slope [slope $m = 0.04803$ in **(A)** Cluster 1, L. Lingual Gyrus BA 18], the RBD slope was lower [$m = 0.01859$ in **(A)** Cluster 1], the PDRBD slope was lower still ($m = 0.003069$ in **(A)** Cluster 1], and the PD slope was negative [$m = -0.06649$ in **(A)** Cluster 1]. This ordered pattern of decreasing slopes across these groups is similar in the other clusters: cluster 5 **(B)**, cluster 6 **(C)**, cluster 8 **(D)**, cluster 9 **(E)**, and cluster 11 **(F)**. Slopes are computed using the average of voxels in each cluster in each subject. Cluster numbers are the same as in **Table 3C**. The color of cluster voxels in the inflated and orthogonal views is arbitrary.

0.02382 [$F_{(1, 30)} = 1.943$, $p = 0.1736$] and the CON individuals also had a nearly flat slope of −0.01479 [$F_{(1, 9)} = 0.2632$, $p = 0.6203$].

DISCUSSION

The main novel finding of the present study is that a positive relationship was found between UA levels and SN resting state functional connectivity with posterior cortical regions in controls. Increased functional connectivity with higher UA levels in controls would appear consistent with the oxidant stress hypothesis, with levels of the antioxidant UA corresponding to stronger connectivity. But this relationship appears to break down in the patients: the positive relationship decreased in patients with RBD, and turned to a negative relationship in patients with PD. If the hypothesis that RBD is a prodromal state of PD, with idiopathic RBD a kind of intermediate state between controls and PD, then the pattern of slopes found with controls highest and positive, RBD in between, and PD lowest and negative may be consistent with more neurodegeneration from RBD to PD corresponding to reduced functional connectivity. This logic assumes that stronger functional connectivity is beneficial while reduced connectivity is detrimental, a relationship in neurodegenerative diseases that is supported by some data but remains a matter of debate especially in regards to functional compared to structural connectivity (67). Previous work has documented reduced resting state functional connectivity between SN and other basal ganglia regions as well as with posterior cortex with controls having highest connectivity, RBD patients in the middle and PD patients with lowest connectivity (38). The present study augments previous work by showing that a UA covariate is associated with changes in SN-posterior cortical connectivity. However, it is important to note that based on the present results nothing can be inferred about the neuroprotective role of UA in disease progression or connectivity. To address those questions, longitudinal imaging with UA levels taken at the same timepoints would need to be done to determine whether higher UA early after the diagnosis of RBD corresponded with a longer duration of time to convert (or never convert) to PD.

The physiological relevance of the altered functional connectivity between SN and lingual gyrus in RBD and PD found in the present study requires further investigation. A recent resting state fMRI reports reduced brain functional connectivity with a multiple posterior cortical regions in RBD patients in whom the connectivity changes correlated with mental processing slowness (50). Earlier studies using metabolic PET imaging (68) and corticometry (49) specifically implicate the lingual gyrus as one posterior cortical area showing reduced glucose tracer uptake and cortical thinning respectively in RBD vs. healthy controls. The lingual gyrus is typically associated with visual function and it has also been documented to show reduced gray matter along with superior parietal lobule in PD patients with visual hallucinations (69). If differences in the relationship between UA and SN functional connectivity in lingual gyrus can distinguish among disease states, that may be important for identifying prodromal biomarkers. However, a cross-sectional study like the present one is limited in this regard and it needs to be followed by future longitudinal imaging to track how functional connectivity changes with UA levels after the diagnosis of RBD and PD.

It is important not to draw firm conclusions regarding the hemispheric laterality of the present findings because patients present with asymmetric symptoms with some more compromised on the left and some more compromised on the right, which implicates opposite hemispheres in the disease pathophysiology. For our sample of PD patients with clinically significant asymmetric movement deficits, 12 had right disease onset and 11 had left disease onset. We attempted to address the issue of laterality in a supplemental *post-hoc* analysis in which the images of 11 patients with left disease onset as confirmed by clinical data were flipped. The rationale for this image flipping was that it would align their relatively more diseased and non-diseased hemispheres with the 12 other patients with opposite asymmetry whose images were not flipped. The prediction for this analysis was that it would increase magnitude of effects because without alignment of the diseased and non-diseased hemispheres, the effects would "average out" to some extent. The result of this subsequent *post-hoc* ANCOVA supported this idea as it showed the biggest increase in spatial extent for the largest cluster encompassing lingual gyrus in one hemisphere, and a smaller expansion in spatial extent of the other smaller cluster encompassing lingual gyrus in the opposite hemisphere. Inspection of regional slopes for the largest cluster revealed a similar pattern, but the negative slope for the PD (RBD–) group become more negative and more significant (**Supplemental Figure 1**).

While previous nigrocortical and cortico-cortical connectivity studies using resting state fMRI have reported left lateralized connectivity changes (38, 50, 70) consistent with left hemispheric predominance of nigrostriatal dysfunction (71), the laterality of connectivity results in the present study require further investigation. Laterality of disease onset is determined by neurologic exam and it is only possible to make these clinical determinations for the PD patients who show motor impairment. Controls and RBD subjects show no lateralized movement impairments, and in the absence of measurements from a gold-standard imaging technique like DaTscan (72) to quantify hemispheric differences in dopamine transporter levels, any lateralized functional connectivity differences in the present study should be interpreted with caution or not be interpreted at all without additional imaging evidence.

It has recently been reported using resting state fMRI that male *de novo* PD patients with higher UA levels had higher cortical functional connectivity in resting state networks including the dorsal attention network, executive control network, and default mode network, while female patients had lower functional connectivity regardless of UA level (73). These findings suggest that resting state networks might be closely and gender-specifically associated with the status of serum UA in *de novo* PD patients. Another study reports increased resting state connectivity between midbrain and cortex in PD (74). The organizational pattern of substantia connections with seven resting state functional networks has also been investigated to show that the medial portion of the SN compacta (mSNc) dominantly connects to limbic and visual cortex, while ventral SN (vSN) mainly connects with fronto-parietal and default mode networks (75). Widespread patterns of SN functional connectivity modulated by UA levels may also have a structural basis as diffusion tensor imaging reveals widespread structural connectivity of SN including primary motor cortex, somatosensory cortex, prefrontal cortex, caudate and putamen, globus pallidus, nucleus accumbens, temporal lobe, amygdala, pontine basis, occipital lobe, anterior and posterior cerebellum, corpus callosum, and external capsule (76).

Our main finding is that SN-posterior cortical resting state connectivity is positively associated with serum UA in male controls, and this relationship decreases in RBD and turns negative in PD. However, we also found a trend toward *increased* SN functional connectivity as a function of UA in frontal cortex and cerebellum in RBD patients (**Supplemental Figure 2**) and in cortex and lentiform nucleus in PD patients with and without RBD **(Supplemental Figures 3**, **4**), but these results did not survive multiple comparisons correction. A limitation of the present study is relatively small sample sizes in the control and PD (RBD–) groups, which limits statistical power. Future studies utilizing larger sample sizes and more powerful longitudinal designs are needed investigate how different patterns of connectivity distinguish among controls, prodromal individuals, and diagnosed PD patients.

CONCLUSION

We conclude that UA and SN functional connectivity among controls, a prodromal idiopathic RBD group, and PD patients with and without RBD is altered differentially in a ventral occipital region previously documented to be metabolically and structurally altered in RBD and PD. Replication in longitudinal designs with larger samples supplemented by dopaminergic imaging is needed to clarify the relevance of these patterns as biomarkers in prodromal Parkinson's disease.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Committee for the Protection of Human Subjects and The University of Texas Health Science Center at Houston. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TE, JS, RC, and MS contributed equally to study conception, design, data collection, edited the paper, and read the final draft. TE and JS analyzed the data. TE wrote the first draft of the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00815/full#supplementary-material

Supplemental Figure 1 | The group-by-uric acid interaction reveals positive SN functional connectivity as a function of uric acid in RBD in four brain regions. Of the 15 clusters identified in the ANCOVA interaction F-map, four exhibited a relationship in which RBD subjects had a positive regional slope (slope $m =$ 0.03231 in panel a Cluster 2, R. Middle Frontal Gyrus BA 8), while CON subjects had negative or flat slope ($m = -0.07525$ in panel a Cluster 2). Slopes are computed using the average of voxels in each cluster in each subject. Cluster numbers are the same as in **Table 3c**. The color of cluster voxels in the inflated and orthogonal slice views in this figure is arbitrary.

Supplemental Figure 2 | The group-by-uric acid interaction reveals positive SN functional connectivity as a function of uric acid in PD (RBD+) in four brain regions. Of the 15 clusters identified in the ANCOVA interaction F-map, four exhibited a relationship in which PD (RBD+) patients had a positive regional slope (slope $m = 0.09794$ in panel a Cluster 3, R. Precentral Gyrus), while the slope for CON subjects was negative ($m = -0.08411$ in panel a Cluster 3). Slopes are computed using the average of voxels in each cluster in each subject. Cluster numbers are the same as in **Table 3c**. The color of cluster voxels in the inflated and orthogonal views is arbitrary.

Supplemental Figure 3 | The group-by-uric acid interaction reveals opposite relationships between SN functional connectivity as a function of uric acid in PD (RBD+) and PD (RBD–) in two brain regions. Of the 15 clusters identified in the ANCOVA interaction F-map, two exhibited a relationship in which the PD (RBD+) patients had a negative regional slope (slope $m = -0.03982$ in panel a Cluster 7, R. Lingual Gyrus), while the slope for PD patients without RBD were positive ($m = 0.1741$ in panel a Cluster 7). Slopes are computed using the average of voxels in each cluster in each subject. Cluster numbers are the same as in **Table 3c**. The color of cluster voxels in the inflated and orthogonal views is arbitrary.

Supplemental Figure 4 | Group-by-uric acid regional interaction for the largest cluster encompassing lingual gyrus in the *post-hoc* left disease onset image flip analysis. In the *post-hoc* ANCOVA with image flipping for left disease onset patients, the largest cluster encompassing lingual gyrus from the original analysis (cluster 1, **Figure 4**) exhibited a similar pattern of slopes with the biggest difference being a more negative and significant slope for the PD (RBD–) group. Slopes are computed using the average of voxels in each cluster in each subject.

Supplemental Table 1 | Brain regions with a group-by-uric acid interaction in the *post-hoc* analysis with image flipping for the left disease onset patients. Regions exceeding a joint threshold (cluster height $p < 0.05$ & cluster size k≥50 voxels) are listed in descending order by k along with the corresponding Montreal Neurological Institute x,y,z coordinate, labeled brain region with Brodmann Area (BA) where applicable, peak F value of the cluster and associated probability value. †Cluster exceeds threshold corrected for multiple comparisons. ‡Cluster is a subset of cluster †in **Table 3b**.

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