

Limbic Vulnerability Characterises REM Sleep Behaviour Disorder Across Prodromal and Established Lewy Body Disease

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Abstract

Isolated rapid eye movement sleep behaviour disorder (iRBD) strongly predicts future Lewy body disease and identifies a more aggressive, non-motor phenotype in Parkinson's disease (PD). Limbic degeneration may contribute to this phenotype with abnormalities reported separately in iRBD and PD with RBD (PD-RBD). However, whether limbic vulnerability reflects RBD status independent of disease stage remains unknown. We investigated multimodal limbic vulnerability across prodromal and established disease using structural and functional MRI from 175 polysomnography-confirmed isolated RBD (iRBD), 79 Parkinson's disease with RBD (PD-RBD), 250 Parkinson's disease without RBD (PD-nRBD) and 54 healthy controls from the Parkinson's Progression Markers Initiative. Hippocampal, amygdala and anterior cingulate measures were integrated into structural, functional and multimodal Limbic Vulnerability Indices. iRBD showed greater structural and multimodal limbic vulnerability than healthy controls and PD-nRBD, while PD-RBD showed greater structural and multimodal vulnerability than PD-nRBD. Greater limbic vulnerability was associated with RBD symptom burden, reduced dopamine transporter binding and cognitive impairment. In iRBD, greater structural and multimodal limbic vulnerability predicted future incident hallucinations. These findings identify limbic vulnerability as a feature associated with the RBD phenotype across disease stages and suggest a role in neuropsychiatric progression.

Introduction

Rapid eye movement (REM) sleep behaviour disorder (RBD) is a parasomnia characterised by recurrent dream enactment behaviours during REM sleep in association with REM sleep without atonia (RSWA).¹ Isolated RBD (iRBD) is an established prodromal manifestation of α -synucleinopathy, with most affected individuals ultimately developing a Lewy body disease such as Parkinson's disease (PD) or dementia with Lewy bodies (DLB), and a small subset converting to multiple system atrophy.²

In addition to preceding the diagnosis, RBD occurs frequently at presentation and throughout the course of these conditions. In fact, the presence and timing of RBD development has played a crucial role in our understanding of the biology underpinning the heterogeneity of Lewy body disease.³ While most patients with DLB have RBD at diagnosis, only around 40–50% with PD will have RBD,^{4,5} although its prevalence increases throughout the disease course.⁶ Additionally, a subset of patients will develop RSWA, while demonstrating no overt dream enactment behaviour. Recent work in early PD has shown that isolated RSWA is not simply a milder form of RBD, with the two groups having distinct clinical characteristics and longitudinal trajectories.⁷ Furthermore, PD patients with RBD consistently exhibit a more aggressive disease phenotype characterised by greater autonomic dysfunction, cognitive impairment, neuropsychiatric symptoms and faster clinical progression.⁸ Consequently, RBD has emerged not simply as a symptom of synucleinopathy, but potentially as a marker of a distinct biological subtype.^{9,10} Understanding the neurobiological changes associated with RBD may provide insight into both disease initiation during the prodromal phase and the heterogeneity of established synucleinopathies.

RSWA is generally attributed to dysfunction of pontine and medullary nuclei responsible for REM sleep regulation.¹¹ However, dysfunction of these circuits may not fully account for the vivid dream mentation, emotional salience and organised dream-enactment behaviours that characterise RBD.⁷ Limbic regions, including the amygdala, hippocampus and anterior cingulate cortex (ACC), are engaged during REM sleep and support emotional processing, memory and salience.^{12,13} Pathological studies consistently implicate the amygdala as an early site of Lewy pathology deposition in PD,^{14–16} and in iRBD, neuroimaging has also identified deficits in the

amygdala,¹⁷ hippocampus¹⁸ and ACC.¹⁹ Additionally, a recently published pathological case report of a DLB patient reported PSG-confirmed RBD in the absence of brainstem α -synuclein pathology, suggesting that limbic involvement alone may be sufficient to generate the RBD phenotype.²⁰ Together, these observations raise the possibility that limbic dysfunction is an important mechanistic contributor to RBD.

Structural and functional neuroimaging provide a means to investigate *in vivo* limbic dysregulation across prodromal and established LBD by indexing regional tissue loss and alterations in functional connectivity. While neuropathological studies have been fundamental in defining the anatomical distribution of pathology, they are necessarily limited to end-stage tissue availability and are particularly challenging in prodromal populations such as iRBD. Moreover, cellular loss indexed by structural atrophy is better associated with clinical manifestations compared to pathological burden itself.²¹ Consistent with this, limbic atrophy including reduced hippocampal, amygdala and ACC volumes has been demonstrated in iRBD relative to healthy controls, while functional imaging has also demonstrated altered limbic connectivity, offering complementary insights into early network-level neurodegeneration.^{17,18,22–26} Comparable results have been separately reported in PD with probable RBD (PD-RBD) relative to PD without probable RBD (PD-nRBD).^{27–31} These findings suggest limbic involvement may accompany RBD at both the prodromal and clinically manifest stages of Lewy body disease. However, because these studies have analysed these conditions separately, it is unclear whether limbic vulnerability is comparable across these stages, and whether iRBD is distinguished from established PD by the presence of RBD rather than by disease stage or severity.

The clinical and pathological significance of these limbic abnormalities also remains uncertain. Structural and functional measures have also rarely been integrated within the same study or related to markers of nigrostriatal degeneration and Alzheimer-related co-pathology. There are likewise few studies showing whether limbic vulnerability tracks the presence of the RBD phenotype across LBD or risk of future cognitive and neuropsychiatric manifestations in prodromal populations.³² This is particularly relevant to visual hallucinations, which are associated with RBD and have themselves been linked to dysfunction of the amygdala and its interactions with visual and attentional systems in PD.³³ However, whether similar dysfunction is present early in iRBD and contributes to the subsequent emergence of visual hallucinations remains unknown. Establishing these relationships could help determine whether limbic dysfunction is simply an accompanying disease stage or a clinically meaningful component of the broader phenotype associated with RBD, including its potential relevance to future neuropsychiatric manifestations.

We therefore investigated multimodal limbic vulnerability across the synucleinopathy spectrum using the Parkinson's Progression Markers Initiative (PPMI), comparing healthy controls (HC), polysomnography (PSG)-confirmed iRBD, PD-nRBD, and PD-RBD. Structural and functional measures of the hippocampus, amygdala and anterior cingulate cortex were integrated into normative indices of limbic vulnerability. We aimed to determine whether limbic vulnerability tracks RBD status across the disease spectrum, whether limbic vulnerability is associated with clinical severity and molecular biomarkers of neurodegeneration. Additionally, we aimed to test whether early limbic vulnerability in prodromal iRBD participants predicts the future development of visual hallucinations. We hypothesised that greater limbic vulnerability would be observed in iRBD and PD with probable RBD relative to healthy controls and PD without probable RBD, consistent with limbic dysfunction forming part of the broader biological phenotype associated with clinically manifest RBD.

Results

Clinical characteristics and demographics

The total cohort consisted of 175 iRBD, 79 PD-RBD, 250 PD-nRBD and 54 healthy control participants. Demographic and clinical characteristics are shown in Table 1. The PD-nRBD group was younger than both control and iRBD groups ($P = 0.002$), with no age difference among the remaining cohorts; disease duration was short and comparable between PD groups. The iRBD group contained a higher proportion of males than the control or PD-nRBD groups. Global cognition was lower in iRBD than controls ($P = 0.010$), and motor severity separated the PD groups from the iRBD and control groups ($P < 0.001$) but was also higher in iRBD compared to controls ($P = 0.021$). Olfaction was impaired in all disease groups relative to controls ($P < 0.001$). As expected, RBDSQ scores were higher in PD-RBD than PD-nRBD and were highest in the iRBD group ($P < 0.001$). Autonomic symptoms were greater in iRBD and PD-RBD than controls, and greater in PD-RBD than PD-nRBD ($P < 0.001$); daytime sleepiness was higher in iRBD than PD-nRBD ($P = 0.007$). Depressive symptoms and anxiety scores did not differ across groups (all $P > 0.1$). Head motion (mean framewise displacement) was comparable across all four groups.

Table 1
Demographic and clinical characteristics

Age (years)	Controls (n = 54)	iRBD (PSG-confirmed) (n = 175)	PD-RBD (n = 79)	PD-nRBD (n = 250)	H / χ^2	P value
	68.7 ± 7.2	67.2 ± 6.6	67.2 ± 7.3	65.1 ± 7.9	15.41	0.002 ^{ce}
Disease duration (years, from Dx)	—	—	1.3 ± 1.8	1.1 ± 1.8	—	0.406
Sex (M:F)	30:24	141:34	54:25	151:99	22.87	< 0.001 ^{ae}
MoCA	27.6 ± 2.0	26.1 ± 2.9	26.7 ± 2.2	26.7 ± 2.5	11.33	0.010 ^a
MDS-UPDRS III	1.5 ± 2.5	4.2 ± 4.7	24.2 ± 11.1	23.8 ± 10.2	358.80	< 0.001 ^{abcde}
RBDSQ	1.6 ± 1.6	9.1 ± 1.7	7.3 ± 1.7	1.4 ± 1.2	428.51	< 0.001 ^{abdef}
UPSIT	32.8 ± 5.1	23.2 ± 8.3	21.6 ± 7.5	22.7 ± 7.3	67.40	< 0.001 ^{abc}
SCOPA-AUT	9.2 ± 5.1	12.7 ± 8.5	14.3 ± 6.9	11.4 ± 6.6	18.18	< 0.001 ^{abf}
ESS	4.6 ± 2.8	6.5 ± 4.5	6.1 ± 3.9	5.1 ± 3.6	12.17	0.007 ^e
GDS	5.3 ± 0.8	5.6 ± 1.3	5.7 ± 1.5	5.4 ± 1.3	5.73	0.125
STAI-Trait	47.1 ± 2.7	46.2 ± 3.9	46.1 ± 4.2	45.9 ± 3.8	4.39	0.222
Framewise displacement	0.11 ± 0.1	0.12 ± 0.1	0.12 ± 0.1	0.12 ± 0.1	0.34	0.952

Post-hoc pairwise comparisons (superscripts on P value)

a Controls vs iRBD; b Controls vs PD-RBD; c Controls vs PD-nRBD; d iRBD vs PD-RBD; e iRBD vs PD-nRBD; f PD-RBD vs PD-nRBD. Demographic and clinical characteristics of the four diagnostic groups. Continuous variables are presented as mean $\pm$ standard deviation and compared across groups using nonparametric permutation tests (10,000 permutations) on the Kruskal–Wallis H statistic; the H statistic is reported with its permutation P value. Sex is presented as the male-to-female count and compared using a χ^2 test. Disease duration was compared between the two Parkinson's disease groups by permutation test. Where the omnibus test was significant, post-hoc pairwise comparisons were performed by permutation test (continuous) or Fisher's exact test (sex), FDR correction applied within each variable; superscripts denote pairs significant at corrected $P < 0.05$ (legend above). MoCA was included only where administered within 6 months of the resting-state scan (per-cell n shown); n for other measures varies slightly with incomplete assessments. *iRBD*, isolated REM sleep behaviour disorder; PD-RBD, Parkinson's disease with probable RBD; PD-nRBD, Parkinson's disease without probable RBD; ESS, Epworth Sleepiness Scale; GDS, Geriatric Depression Scale; MDS-UPDRS III, MDS Unified Parkinson's Disease Rating Scale Part III; MoCA, Montreal Cognitive Assessment; RBDSQ, REM Sleep Behaviour Disorder Screening Questionnaire; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease–Autonomic; STAI, State–Trait Anxiety Inventory; UPSIT, University of Pennsylvania Smell Identification Test.

Limbic vulnerability is associated with RBD status across the disease spectrum

To quantify limbic degeneration, structural grey matter volumes and functional connectivity measures of the hippocampus, amygdala and ACC were integrated into multimodal LVIs, each expressed as a control-referenced w-score oriented so that higher values denote greater vulnerability. Relative to controls, iRBD showed higher structural, functional and multimodal LVI, with the largest effect observed for the multimodal index ($\beta = +0.48$, $P = 0.002$; Table 2). Participants with iRBD also had higher LVIs than those with PD-nRBD (multimodal LVI: $\beta = +0.39$, $P < 0.001$, functional LVI: $\beta = +0.21$, $P = 0.038$). Within PD, participants with RBD had higher structural ($\beta = +0.28$, $P = 0.027$) and multimodal LVI ($\beta = +0.25$, $P = 0.049$) than PD-nRBD, with no difference in functional LVI. iRBD and PD-RBD did not differ on any index. Overall, elevated limbic vulnerability was more consistently observed in groups with RBD, with the difference between the PD groups driven primarily by the structural domain.

At the regional level, structural vulnerability localised to the amygdala and ACC. Participants with iRBD had smaller bilateral amygdala volume than controls but did this not survive correction ($\beta = -0.30$, $P = 0.026$, $P_{FDR} = 0.079$). Bilateral amygdala volume was reduced in iRBD relative to PD-nRBD ($\beta = -0.21$, $P = 0.017$, $P_{FDR} = 0.026$), driven predominantly by the left amygdala ($\beta = -0.20$, $P_{FDR} = 0.047$). Bilateral ACC also showed a larger reduction in iRBD compared to PD-nRBD ($\beta = -0.44$, $P_{FDR} < 0.001$), with differences present in both hemispheres (both $P_{FDR} < 0.001$). Bilateral ACC volume showed a trend towards being lower in PD-RBD compared to PD-nRBD, but did not reach significance after correction for multiple comparisons ($\beta = -0.28$, $P = 0.022$, $P_{FDR} = 0.065$).

Within the limbic subnetwork, participants with iRBD had lower amygdala-to-limbic connectivity than healthy controls (AMY-LIM $\beta = -0.44$, $P = 0.005$, $P_{FDR} = 0.028$), with a trend towards lower hippocampal-to-limbic connectivity (HIP-LIM $\beta = -0.34$, $P = 0.030$, $P_{FDR} = 0.091$) (Fig. 1). Relative to PD-nRBD, iRBD was associated with reduced hippocampal-to-ACC and amygdala-to-ACC connectivity ($P_{FDR} < 0.050$), with a trend towards lower ACC-to-limbic connectivity ($\beta = -0.22$, $P = 0.029$, $P_{FDR} = 0.057$).

Limbic-to-cortex connectivity was broadly reduced in iRBD relative to controls, including whole-cortex, visual, somatomotor and ventral attention connectivity (all $P_{FDR} < 0.05$). Relative to PD-nRBD, iRBD had reductions in whole-cortex connectivity and all six cortical networks examined (whole-cortex, visual, somatomotor, dorsal and ventral attention, frontoparietal and default mode; all $P_{FDR} < 0.05$). Cortical dysconnectivity was most pronounced in iRBD compared to controls. No functional-connectivity measure survived FDR correction between iRBD and PD-RBD or between PD-RBD and PD-nRBD. Full pairwise comparisons are provided in Supplementary Table 1.

Table 2
Structural and functional measures of limbic degeneration in iRBD and PD-RBD

<i>Limbic vulnerability indices</i>											
Measure	Group Statistic		iRBD compared to HC			iRBD compared to PD-nRBD			PD-RBD compared to PD-nRBD		
	F	P	β	P		β	P		β	P	
LVI structural	5.19	0.002	+ 0.31	0.043		+ 0.36	< 0.001		+ 0.28	0.027	
LVI functional	2.55	0.055	+ 0.39	0.014		+ 0.21	0.038		+ 0.09	0.505	
LVI multimodal	6.68	< 0.001	+ 0.48	0.002		+ 0.39	< 0.001		+ 0.25	0.049	
<i>Bilateral structural volumes</i>											
Measure	Group Statistic		iRBD compared to HC			iRBD compared to PD-nRBD			PD-RBD compared to PD-nRBD		
	F	P	B	P	P _{FDR}	B	P	P _{FDR}	β	P	P _{FDR}
Bilateral AMY	2.61	0.051	-0.30	0.026	0.079	-0.21	0.017	0.026	-0.10	0.391	0.391
Bilateral HIP	1.05	0.369	-0.09	0.516	0.516	-0.07	0.401	0.401	-0.18	0.098	0.147
Bilateral ACC	7.44	< 0.001	-0.23	0.126	0.190	-0.44	< 0.001	< 0.001	-0.28	0.022	0.065
<i>Lateralised structural volumes</i>											
Measure	Group Statistic		iRBD compared to HC			iRBD compared to PD-nRBD			PD-RBD compared to PD-nRBD		
	F	P	B	P	P _{FDR}	β	P	P _{FDR}	β	P	P _{FDR}
Left AMY	2.44	0.064	-0.29	0.030	0.177	-0.20	0.023	0.047	-0.03	0.811	0.811
Right AMY	2.17	0.090	-0.27	0.060	0.179	-0.12	0.037	0.055	-0.16	0.181	0.218
Left HIP	1.04	0.374	-0.12	0.403	0.484	-0.07	0.524	0.524	-0.17	0.122	0.191
Right HIP	0.85	0.464	-0.06	0.706	0.706	-0.09	0.359	0.431	-0.17	0.127	0.191
Left ACC	5.92	< 0.001	-0.26	0.090	0.179	-0.40	< 0.001	< 0.001	-0.27	0.027	0.111
Right ACC	7.05	< 0.001	-0.17	0.231	0.347	-0.43	< 0.001	< 0.001	-0.26	0.037	0.111
<i>Subcortical connectivity</i>											
Measure	Group Statistic		iRBD compared to HC			iRBD compared to PD-nRBD			PD-RBD compared to PD-nRBD		
	F	P	β	P	P _{FDR}	β	P	P _{FDR}	β	P	P _{FDR}

<i>Limbic vulnerability indices</i>											
HIP-LIM	1.62	0.184	-0.34	0.030	0.091	-0.05	0.628	0.753	+0.02	0.902	0.902
AMY-LIM	2.96	0.032	-0.44	0.005	0.028	-0.14	0.153	0.230	-0.12	0.358	0.782
ACC-LIM	1.61	0.186	-0.13	0.405	0.486	-0.22	0.029	0.057	-0.08	0.543	0.782
HIP-ACC	2.03	0.109	-0.15	0.343	0.486	-0.25	0.014	0.050	-0.09	0.509	0.782
HIP-AMY	0.38	0.770	-0.08	0.596	0.596	-0.01	0.941	0.941	-0.10	0.420	0.782
AMY-ACC	1.97	0.118	-0.14	0.358	0.486	-0.24	0.017	0.050	-0.06	0.651	0.782
<i>Cortical connectivity</i>											
	Group Statistic		iRBD compared to HC			iRBD compared to PD-nRBD			PD-RBD compared to PD-nRBD		
	F	P	β	P	P _{FDR}	β	P	P _{FDR}	β	P	P _{FDR}
LIM-WC	5.32	0.001	-0.43	0.005	0.017	-0.36	< 0.001	0.002	-0.13	0.315	0.665
LIM-VIS	4.42	0.004	-0.42	0.007	0.017	-0.30	0.003	0.009	-0.24	0.065	0.456
LIM-SMN	3.64	0.013	-0.43	0.005	0.017	-0.27	0.007	0.010	-0.07	0.570	0.665
LIM-DAN	2.75	0.042	-0.33	0.036	0.051	-0.26	0.009	0.011	-0.11	0.403	0.665
LIM-VAN	3.31	0.020	-0.37	0.017	0.030	-0.27	0.006	0.010	-0.15	0.232	0.665
LIM-FPN	2.35	0.072	-0.13	0.410	0.410	-0.22	0.029	0.029	+0.09	0.490	0.665
LIM-DMN	2.71	0.044	-0.18	0.259	0.302	-0.28	0.006	0.010	-0.04	0.765	0.765

Structural and functional measures of limbic degeneration across diagnostic groups. Values are standardised β coefficients from age- and sex-adjusted general linear models fitted across all four groups (HC, n = 54; iRBD, n = 175; PD-nRBD, n = 250; PD-RBD, n = 79), with each measure as the outcome and diagnostic group as the predictor. β is the difference for the first-named group relative to the second, in standard deviation units, with its uncorrected P value; a positive β denotes a higher value in the first-named group. The Limbic Vulnerability Indices are oriented so that higher values indicate greater vulnerability, whereas individual volumes and connectivity measures are in their native direction, such that negative β denotes lower volume or connectivity in the first-named group. The Group Statistic columns give the omnibus four-group effect for each measure (age/sex-adjusted F and its P value); this test is not FDR-corrected. For all measures other than the Limbic Vulnerability Indices, the Benjamini-Hochberg false discovery rate P value (PFDR) is also reported, with families defined as bilateral structural volumes, lateralised structural volumes, subcortical connectivity and cortical connectivity. The Limbic Vulnerability Indices, as pre-specified composite outcomes, are interpreted on uncorrected P. Significant effects are shown in bold (uncorrected P < 0.05 for the Limbic Vulnerability Indices; PFDR < 0.05 for all other measures). ACC, anterior cingulate cortex; AMY, amygdala; DAN, dorsal attention; DMN, default mode; FPN, frontoparietal; HC, healthy control; HIP, hippocampus; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; PD, Parkinson's disease; PD-nRBD, PD without RBD; PD-RBD, PD with RBD; RBD, REM sleep behaviour disorder; SMN, somatomotor; VAN, ventral attention; VIS, visual; WC, whole cortex.

Regions are coloured where connectivity with the limbic network was significantly lower in isolated REM sleep behaviour disorder (iRBD) than in healthy controls (HC). **(A)** Cortical networks showing reduced limbic connectivity in iRBD, spanning the visual, somatomotor and ventral attention networks alongside a broad whole-cortex reduction. **(B)** Reduced FC between the amygdala and the limbic network. HC, healthy control; iRBD, isolated REM sleep behaviour disorder.

Associations between clinical measures and limbic imaging in iRBD

We next examined whether limbic imaging measures tracked clinical severity within the iRBD group. In iRBD, MoCA was associated with reduced limbic-to-visual connectivity ($\beta = +0.257$, $P_{FDR} = 0.007$) and trended with smaller hippocampal volume ($P = 0.041$, $P_{FDR} = 0.122$; Fig. 2A; Supplementary Table 2). Higher depressive symptom burden was associated with lower hippocampal-to-limbic connectivity ($\beta = -0.221$, $P_{FDR} = 0.023$), and with higher functional and multimodal LVI (LVI functional $\beta = +0.214$, $P = 0.005$; LVI multimodal $\beta = +0.171$, $P = 0.025$). Higher RBDSQ scores were associated with lower limbic-to-frontoparietal connectivity ($\beta = -0.208$, $P_{FDR} = 0.049$). Greater motor severity was associated with reduced bilateral amygdala ($\beta = -0.156$, $P = 0.036$), although did not survive FDR correction. No associations with autonomic symptoms, daytime sleepiness, state or trait anxiety, or olfaction survived correction.

Associations between clinical measures and limbic imaging in PD

The same associations were examined within the established PD group. Within PD, greater motor severity was associated with higher structural and multimodal LVI ($\beta = +0.158$, $P = 0.005$ and $\beta = +0.185$, $P = 0.001$). At the regional level, this was associated with smaller hippocampal volume (bilateral; $\beta = -0.158$, $P_{FDR} = 0.003$; left and right both; $P_{FDR} = 0.011$) and reduced limbic-to-visual functional connectivity ($\beta = -0.162$, $P_{FDR} = 0.036$; Fig. 2B; Supplementary Table 3). Higher daytime sleepiness was associated with bilateral amygdala volume ($P_{FDR} = 0.013$), particularly involving the right amygdala ($P_{FDR} < 0.001$). Higher RBDSQ was associated with higher structural and multimodal LVI ($\beta = +0.114$, $P = 0.034$ and $\beta = +0.116$, $P = 0.035$, respectively). Lower MoCA was associated with higher functional and multimodal LVI ($\beta = -0.150$, $P = 0.007$ and $\beta = -0.149$, $P = 0.006$), and reduced hippocampal and amygdala functional connectivity to the limbic network (HIP-LIM; $\beta = +0.151$, $P_{FDR} = 0.019$ and AMY-LIM; $\beta = +0.188$, $P_{FDR} = 0.004$). No individual regional or connectivity association with RBDSQ survived FDR correction.

Associations between clinical measures and limbic imaging in the combined iRBD and PD group

To identify associations shared across the disease spectrum, we repeated the analysis in the pooled iRBD and the entire PD sample and found that the strongest and most distributed associations were with RBD symptom severity (Fig. 2C; Supplementary Table 4). Higher RBDSQ was associated with higher structural, functional and multimodal LVI (LVI multimodal $P < 0.001$). Structurally this localised to the bilateral anterior cingulate and amygdala ($P_{FDR} < 0.001$ and $P_{FDR} = 0.032$). Functionally, higher RBDSQ scores were associated with reduced limbic-to-whole-cortex connectivity ($\beta = -0.172$, $P_{FDR} < 0.001$) and with reduced connectivity across every limbic-to-network edge (visual, somatomotor, dorsal and ventral attention, frontoparietal and default mode, all $P_{FDR} \leq$

0.012), together with reduced anterior cingulate connectivity (ACC-LIM, HIP-ACC and AMY-ACC, all $P_{FDR} = 0.016$). Higher ESS scores were associated with higher multimodal LVI ($\beta = +0.099$, $P = 0.024$) and smaller right amygdala volume ($\beta = -0.120$, $P_{FDR} = 0.022$). Lower MoCA scores, poorer olfaction and higher depressive symptom scores were each associated with higher functional or multimodal LVI (MoCA LVI multimodal $\beta = -0.101$, $P = 0.023$; UPSIT LVI functional $\beta = -0.139$, $P = 0.003$; GDS LVI multimodal $\beta = +0.092$, $P = 0.036$).

Each cell is an age- and sex-adjusted standardised β (a partial correlation) from a linear model of one limbic measure on one clinical variable, both z-scored, fitted separately within polysomnography-confirmed iRBD (**A**; $n = 175$), established PD (**B**; PD-RBD + PD-nRBD, $n = 329$), and the two pooled (**C**; iRBD + PD, $n = 504$). Rows are grouped into the Limbic Vulnerability Indices, regional volumes, subcortical connectivity, and cortical connectivity; columns are clinical measures of cognition, motor severity, RBD symptom burden, olfaction, autonomic function, daytime sleepiness, depression, and trait anxiety. All cells are oriented to a common severity axis: measures are signed so that higher indicates greater vulnerability (regional volumes and connectivity are reversed, since lower values denote greater degeneration; the Limbic Vulnerability Indices retain their native direction), and clinical scores are signed so that higher indicates worse status (MoCA and UPSIT are reversed, since higher scores are better). A positive (warm) cell therefore indicates that greater limbic vulnerability, reduced volume or reduced FC is associated with worse clinical status, and a negative (cool) cell the reverse. Asterisks denote significance within each clinical column: ** Benjamini-Hochberg FDR $q < 0.05$, computed down each column separately within each sample; * uncorrected $P < 0.05$. Sample sizes are the maximum complete-case n per panel and vary by cell with missing data. ACC, anterior cingulate cortex; AMY, amygdala; DAN, dorsal attention; DMN, default mode; ESS, Epworth Sleepiness Scale; FC, functional connectivity; FPN, frontoparietal; GDS, Geriatric Depression Scale; HIP, hippocampus; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; LVI, Limbic Vulnerability Index; MDS-UPDRS III, Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; RBDSQ, REM Sleep Behaviour Disorder Screening Questionnaire; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease–Autonomic; SMN, somatomotor; STAI-T, State-Trait Anxiety Inventory, trait; UPSIT, University of Pennsylvania Smell Identification Test; VAN, ventral attention; VIS, visual.

Association between limbic imaging and biomarkers

To examine whether limbic imaging abnormalities were associated with biomarkers of PD and Alzheimer's disease pathology, we tested associations with striatal and putaminal dopamine transporter (DAT) binding, CSF A β 42/40 and plasma p-tau217. CSF A β 42/40 was not associated with any LVI, regional volume or connectivity measure in either iRBD or PD (Supplementary Tables 5 and 6). Plasma p-tau217, available only in iRBD, was not associated with the LVIs or functional-connectivity measures. However, higher p-tau217 was associated with smaller bilateral hippocampal volume ($\rho = -0.252$, $P_{FDR} = 0.035$) with a stronger association with the left hippocampus ($\rho = -0.338$, $P_{FDR} = 0.003$).

Dopaminergic function was the principal correlate of limbic vulnerability in both disease groups, although the pattern was more extensive in PD. In iRBD, lower striatal DAT binding was associated with higher functional and multimodal LVI (functional $\rho = -0.221$, $P = 0.004$; multimodal $\rho = -0.216$, $P = 0.004$) and with smaller hippocampal volume (bilateral $\rho = +0.195$, $P_{FDR} = 0.031$). Putaminal DAT binding showed the same pattern (functional LVI $\rho = -0.182$, $P = 0.017$; multimodal LVI $\rho = -0.188$, $P = 0.013$). Within PD, lower striatal DAT binding was associated with higher structural, functional and multimodal LVI (multimodal $\rho = -0.198$, $P < 0.001$) and smaller volumes across

every limbic region, all surviving correction: bilateral hippocampus ($\rho = +0.205$, $P_{FDR} < 0.001$), bilateral amygdala ($\rho = +0.157$, $P_{FDR} = 0.005$), and bilateral ACC ($\rho = +0.164$, $P_{FDR} = 0.005$). Each lateralised volume was also significant, most strongly the left hippocampus ($\rho = +0.233$, $P_{FDR} < 0.001$) and left anterior cingulate ($\rho = +0.197$, $P_{FDR} = 0.001$). Lower striatal DAT binding was also associated with reduced limbic-to-default-mode-network connectivity ($P_{FDR} = 0.010$). Putaminal DAT binding was associated with all three LVIs (multimodal $\rho = -0.187$, $P < 0.001$) but showed a more focal structural pattern, surviving correction only for the left anterior cingulate ($\rho = +0.170$, $P_{FDR} = 0.014$).

Prediction of clinical endpoints in iRBD

Finally, we tested whether baseline limbic measures predicted incident visual hallucinations in iRBD using Cox proportional hazards models. Among 175 participants with iRBD, 151 had usable follow-up data and no incidental hallucinations or psychotic phenomena at baseline, and of those, 24 developed an MDS-UPDRS Part I hallucinations item score above zero during follow-up. Each standard-deviation increase in baseline structural LVI was associated with a 67% higher hazard of hallucinations ($HR = 1.67$, 95% CI 1.10–2.54, $P_{FDR} = 0.045$). Higher multimodal LVI was similarly associated with greater incidence of hallucinations ($HR = 1.67$, 95% CI 1.05–2.67, $P_{FDR} = 0.045$) (Fig. 3A; Supplementary Table 7). At the regional level, higher hallucination risk was associated with smaller bilateral amygdala volume (bilateral $HR = 1.78$, 95% CI 1.14–2.79, $P_{FDR} = 0.036$) (Fig. 3B) and also had trending association with reduced ACC volume that was not significant after correction (bilateral ACC $HR = 1.68$, 95% CI 1.02–2.77, $P_{unc} = 0.041$). Reduced limbic network connectivity to the somatomotor and dorsal attentional networks was similarly associated with higher hallucination risk, however also did not pass correction for multiple comparisons (LIM-SMN $HR = 1.55$, 95% CI 1.07–2.25 $P_{unc} = 0.021$; LIM-DAN $HR = 1.49$, 95% CI 1.02–2.17, $P_{unc} = 0.041$). The proportional hazards assumption was satisfied for all reported models, as verified by the tests of scaled Schoenfeld residuals (all $P > 0.10$).

(A) Hazard ratios (per standard deviation, with 95% CI) for each limbic structural and functional measure as a predictor of incident hallucinations, from age- and sex-adjusted Cox proportional hazards models within the polysomnography-confirmed iRBD cohort. Hazard ratios are oriented so that values above 1 denote greater risk with greater limbic vulnerability: for the Limbic Vulnerability Indices this corresponds to a per-SD increase, and for regional volumes and connectivity measures to a per-SD decrease. The dashed line marks a hazard ratio of 1 (no association); estimates whose 95% CI excludes 1 are shown in red. Bilateral amygdala volume (boxed) was the strongest structural predictor. **(B)** Kaplan-Meier hallucination-free survival stratified by bilateral amygdala volume, dichotomised at the sample. Shaded bands are 95% confidence intervals and the table below gives the number at risk at each interval. Participants with lower amygdala volume showed shorter hallucination-free survival. ACC, anterior cingulate cortex; AMY, amygdala; CI, confidence interval; DAN, dorsal attention; DMN, default mode; HIP, hippocampus; HR, hazard ratio; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; LVI, Limbic Vulnerability Index; SD, standard deviation; SMN, somatomotor; VAN, ventral attention; VIS, visual; WC, whole cortex.

Discussion

In this study, we examined structural and functional limbic integrity across prodromal and established Lewy body disease. We found that limbic vulnerability was closely associated with the presence of RBD across groups.

Indeed, participants with iRBD had greater structural, functional and multimodal limbic vulnerability than healthy controls and participants with PD without probable RBD, despite their differences in disease stage. Within PD, participants with probable RBD had greater structural and multimodal vulnerability than those without probable RBD, although functional vulnerability did not differ. Greater baseline limbic vulnerability was also associated with RBD symptom burden and with subsequent development of hallucinatory phenomena. Together, these findings suggest that limbic involvement may represent a feature of the RBD phenotype across the Lewy body disease spectrum, rather than simply a consequence of established PD, and may have prognostic implications.

Limbic vulnerability in early disease

Our findings extend neuropathological evidence showing that limbic structures may be affected during the prodromal phase of Lewy body disease. Established pathological staging frameworks place limbic involvement alongside the early stages of nigrostriatal degeneration, with regions including the ACC, hippocampus and amygdala affected before widespread cortical involvement.^{11,34} Recent post-mortem observations in individuals with iRBD provide further support, demonstrating substantial Lewy pathology and neuronal loss within limbic associated regions prior to phenoconversion to progressed clinical disease.³⁵ Our research complements these findings by revealing that limbic abnormalities can be detected *in vivo* in a large cohort with PSG-confirmed iRBD.

Functionally, the most prominent finding was a widespread reduction in connectivity between the limbic network and visual, somatomotor and ventral attentional networks in iRBD compared to HC, accompanied by reduced amygdala-to-limbic connectivity. Rather than representing focal disruption, this widespread hypoconnectivity is consistent with a broader shift to a more segregated network architecture previously described in iRBD.³⁶ This decoupling of the limbic network from sensory and attentional networks may represent a loss of functional integration that supports flexible cognitive function. Consistent with this interpretation, reduced limbic-to-visual connectivity was associated with poorer global cognition within the iRBD group. Previous functional imaging studies have similarly reported reduced FC between limbic regions to parietal and occipital lobes,²⁵ and reduced ACC-cortical connectivity, which has been linked with cognitive impairment in iRBD.³⁷

Our structural findings were also consistent with previous neuroimaging studies reporting reduced hippocampal,^{17,38,39} amygdala^{17,38,39} and ACC^{19,40} volumes in iRBD. In our cohort, the structural abnormalities became more evident when considered collectively across multiple regions. This may indicate that limbic degeneration is distributed across multiple interconnected structures rather than dominated by a single vulnerable locus. Such a pattern is consistent with the concept of Lewy body disease as a network-level disorder, in which relatively subtle abnormalities across interconnected regions may collectively represent a meaningful disease signature. This early limbic disruption may be driven in part by neuromodulatory dysfunction, particularly degeneration of the cholinergic basal forebrain and the noradrenergic locus coeruleus. Basal forebrain atrophy parallels limbic degeneration in iRBD, with reduced volume reported alongside and correlated with reduced amygdala and ACC volumes.⁴¹ Mediation analyses also indicate that basal forebrain degeneration influences episodic memory decline through limbic atrophy, potentially highlighting limbic cholinergic denervation as a marker of DLB conversion.⁴² Given that nucleus basalis of Meynert provides widespread cholinergic innervation to the cortex, amygdala and hippocampus, its early degeneration offers a plausible substrate for the widespread functional disruptions of the limbic system observed in iRBD patients.^{43,44} Future multimodal studies incorporating functional and molecular imaging methods will help clarify this association.

Limbic involvement and the RBD phenotype across Lewy body disease

Importantly, our findings demonstrate a close relationship between limbic vulnerability and RBD across the Lewy body disease spectrum. Individuals with iRBD showed significant limbic alterations compared to PD-nRBD patients, while iRBD and PD-RBD showed no differences in limbic vulnerability despite their differences in clinical stage. This pattern suggests that limbic involvement may represent a biological feature that is specifically associated with the RBD phenotype rather than a consequence of PD progression. RBD has now been recognised as a clinical marker integral in understanding the heterogeneity in PD,⁴⁵ with PD-RBD patients consistently demonstrating greater autonomic, cognitive, psychiatric and motor disease burden than those without RBD.⁸ Our finding extends this distinction to the limbic system, identifying neurobiological differences that distinguish PD-RBD and PD-nRBD, while also exhibiting associations between limbic dysfunction and cognitive and motor impairment within PD.

Prior neuroimaging studies have reported reduced amygdala, hippocampal and ACC volumes^{29–31}, together with reduced amygdala and cingulate functional connectivity,^{27,28} in patients with PD-RBD compared to PD-nRBD. More recent work examining brain-first and body-first PD subtypes has similarly identified greater hippocampal and amygdala volume loss in PD-RBD, supporting limbic degeneration as a component of the RBD-related phenotype.⁴⁶ The present findings broadly support this literature while placing the PD differences alongside those observed in iRBD. Cholinergic reductions have also been observed in limbic regions in PD-RBD compared to PD-nRBD, and are more pronounced in DLB than in PD.⁴⁷ The extent of limbic vulnerability observed in iRBD may also be relevant to the heterogeneous disease trajectories that follow the prodromal stage. Around 40% of individuals with iRBD will ultimately develop DLB,⁴⁸ a phenotype characterised by more prominent cognitive, neuropsychiatric and visuoperceptual dysfunction. Interestingly, vulnerability of the limbic system has been extensively demonstrated in DLB patients, with data-driven subtype analysis predominantly tying DLB type patterns of atrophy with high limbic vulnerability.³² Therefore, the strong results we observe in iRBD patients may in part be driven by those on the DLB conversion trajectory. Together with our findings, these observations suggest that limbic involvement may not solely be restricted to late-stage disease but may emerge early, be closely associated with RBD and may be tied to non-motor phenotypes following phenoconversion.³²

Across our Lewy body disease cohort, greater limbic vulnerability was associated with higher RBDSQ scores, poorer olfactory function and greater excessive daytime sleepiness, suggesting that limbic dysfunction may be directly linked to RBD status and the severity of its broader clinical expression. Although the loss of REM atonia is attributed principally to α -synuclein-mediated degeneration of brainstem nuclei regulating REM sleep,^{49,50} the limbic system may contribute more directly to the clinical expression of RBD than a purely downstream role would suggest. Limbic structures are active during REM sleep and contribute to emotional salience, memory and the integration of internally generated experiences.^{51,52} Therefore, limbic dysfunction could plausibly contribute to the vivid, often threatening, dream content characteristic of RBD,⁵³ rather than indexing disease severity alone. Consistent with a limbic contribution, rare clinicopathological cases have demonstrated clinically manifest RBD in DLB with extensive limbic degeneration despite an absence of α -synuclein brainstem pathology.^{20,35} Together, these observations raise the possibility that limbic pathology may contribute to the clinical expression of RBD, in addition to, or in spite of, the established brainstem mechanisms underlying RSWA. These brainstem changes may play a more permissive role, releasing behaviours whose emotional and narrative content is shaped by limbic

dysfunction. Importantly, the present imaging was acquired during wakefulness and cannot demonstrate that limbic dysfunction directly shapes REM mentation or dream enactment. Functional imaging acquired directly during REM sleep will be required to establish whether limbic dysfunction shapes RBD phenomenology directly or reflects broader disease burden.

Clinical and prognostic relevance of limbic vulnerability

The clinical associations provide preliminary evidence that limbic abnormalities are relevant beyond diagnostic-group differences. Within iRBD, lower limbic-to-visual connectivity was associated with poorer cognition, while greater depressive symptom burden was associated with lower hippocampal-to-limbic connectivity. Within PD, greater motor severity was associated with higher structural and multimodal LVI, smaller hippocampal volume and lower limbic-to-visual connectivity. These relationships were generally modest and varied between cohorts, suggesting that limbic vulnerability contributes to, but does not alone determine, the clinical heterogeneity of Lewy body disease.

Furthermore, in our longitudinal analysis, baseline structural limbic vulnerability and bilateral amygdala degeneration were associated with an increased risk of developing hallucinatory phenomena in iRBD. In PD, amygdala atrophy and altered connectivity with visual and attention networks have been associated with visual hallucinations.^{32,54,55} Amygdala dysfunction may also interact with degeneration of subcortical cholinergic and broader attentional networks, altering the balance between externally driven sensory information and internally generated representations to produce visual hallucinations.^{33,56} This provides a plausible framework through which early structural limbic vulnerability could increase susceptibility to later perceptual disturbances as seen in our study.

Biomarker associations of limbic vulnerability

The association between limbic vulnerability and reduced striatal dopamine transporter binding links limbic abnormalities to the wider neurodegenerative process. Lower DAT binding was associated with functional and multimodal vulnerability in iRBD and with more distributed structural and functional abnormalities in PD. As DAT binding indexes nigrostriatal degeneration, we interpret its association with more widespread limbic vulnerability as reflecting greater synucleinopathy-related disease burden.

In contrast, biomarkers of Alzheimer's disease co-pathology showed a more restricted pattern. CSF A β 42/40 was not associated with limbic imaging in either iRBD or PD. Within iRBD, higher plasma p-tau217 was associated with smaller hippocampal volume but not with the LVIs or functional connectivity. Although only available in a subset of our cohort, this preliminary finding raises the possibility that tau co-pathology contributes to a relatively focal hippocampal component of limbic vulnerability. Tau and amyloid co-pathology are common across the Lewy body continuum and are detectable from the prodromal stage.⁵⁷ Elevated phosphorylated tau levels have been associated with phenoconversion, especially to a dementia phenotype.^{57–59} Post-mortem iRBD cohorts have demonstrated tau deposition within the hippocampus, alongside α -synuclein but in the absence of amyloid pathology³⁵. Together, these observations suggest that tau-related processes may contribute to a focal hippocampal pathway in a subset of individuals with iRBD, potentially increasing vulnerability to dementia-dominant phenoconversion.

Strengths, limitations and future work

Key strengths of this study include the large, well-characterised cohort spanning prodromal and established Lewy body disease, the inclusion of PSG-confirmed iRBD, and the integration of structural and functional imaging with molecular biomarkers and longitudinal follow-up. However, several limitations should also be considered. First, the iRBD cohort contained relatively few individuals who had phenoconverted at the time of analysis, preventing direct assessment of whether limbic vulnerability distinguishes future PD from DLB conversion. Continued follow-up and validation in larger longitudinal cohorts will be required. Second, DLB was not represented as a distinct clinical group, limiting our ability to determine whether the observed limbic vulnerability preferentially predicts dementia-dominant disease. This should be addressed through future comparisons incorporating established DLB. In our study, LVI provided an interpretable summary of multimodal limbic abnormalities, and this approach should be extended to DLB, to characterise limbic dysfunction across Lewy body diseases and to clarify its role in phenoconversion in iRBD, using larger HC samples from independent normative cohorts. Having shown that limbic vulnerability is critical in the RBD spectrum, future work should characterise the temporal evolution of structural and functional dysfunction, and incorporate diffusion imaging to model white matter degeneration of these limbic structures. Finally, the classification of RBD differed across groups: the iRBD cohort was polysomnography-confirmed, whereas RBD status in the Parkinson's disease groups was defined by questionnaire (RBDSQ ≥ 5). Future studies incorporating PSG and quantitative RSWA measures across all groups will help distinguish the neural correlates of RSWA from those of overt RBD.

Conclusions

Our findings identify structural and functional limbic disruption in iRBD and demonstrate that this vulnerability is associated with the presence of RBD across the Lewy body disease spectrum. The convergence of limbic abnormalities in iRBD and PD-RBD, together with their association with RBD symptom burden, dopaminergic degeneration and subsequent visual hallucinations, suggests that limbic dysfunction represents an important component of the biological phenotype associated with RBD. These findings extend neuropathological evidence and highlight multimodal limbic vulnerability as a potential framework for understanding why RBD is associated with distinct clinical trajectories across Lewy body disease.

Methods

Participants

Participants with baseline resting-state functional MRI (fMRI) and clinical data were drawn from the PPMI (<https://www.ppmi-info.org/access-data-specimens/download-data>; RRID: SCR_006431) in March 2026 and comprised four diagnostic groups: HC, PSG-confirmed iRBD; PD patients without suspected RBD defined as an RBDSQ score below 5 (PD-nRBD); PD patients with probable RBD defined as an RBDSQ score of 5 or greater (PD-RBD). Two pre-specified exclusion criteria were applied to all participants: excessive head motion, defined as mean framewise displacement exceeding 0.5 mm, and age below 50 years at the time of scanning, the latter to limit young-onset presentations that may reflect secondary RBD (in the case of iRBD) or genetic PD. Healthy controls with an RBDSQ score above 5 were excluded. Of 753 participants assessed, 44 were excluded on these criteria (34 for age, 6 for head motion, and 4 HC with an elevated RBDSQ), and a further 151 iRBD participants for not being PSG-confirmed. The final analytic sample comprised 558 participants (HC, $n = 54$; polysomnography-confirmed iRBD, $n = 175$; PD-nRBD, $n = 250$; PD-RBD, $n = 79$). Group-level exclusions by criterion are shown in Fig. 4A. All patients included in this study gave written informed consent according to the Declaration of Helsinki

and all ethics approvals were handled by the local institutional boards at respective recruitment sites. The original PPMI protocol (NCT01141023) was approved by the University of Rochester Research Subjects Review Board and by the institutional review board or ethics committee at each participating site. The current PPMI Clinical protocol (Protocol 002; NCT04477785) is approved by WCG IRB (IRB Tracking #20200597), with local ethics approval at each site. Data analysis was approved by the Sydney University Human Research Ethics Committee (HREC No. 2013/HE000945).

Clinical and Neuropsychological Testing

Participants underwent standardised clinical and neuropsychological assessment at baseline, with continued annual follow-up. Global cognition was measured using the Montreal Cognitive Assessment (MoCA), and motor severity using the Movement Disorders Society Unified Parkinson's Disease Rating Scale, Part III (MDS-UPDRS III). Autonomic symptom burden was indexed by the Scale for Outcomes in Parkinson's Disease – Autonomic Dysfunction (SCOPA-AUT), daytime sleepiness by the Epworth Sleepiness Scale (ESS), depressive symptoms by the Geriatric Depression Scale (GDS), and anxiety by the State-Trait Anxiety Inventory (STAI). RBD symptom severity was quantified with the RBD Screening Questionnaire (RBDSQ).

MRI Acquisition and Preprocessing

Whole-brain T1-weighted anatomical and resting-state BOLD functional images were acquired on 3T MRI using standardised PPMI protocols available at (<https://www.ppmi-info.org/study-design/research-documents-and-sops>). Image preprocessing was performed with fMRIPrep (version 24.1.1), including intensity non-uniformity correction, skull stripping, tissue segmentation, cortical surface reconstruction, FreeSurfer subcortical segmentation and cortical parcellation, spatial normalisation to MNI152NLin6Asym space, head-motion estimation, boundary-based co-registration of the functional to the anatomical image, and confound estimation (details in the Supplementary Methods).

Grey Matter Volumetric Analysis

Cortical and subcortical volumes were derived from the T1-weighted images using FreeSurfer 7.3.2. Amygdala and hippocampal volumes were obtained from the subcortical (aseg) segmentation and anterior cingulate cortex volume from the Destrieux (aparc.a2009s) parcellation⁶⁰ and were extracted for each hemisphere and summed to yield bilateral estimates. To account for differences in head size, each regional volume was adjusted for intracranial volume using a regression-based residual approach (Fig. 4B).⁶¹

Functional Connectivity Analysis

To facilitate comparable functional and structural relationships, the hippocampus, amygdala, and anterior cingulate cortex were defined from each participant's own FreeSurfer segmentation, so that functional and structural measures were derived from identical, participant-specific anatomy. Each participant's segmentation was resampled from FreeSurfer space into functional space (MNI152NLin6Asym, 2 mm isotropic), and the anatomical fidelity of every resampled region was confirmed. Regional time series were extracted in

MNI152Nlin6Asym space using a combined parcellation comprising these subcortical regions and the 400-region Schaefer 7-network cortical atlas.⁶² Nuisance regression, temporal filtering, and standardisation were applied in a single step during extraction. Confounds comprised the six rigid-body motion parameters and their temporal derivatives, and the mean white matter, cerebrospinal fluid, and whole-brain global signals; time series were simultaneously linearly detrended, band-pass filtered (0.01–0.1 Hz), and z-scored. Functional connectivity was estimated as the Fisher z-transformed Pearson correlation between each pair of regional time series. Cortical regions were assigned to the seven Yeo networks, and network-level connectivity was summarised as the mean connectivity of the limbic network with each cortical network and with the whole cortex. For the subcortical analysis, functional connectivity was computed between the hippocampus, amygdala, and anterior cingulate cortex, both pairwise and to the limbic network (Fig. 4C). A comprehensive readout of anatomical and functional MRI preprocessing steps are located in the Supplementary Methods.

(A) Participant selection from PPMI. Group-specific exclusions applied for age, motion (mean FD > 0.5 mm), and, for iRBD, absence of PSG confirmation. Final sample: n = 558 (HC = 54, iRBD = 175, PD-nRBD = 250, PD-RBD = 79). **(B)** Structural MRI: FreeSurfer aseg and Destrieux-atlas grey matter volumes for the amygdala, hippocampus, and ACC. **(C)** Functional MRI: subject-specific hippocampus, amygdala, and ACC seeds used for subcortical connectivity, and a cortical limbic network used to compute connectivity to the six Yeo resting-state networks. **(D)** Limbic Vulnerability Indices (LVI): each structural and functional measure expressed as a w-score relative to age- and sex-adjusted healthy-control norms, with higher values indicating greater vulnerability. LVI-Functional and LVI-Structural were computed separately and averaged to give LVI-Multimodal. ACC, anterior cingulate cortex; AMY, amygdala; DAN, dorsal attention network; DMN, default mode network; FD, framewise displacement; FPN, frontoparietal network; HC, healthy controls; HIP, hippocampus; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; LVI, Limbic Vulnerability Index; PD-nRBD, Parkinson's disease without probable RBD; PD-RBD, Parkinson's disease with probable RBD; PPMI, Parkinson's Progression Markers Initiative; PSG, polysomnography; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; SMN, somatomotor network; VAN, ventral attention network; VIS, visual network.

Limbic vulnerability indices

To summarise distributed limbic abnormalities while preserving the distinction between structural and functional modalities, we constructed structural, functional and multimodal Limbic Vulnerability Indices (LVIs). The structural LVI comprised three intracranial-volume-adjusted measures: the bilateral amygdala, the bilateral hippocampus, and the bilateral anterior cingulate cortex. The functional LVI comprised four resting-state measures: limbic network to the whole cortex FC, and FC of the hippocampus, amygdala, and anterior cingulate cortex with the limbic network.

Each constituent measure was converted to a control referenced w-score. For each measure, an ordinary least squares model was fitted in HC with age and sex as predictors. The expected value for each participant was estimated from this control model, and the deficit-oriented w-score was calculated as the model-predicted value minus the observed value, divided by the standard deviation of the control-model residuals. Scores were therefore oriented so that higher values denote greater departure from healthy expectation (i.e., lower-than-expected volume or functional connectivity). These scores represent the direction and magnitude of a deficit relative to the healthy-control reference model, rather than the absolute magnitude of any deviation from normality. Thus greater/higher scores represented a greater magnitude of deficit compared to controls (increased “vulnerability”).

The structural LVI was calculated as the mean of the three structural w-scores, and the functional LVI as the mean of the four functional w-scores. The multimodal LVI was then calculated as the mean of the structural and functional LVIs multimodal (Fig. 4D). This approach gave equal weight to the structural and functional domains, regardless of the number of measures in each. Structural and functional LVIs were retained as domain-specific measures, while the multimodal LVI provided an overall summary.

Statistical Analysis

Group differences in limbic imaging measures and in the LVIs were assessed across the four diagnostic groups using general linear models, with each measure as the outcome, diagnostic group as a four-level predictor, and age and sex as covariates. Relationships with clinical variables were calculated using a linear regression, with age and sex as covariates. For each measure we fit an omnibus model testing the overall group effect (F statistic). Benjamini-Hochberg false discovery rate (P_{FDR}) correction was applied within each measure family.

Relationships between limbic imaging measures and molecular markers were examined within the disease groups. Each biomarker was matched to within 6 months of the visit at which the MRI was acquired. Spearman's correlations were tested for the individual regional volumes and connectivity measures and, separately, for the structural, functional, and combined LVIs, against CSF amyloid- β 42, plasma p-tau217, and striatal and putaminal dopamine transporter binding ratios.

Longitudinal outcomes were examined within the iRBD group using Cox proportional hazards models for incident hallucinations (MDS-UPDRS Part I hallucinations item score above zero). Participants meeting an endpoint at baseline were excluded from that model, and time was measured from the baseline scan to the first assessment meeting the endpoint, or to the last available assessment otherwise. Each imaging measure was entered as a separate predictor, standardised and adjusted for age and sex, with hazard ratios expressed per standard deviation. Hazard ratios are oriented so that values above one uniformly denote reduced volume and functional connectivity being associated with higher risk of developing hallucinations.

Declarations

Conflicts of Interest:

The authors declare no conflict of interest.

Competing interests

The authors declare no competing interests.

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Author Contribution

Author roles included: conception and design of the study (LC., S.J.G.L., E.M.); pre-processing and analysis of data (L.C., A.K., J.A., A.I.); and final approval of manuscript and/or figures (L.C., A.K., J.A., A.I., S.J.G.L., E.M.). All authors declare accountability for the work contained in the manuscript.

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Data Availability

Data availability A key resources table, de-identified derived data supporting the reported findings, data dictionary and analysis code are available on Zenodo at <https://doi.org/10.5281/zenodo.22313779>. Raw participant-level data are openly available to researchers upon request on the PPMI database (<https://www.ppmi-info.org/access-data-specimens/data>).

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Figures

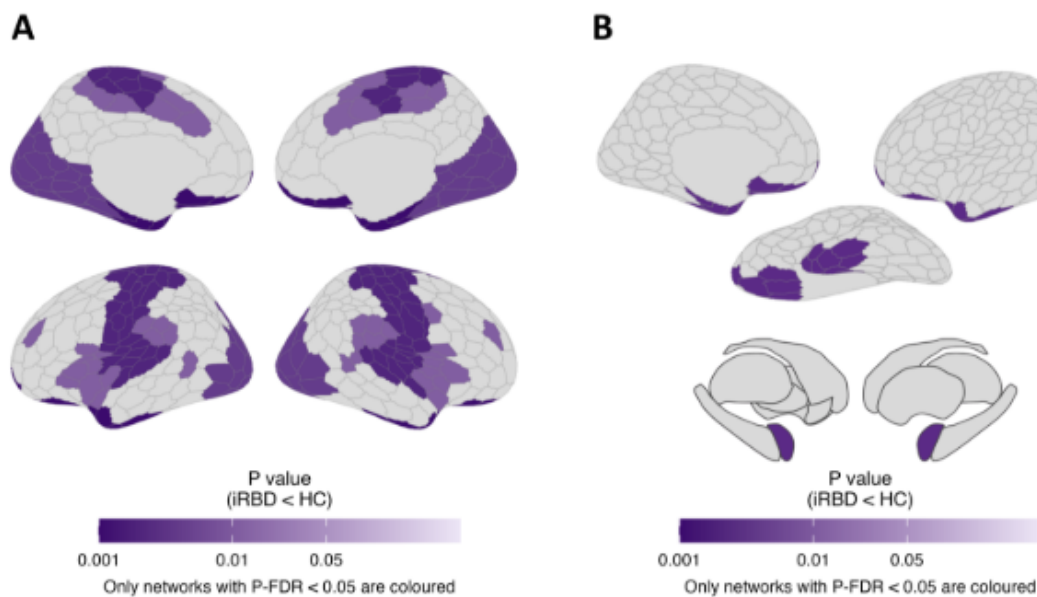


Figure 1

Reduced limbic connectivity in iRBD relative to healthy controls.

Regions are coloured where connectivity with the limbic network was significantly lower in isolated REM sleep behaviour disorder (iRBD) than in healthy controls (HC). **(A)** Cortical networks showing reduced limbic connectivity in iRBD, spanning the visual, somatomotor and ventral attention networks alongside a broad whole-cortex reduction. **(B)** Reduced FC between the amygdala and the limbic network. HC, healthy control; iRBD, isolated REM sleep behaviour disorder.

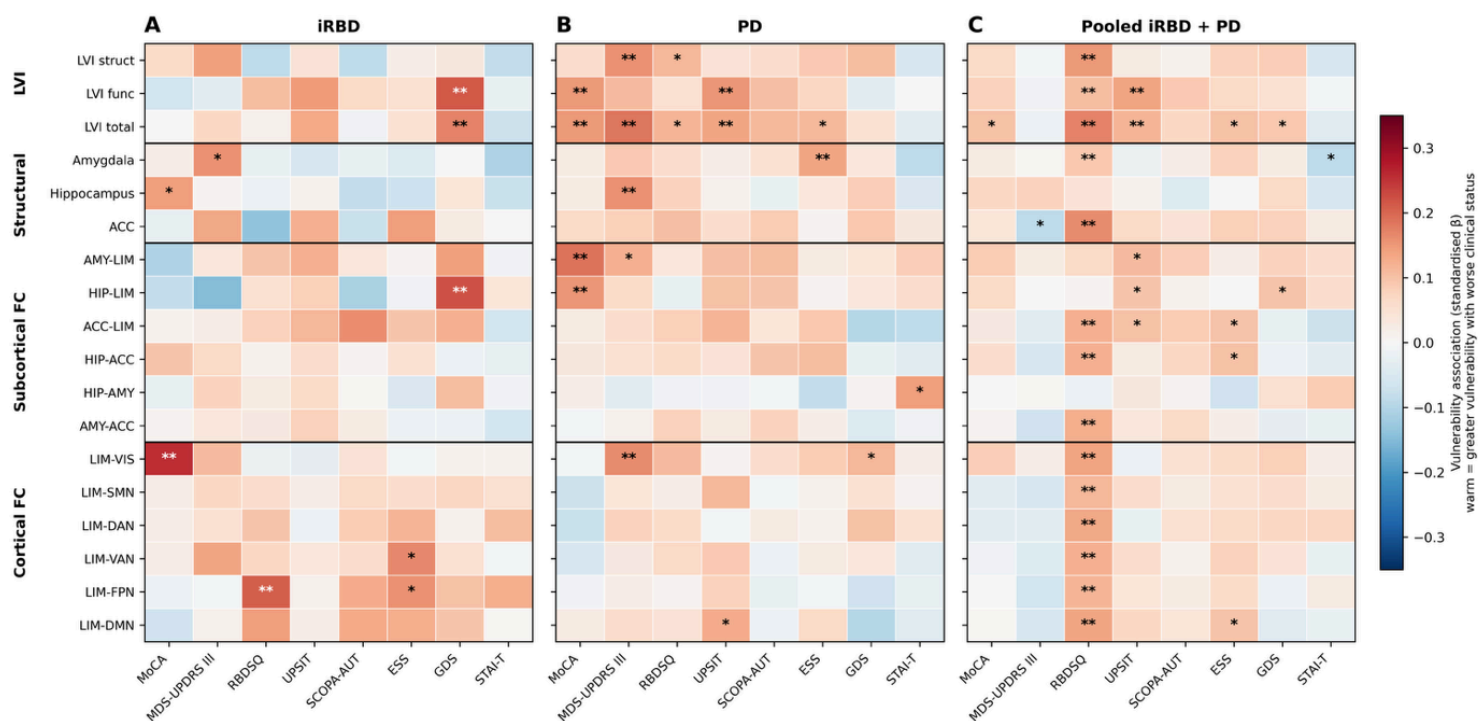


Figure 2

Associations between limbic vulnerability and clinical severity within iRBD, within PD, and in the pooled patient sample.

Each cell is an age- and sex-adjusted standardised β (a partial correlation) from a linear model of one limbic measure on one clinical variable, both z-scored, fitted separately within polysomnography-confirmed iRBD (**A**; $n = 175$), established PD (**B**; PD-RBD + PD-nRBD, $n = 329$), and the two pooled (**C**; iRBD + PD, $n = 504$). Rows are grouped into the Limbic Vulnerability Indices, regional volumes, subcortical connectivity, and cortical connectivity; columns are clinical measures of cognition, motor severity, RBD symptom burden, olfaction, autonomic function, daytime sleepiness, depression, and trait anxiety. All cells are oriented to a common severity axis: measures are signed so that higher indicates greater vulnerability (regional volumes and connectivity are reversed, since lower values denote greater degeneration; the Limbic Vulnerability Indices retain their native direction), and clinical scores are signed so that higher indicates worse status (MoCA and UPSIT are reversed, since higher scores are better). A positive (warm) cell therefore indicates that greater limbic vulnerability, reduced volume or reduced FC is associated with worse clinical status, and a negative (cool) cell the reverse. Asterisks denote significance within each clinical column: ** Benjamini-Hochberg FDR $q < 0.05$, computed down each column separately within each sample; * uncorrected $P < 0.05$. Sample sizes are the maximum complete-case n per panel and vary by cell with missing data. ACC, anterior cingulate cortex; AMY, amygdala; DAN, dorsal attention; DMN, default mode; ESS, Epworth Sleepiness Scale; FC, functional connectivity; FPN, frontoparietal; GDS, Geriatric Depression Scale; HIP, hippocampus; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; LVI, Limbic Vulnerability Index; MDS-UPDRS III, Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; MoCA, Montreal Cognitive Assessment; PD, Parkinson's disease; RBDSQ, REM Sleep Behaviour Disorder Screening Questionnaire; SCOPA-AUT, Scales for Outcomes in Parkinson's Disease–Autonomic; SMN, somatomotor; STAI-T, State-Trait Anxiety Inventory, trait; UPSIT, University of Pennsylvania Smell Identification Test; VAN, ventral attention; VIS, visual.

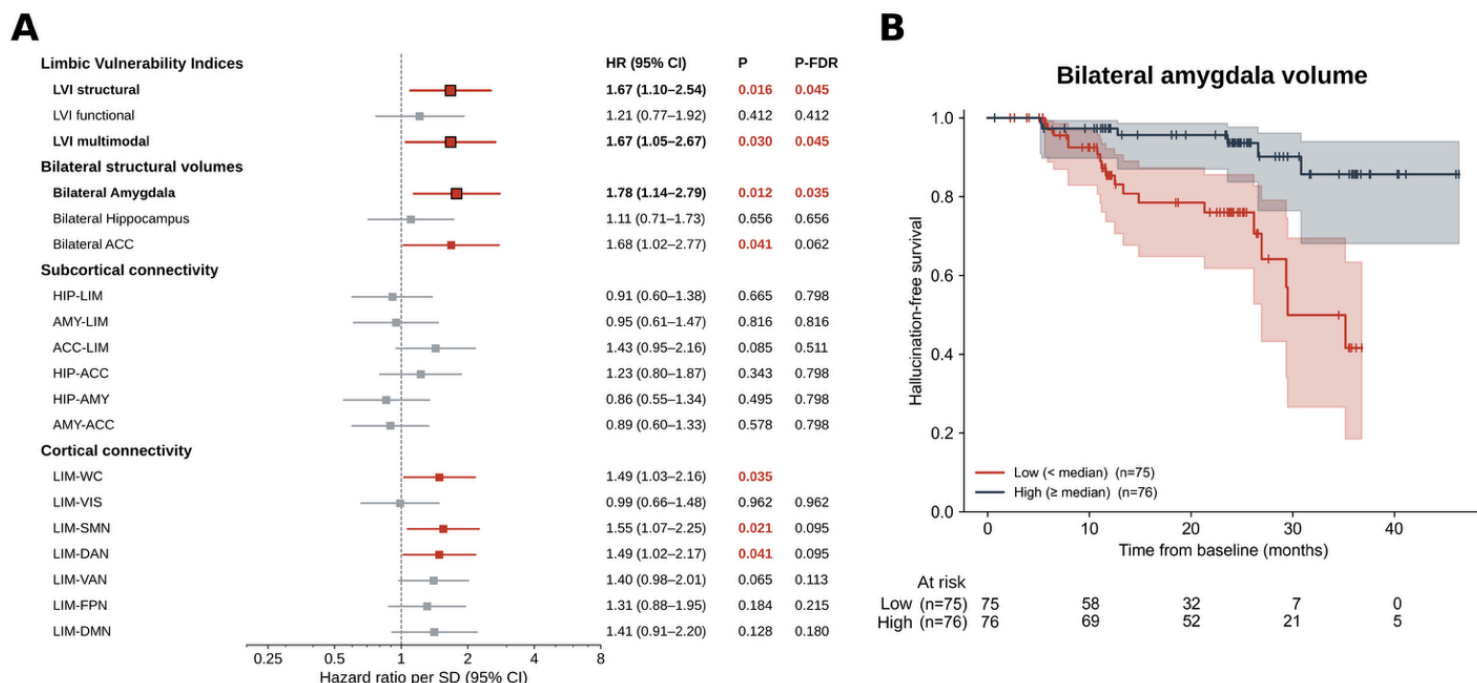


Figure 3

Limbic degeneration predicts incident hallucinations in iRBD.

(A) Hazard ratios (per standard deviation, with 95% CI) for each limbic structural and functional measure as a predictor of incident hallucinations, from age- and sex-adjusted Cox proportional hazards models within the polysomnography-confirmed iRBD cohort. Hazard ratios are oriented so that values above 1 denote greater risk with greater limbic vulnerability: for the Limbic Vulnerability Indices this corresponds to a per-SD increase, and for regional volumes and connectivity measures to a per-SD decrease. The dashed line marks a hazard ratio of 1 (no association); estimates whose 95% CI excludes 1 are shown in red. Bilateral amygdala volume (boxed) was the strongest structural predictor. **(B)** Kaplan-Meier hallucination-free survival stratified by bilateral amygdala volume, dichotomised at the sample. Shaded bands are 95% confidence intervals and the table below gives the number at risk at each interval. Participants with lower amygdala volume showed shorter hallucination-free survival. ACC, anterior cingulate cortex; AMY, amygdala; CI, confidence interval; DAN, dorsal attention; DMN, default mode; HIP, hippocampus; HR, hazard ratio; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; LVI, Limbic Vulnerability Index; SD, standard deviation; SMN, somatomotor; VAN, ventral attention; VIS, visual; WC, whole cortex.

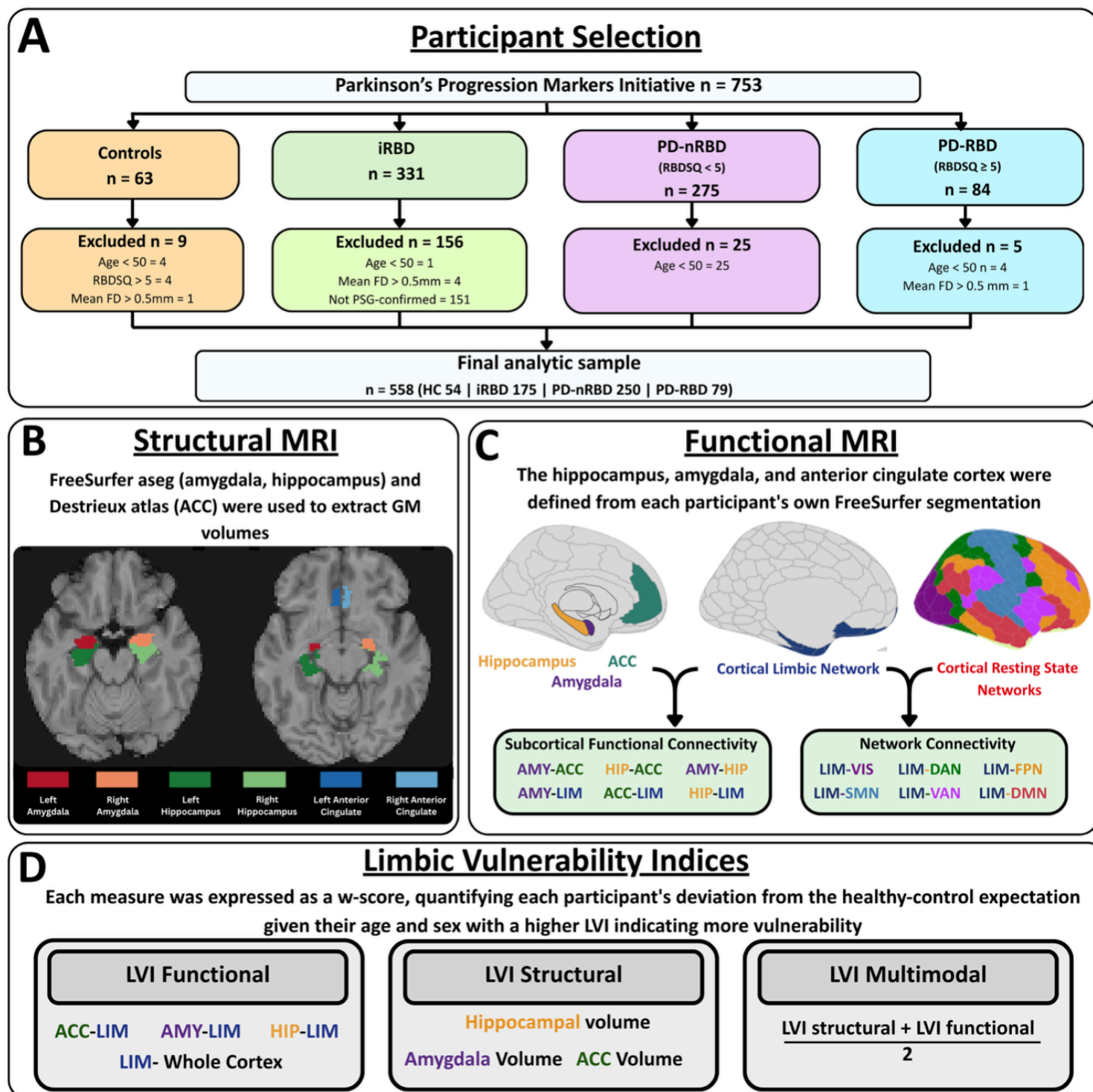


Figure 4

Cohort construction and limbic imaging pipeline.

(A) Participant selection from PPMI. Group-specific exclusions applied for age, motion (mean FD > 0.5 mm), and, for iRBD, absence of PSG confirmation. Final sample: n = 558 (HC = 54, iRBD = 175, PD-nRBD = 250, PD-RBD = 79). (B) Structural MRI: FreeSurfer aseg and Destrieux-atlas grey matter volumes for the amygdala, hippocampus, and ACC. (C) Functional MRI: subject-specific hippocampus, amygdala, and ACC seeds used for subcortical connectivity, and a cortical limbic network used to compute connectivity to the six Yeo resting-state networks. (D)

Limbic Vulnerability Indices (LVI): each structural and functional measure expressed as a w-score relative to age- and sex-adjusted healthy-control norms, with higher values indicating greater vulnerability. LVI-Functional and LVI-Structural were computed separately and averaged to give LVI-Multimodal. ACC, anterior cingulate cortex; AMY, amygdala; DAN, dorsal attention network; DMN, default mode network; FD, framewise displacement; FPN, frontoparietal network; HC, healthy controls; HIP, hippocampus; iRBD, isolated REM sleep behaviour disorder; LIM, limbic network; LVI, Limbic Vulnerability Index; PD-nRBD, Parkinson's disease without probable RBD; PD-RBD, Parkinson's disease with probable RBD; PPMI, Parkinson's Progression Markers Initiative; PSG, polysomnography; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; SMN, somatomotor network; VAN, ventral attention network; VIS, visual network.

Supplementary Files

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- [SupplementaryLimbicNetworkDysfunctioninRBD.docx](#)