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Parkinson's Disease, Cortical Dysfunction, and Alpha-Synuclein

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Abstract

Background—The ability to understand how Parkinson's disease (PD) neurodegeneration leads to cortical dysfunction will be critical for developing therapeutic advances in PD dementia (PD-D). The overall purpose of this project was to study the small amplitude cortical myoclonus in PD as an *in vivo* model of focal cortical dysfunction secondary to PD neurodegeneration. The objectives were to test the hypothesis that cortical myoclonus in PD is linked to abnormal levels of α-synuclein in primary motor cortex and to define its relationship to various biochemical, clinical, and pathological measures.

Methods—Primary motor cortex was evaluated for 11 PD subjects with (PD+Myoclonus group) and 8 without (PD group) electrophysiologically confirmed cortical myoclonus who had premortem movement and cognitive testing. Similarly assessed 9 controls were used for comparison. Measurements for α-synuclein, Aβ-42 peptide, and other biochemical measures were made in primary motor cortex.

Results—A 36% increase in α-synuclein was found in the motor cortex of PD+Myoclonus cases when compared to PD without myoclonus. This occurred without significant differences in insoluble α-synuclein, phosphorylated to total α-synuclein ratio, or Aβ-42 peptide levels. Higher total motor cortex α-synuclein levels significantly correlated with the presence of cortical myoclonus but did not correlate with multiple clinical or pathological findings.

Conclusions—These results suggest an association between elevated α-synuclein and the dysfunctional physiology arising from the motor cortex in PD+Myoclonus cases. Alzheimer's disease pathology was not associated with cortical myoclonus in PD. Cortical myoclonus arising from motor cortex is a model to study cortical dysfunction in PD.

INTRODUCTION

A prerequisite for developing effective treatments of Parkinson's disease (PD) dementia (PD-D) is to determine the mechanism(s) through which PD neurodegeneration *causes* cerebral cortical dysfunction in humans (1–9). Braak and others have associated PD-D with pathology spread to neocortical areas (3–9). This highlights the need for biomarker models to study cortical dysfunction in PD patients as well as to complement and validate findings from animal models.

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The small amplitude cortical myoclonus of PD indicates *in vivo* dysfunction of the primary

motor cortex in PD patients (10). We have characterized this myoclonus in our laboratory, both clinically and electrophysiologically (10). The mechanism for neuronal dysfunction caused by PD in the primary motor cortex of PD patients with myoclonus should have strong similarity to the pathophysiology in other neocortical areas. Instead of associating a type of diffuse dysfunction (i.e. dementia) with *focal* tissue samples from cortex regions with unknown local functional integrity, this biomarker allows specific correlation of primary motor cortex biochemistry with the known presence or absence of abnormal physiology (i.e. cortical myoclonus) in the primary motor cortex per se.

In this study, we tested the hypothesis that the small amplitude cortical myoclonus in PD is linked to abnormal levels of α-synuclein in primary motor cortex. The presence of cortical myoclonus in PD was correlated with biochemical, clinical, and pathological measures.

METHODS

Cases Studied

Biochemical studies were performed on 11 PD cases with small amplitude cortical myoclonus (PD+Myoclonus group) and 8 PD cases without myoclonus (PD group). For comparison, 9 Control cases were also studied. All subjects studied were from the Banner Sun Health Research Institute (SHRI) Brain and Body Donation Program (4,10–12). All subjects signed informed consent; were followed antemortem with standardized medical history; received movement, cognitive, electrophysiological assessments; were autopsied within 4 hours of death; and received a final diagnosis based on clinicopathologic correlation as per our previous reports (4,10–12). The neuropsychological battery included Folstein MMSE, long-term memory score on the Auditory-Verbal Learning Test (AVLT-LTM), Controlled Oral Word Association Test (COWAT), Stroop Interference, Trails B, WAIS-III Digit Span, and Judgment of Line Orientation test (JLO). DSM-IV criteria for dementia were used: abnormalities in memory and one other domain of cognition, functional decline related to cognitive deficit(s), and preservation of consciousness. Abnormal performance in any cognitive domain was determined by a consistent pattern of impaired performance on neuropsychological measures that load on that cognitive domain. Additionally, these subjects did not meet clinical criteria for dementia with Lewy bodies (13). Those in the PD+Myoclonus group had the clinical and electrophysiologic demonstration of bilateral small amplitude cortical myoclonus as previously described, within 2 years of death, including documentation of a back-averaged pre-myoclonus electroencephalographic (EEG) transient (10). Using CURRY software (Neuroscan, Charlotte, NC, USA), all PD+Myoclonus cases were confirmed to have a primary motor cortex myoclonus source as demonstrated in Figure 1 with EEG dipole source localization and mapping on the averaged MRI within the CURRY program. PD group cases were confirmed not to have myoclonus within 2 years of death. Controls were defined as having the absence of pre-mortem clinical or post-mortem pathological evidence of dementia or movement disorder secondary to neurodegenerative diseases. Control subjects did not have clinical myoclonus and did not received electrophysiological investigation.

Pathology Methods

Standardized neuropathology methods and diagnostic criteria for the SHRI Brain Bank were performed and have been described (11,12,). In brief, dissected brain tissue was frozen as coronal sections (right hemisphere) on dry ice and stored at −80°C. The opposite hemisphere was fixed for 48 hours in 4% paraformaldehyde and sectioned for histological studies. Each brain received a full neuropathological diagnosis by standard criteria for PD and Alzheimer's disease (AD) (4). These procedures included assignment of a Lewy-related pathology (LRP) staging score (1 through 4) using immunohistochemical staining of anti-

alpha synuclein (antibody LB509, Invitrogen, Carlsbad, CA) for each defined region as described in the consensus publication on dementia with Lewy bodies (13). As a measure of global Lewy related pathology, a total staging score was calculated of all regions and also summed separately for the three neocortical regions of frontal, temporal, and parietal. For primary motor cortex, sections were taken from dorsolateral arm area within precentral gyrus. LRP staging, senile plaques and neurofibrillary tangles were also scored in the primary motor cortex (dorsolateral precentral gyrus) with the same staining and scoring methods. We also assign a Unified Staging system score for LB stage which is along the lines of Braak (4).

Protein Studies

Alpha-synuclein and Aβ-42 peptide ELISA—To measure total levels of total α-synuclein and Aβ-42 peptide, samples of primary motor cortex gray matter were extracted in 4 volumes of 5 M guanidine hydrochloride (GHCL) (Thermo Scientific, Pierce) buffered in 50 mM Tris-HCl (pH7.4) to solubilize α-synuclein or Aβ (14). To measure concentrations of total α-synuclein and Aβ-42 peptide, commercial ELISA kits specific for human α-synuclein and Aβ-42 (Invitrogen) were utilized. ELISA was performed according to manufacturer's instructions; results were calculated as pg/mg protein (Aβ-42) or ng/mg protein (α-synuclein).

ELISA for parvalbumin, synaptophysin, and glial fibrillary acidic protein (GFAP)—We developed in our laboratory ELISAs for measuring concentrations of parvalbumin (ng/mg), synaptophysin (ng/mg), and GFAP (μg/mg). For these assays, ELISA plates were coated with the optimal concentration of capture antibody; for synaptophysin, SY17 (IgM monoclonal, Covance) was used; for parvalbumin, affinity purified polyclonal (R&D Systems) was used; for GFAP, a cocktail of 3 monoclonal antibodies (BD Biosciences) was used. Purified proteins for each were available for use in preparation of standard curves (synaptophysin; ABNOVA, Taiwan; parvalbumin – AbCAM, MA; GFAP; Calbiochem, NJ). Samples were prepared by extraction of primary motor cortex gray matter with RIPA buffer. Bound proteins were detected with the appropriate detection antibody (Synaptophysin – rabbit polyclonal, Chemicon; parvalbumin – rabbit polyclonal – AbCAM; GFAP – Calibiochem) and followed by the appropriate horseradish peroxidase labeled secondary antibody. Bound proteins (sample and standards) were detected using tetramethylbenzidine (TMB) peroxidase substrate (R & D Systems). Results were measured using a plate reader (Absorbance, 570 nm) and calculated against the generated standard curves.

Western blots—Western blot analysis of the insoluble fraction of α-synuclein in primary motor cortex gray matter extracts was performed in 6 M urea/2% SDS extracted samples. To measure concentrations of phosphorylated α-synuclein, aliquots of RIPA extracts were dissolved in 4× LDS gel sample buffer, and separated by electrophoresis on 4–12% NuPAGE Bis Tris gels (Invitrogen). The blots of brain samples were probed with an antibody to phosphorylated α-synuclein (p-ser 129), the most abundant form of p-synuclein (15,16). Blots were reprobed with an antibody to unmodified α-synuclein (BD Biosciences). This analysis yielded values from western blot for both phosphorylated (pSyn) and total α-synuclein (tSyn) expressed as a ratio (pSyn:tSyn).

Data Analysis and Statistical methods

The mean and prevalence of characteristics for the PD+Myoclonus, PD, and Control groups were compared. Our hypotheses were related to differences between the PD+Myoclonus and PD groups, where the only difference was the presence of small amplitude cortical myoclonus in the PD+Myoclonus group. The Control group had different exclusions,

especially the presence of dementia and any evidence of neurodegenerative disease, etc. Thus, we designated PD+Myoclonus versus PD group as the primary analysis and all other comparisons secondary, such as PD+Myoclonus versus Control group. Statistical significance was calculated by using the two-sample *t*-test or Fisher exact test. Distribution assumptions were verified by using the permutation test. The effect of characteristics that are observable in living subjects (sex, age, postmortem interval (PMI)), PD duration, Unified Parkinson's Disease Rating Scale (UPDRS) part III (motor score) and Hoehn and Yahr (H&Y) at last clinical assessment, Levodopa (LD) dose equivalents, dementia, neuropsychological test battery variables, and ApoE4 gene status on the relationship between total α-synuclein and the occurrence of cortical myoclonus was assessed by using a general linear model with terms for cortical myoclonus and the covariate. The relationships of different measures with total α-synuclein were quantified by using the Pearson correlation. Significance was set at $P<0.05$.

RESULTS

a) Demographic, clinical and pathological comparisons of disease groups

The clinical and pathological features are presented in Tables 1 and 2. Two subjects did not have a full UPDRS motor score or H&Y staging completed before death. Unavailable samples from certain areas caused N values to be <19 for "Neocortical" LRP Classification (N=18), LRP Stage Total (N=17), and Neocortical LRP Stage Total (N=17). There were no demographic or clinical differences between the groups for gender, age, PD duration, UPDRS motor score, H&Y stages, LD dose equivalents, presence of dementia, and time since electrophysiological study (Table 1). Pathology findings (Table 2), including multiple Lewy-related and AD pathology measures and postmortem interval did not differ between groups. There was little Lewy-related pathology as the highest primary motor cortex stage was only grade 1 (sparse Lewy bodies or Lewy neurites) for any PD+Myoclonus or PD case, with many cases receiving a grade 0 (no Lewy bodies nor Lewy neurites).

b) Protein measurements (Table 3)

There was a 36% increase in total α-synuclein in the PD+Myoclonus group primary motor cortex compared to the PD group (P=0.02), and was also increased compared to the Control group (P=0.02), suggesting that increased α-synuclein may play an important role in the neocortical neuronal dysfunction in primary motor cortex that produces PD+Myoclonus. Based on the relative exposure time for α-synuclein western blots between soluble and insoluble forms, the levels of insoluble α-synuclein in these samples were very low (data not shown). The parvalbumin, synaptophysin, and GFAP values did not differ between groups. There was significantly less Aβ-42 between the Control and PD and PD+Myoclonus groups. Despite a higher nonsignificant mean value for the Aβ-42 peptide in the PD+Myoclonus group, the high variability and very low values in some PD+Myoclonus cases suggests that Aβ-42 and PD+Myoclonus are not consistently linked. There was no difference between the PD and PD+Myoclonus groups for the relative proportion of phosphorylated to total α-synuclein as expressed in a ratio.

Table 4 shows the correlation of total primary motor cortex α-synuclein values with demographic, clinical, pathological, and Apo-E4 findings. Cortical myoclonus had the strongest and the only significant correlation with total α-synuclein. None of the clinical characteristics found during life affected the relationship between cortical myoclonus and total α-synuclein by more than 20%.

DISCUSSION

Our findings indicate that the mean α-synuclein level measured by ELISA is increased 36% in the primary motor cortex of PD+Myoclonus cases when compared to PD without myoclonus subjects. This measure represents the total pool of extractable α-synuclein. It is remarkable that this did not occur with group differences, or with much presence in either group of insoluble α-synuclein, as assessed by western blot analysis and by Lewy body/neurite presence. As the primary motor cortex is the myoclonus source in our PD +Myoclonus cases, our results suggest a possible association between elevated α-synuclein and the dysfunctional physiology arising from this localized area of the cerebral cortex. The association of cortical myoclonus with higher α-synuclein levels is consistent with the concept that abnormal accumulation of α-synuclein (even when not insoluble) is pathogenic in PD. Moreover, higher total α-synuclein levels significantly correlated with PD +Myoclonus but did not correlate with multiple clinical or pathological findings (see table 4).

Other biochemical findings did not differ between the PD+Myoclonus and PD groups. In particular, the loss of important inhibitory influences in the primary motor cortex is a plausible explanation for the increased excitability of primary motor cortex neuronal circuits causing myoclonus, but no group differences in parvalbumin protein suggests that a loss of major inhibitory GABA neurons in the primary motor cortex is not responsible for cortical myoclonus generation in PD+Myoclonus cases. Both basket cells and chandelier cells can be identified with immunocytochemical stains for parvalbumin in mammalian (including primate) motor cortex (DeFelipe, 1998; Porter, 2000). However, parvalbumin staining neurons are known to be more powerful in inhibiting target neurons than calbindin- and calretinin-staining neurons (Thomson, 1997, DeFelipe, 1999).

We suggest that small amplitude cortical myoclonus is appropriate for study as a model of localized PD cortical dysfunction in the primary motor cortex. Autopsy studies of PD-D have shown that the relative proportions of pathological involvement between different cortical areas is not always consistent across all cases (3,4,6–9). For biochemical studies of PD neocortex, the state of “dementia” is commonly used as the independent variable. However, when focal tissue samples are obtained from PD neocortex (e.g. cingulate gyrus, etc.) in demented patients, it is difficult to know whether that focal tissue location is experiencing the same pathological dysfunction that truly represents all cortical areas, especially those affecting the clinical cognitive state. Likewise, it is tricky to assume that “non-dementia” tissue samples are free from localized neocortical dysfunction in PD without having a measure of function for that focal cortical area from which the sample is taken. In this study, the primary motor cortex elevated α-synuclein correlation was significant for the presence of cortical myoclonus but *not* for the more global measure of clinical dementia presence. Measurement of localized neocortical dysfunction through electrophysiological detection of abnormal physiology (e.g. PD+Myoclonus) may provide a more accurate assessment of disease-affected tissue samples than the simple presence or absence of generalized clinical dysfunction (dementia) or global neocortical Lewy-related pathology staging systems.

This study provides verification of small amplitude cortical myoclonus in 11 more cases of pathologically proven idiopathic Lewy body PD (10). Parkinsonism severity and medication doses were not different between the PD and PD+Myoclonus groups, and these results are consistent with our previous report on the clinical characteristics of small amplitude cortical myoclonus in PD (10). Cortical myoclonus is also seen in other disorders that have cortical Lewy bodies, such as dementia with Lewy bodies (DLB) and hereditary Lewy body disease due to α-synuclein triplication (21–23). We have previously demonstrated that PD

+Myoclonus in these cortical Lewy body disorders show identical electrophysiological localization and characteristics to the small amplitude cortical myoclonus in PD (21–23). It is not known whether our present results may be generalized to cases with other cortical Lewy body disorders but this remains an interesting possibility.

There are several findings that suggest cortical myoclonus in PD is not associated with AD pathological changes. Myoclonus is common in AD and has been associated with severe primary motor cortex pathology of AD patients (24,25). Since AD pathology changes in the primary motor cortex seemed a possible explanation for the cortical myoclonus in PD, several variables were examined in the primary motor cortex of our PD groups for this purpose. First, Aβ-42 peptide mean levels did not show a significant increase or difference in PD+Myoclonus subjects. These levels were more variable from case to case in both PD subject groups than the α-synuclein levels. The Control group had less Aβ-42 peptide than either the PD+Myoclonus or PD groups. Second, the presence of senile plaques in the primary motor cortex did not differ between the groups. Third, no neurofibrillary tangles were present in primary motor cortex of any subject. Fourth, synaptophysin which has been found to be reproducibly decreased in AD was not different for protein level in PD +Myoclonus cases. Lastly, most PD+Myoclonus cases did not have histological criteria for AD; nor did they differ in Braak staging for AD. These data therefore suggest that AD pathology is not necessary to produce the physiologic dysfunction of PD+Myoclonus, but given the high Aβ-42 variability found in both PD groups, a possible role for AD pathology in some cases can not be ruled out.

There are limitations of this study. Higher numbers of autopsied subjects may have found differences between PD+Myoclonus and PD groups for more measures. In particular, there were possible trends for the PD+Myoclonus group to be older, have longer duration and more severe PD requiring more medication. More subjects studied may have found these trends to be significant. Such a possible trend may possibly suggest that PD+Myoclonus cases correlate with more severe disease. The monoamines serotonin and dopamine have been implicated in human myoclonus and animal models. Although not examined in this study, markers for these monoamines, and calbindinand calretinin GABA neuron markers, are worthy of study in this PD+Myoclonus model (26,27).

The mechanism by which higher α-synuclein levels could directly cause or be associated with primary motor cortex neuron toxicity is currently unknown. Several mechanisms have been proposed for α-synuclein neuronal toxicity and include membrane disruption; interference with signaling pathways; altering vesicle trafficking; post-translational modification; and others (28–31). Phosphorylation is an example of post-translational modification and has been proposed to be associated with toxicity and α-synuclein aggregation. However, the ratio of phosphorylated to total α-synuclein was not altered in our PD+Myoclonus cases, and other investigators have evidence that phosphorylation is not directly responsible for α-synuclein toxicity (31–33). Membrane disruption of α-synuclein can alter the electrical properties of neurons, causing abnormal neuron firing (21,34–38). This phenomenon in pyramidal neurons could potentially cause the abnormal paroxysmal discharges associated with cortical myoclonus. More research is needed to determine how α-synuclein may be toxic to cortical neuron function.

In summary, small amplitude cortical myoclonus in PD may serve as a useful model of *in vivo* PD cortical dysfunction in humans. This cortical dysfunction is associated with higher total α-synuclein levels rather than measures of insoluble α-synuclein. The mechanism for this dysfunction should have relevance for other cortical areas in PD. Such a model may provide a more specific correlation for studying focal brain tissue sample abnormalities than

generalized dementia. Finally, the clinical biomarker significance of small amplitude cortical myoclonus in PD deserves further study.

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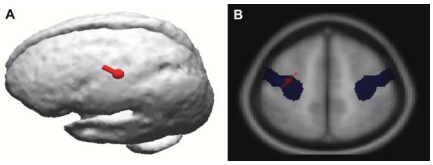

Figure 1.

A-(left) shows dipole localization (red) of the abnormal electrical activity (represented by a dipole) from the cortical myoclonus generation in a PD+Myoclonus subject from the right hand/wrist. This shows that the physiologic abnormality that produces the cortical myoclonus is a highly focal neocortical location. B-(right) shows that the same cortical myoclonus electrical activity (red) also overlaps the Talairach coordinates (blue) of the precentral gyrus (primary motor cortex) on MRI. These data provide evidence that the primary motor cortex is the location of the pathology that produces the cortical myoclonus in PD.

Table 1

Demographic and clinical findings[a]. Total UPDRS and H&Y scores were not assessed for two PD+Myoclonus subjects.

	PD+Myoclonus	PD	Control
Female; n/N (%)	3/11 (27%)	3/8 (38%)	3/9 (33%)
Age (y); mean (SD)	82.6 (6.5)	78.6 (9.0)	85.1 (6.8)
Duration of PD (y); mean (SD)	13.6 (5.1)	10.0 (4.6)	N/A
UPDRS III; mean (SD)	33 (14), N=9	22 (13)	N/A
H&Y; mean (SD)	3.2 (1.1), N=9	2.75 (0.71)	N/A
Levodopa Dose Equivalents (mg); mean (SD)	690 (370)	560 (290)	N/A
Dementia; n/N (%)	6/11 (55%)	5/8 (62%)	0/9
Time since EP (y); mean (SD)	1.48 (0.85)	1.31 (0.47)	1.4 (1.2)
Post-Mortem Interval (hr); mean (SD)	3.4 (2.4)	4.9 (5.6)	2.55 (0.46)

[a]No values were significantly different for PD+Myoclonus versus PD or versus Control for Female predominance, Age, Time since EP, and Post-Mortem Interval; two-sample *t*-test or Fisher exact test. H&Y=Hoehn and Yahr score; EP=electrophysiological study

Table 2

Pathology findings[a]. Unavailable samples from certain areas caused N values to be <19 for "Neocortical" LRP Classification (N=18), LRP Stage Total (N=17), and Neocortical LRP Stage Total (N=17).

	PD+Myoclonus	PD
Primary Motor Cortex LRP non-zero stage; n/N (%)	7/11 (64%)	4/8 (50%)
"Neocortical" LRP Classification; n/N (%)	5/10 (50%)	1/8 (12%)
LRP Stage Total (all areas); mean (SD)	23.4 (5.0)	22.1 (8.4)
Unified LB Stage; mean (SD)	3.00 (0.94)	3.00 (0.76)
Neocortical LRP Stage Total; mean (SD)	3.7 (1.5)	3.6 (3.6)
AD Pathology Criteria; n/N (%)	4/11 (36%)	2/8 (25%)
Braak Stage; mean (SD)	3.33 (0.71)	2.71 (0.76)
Primary Motor Cortex Senile Plaques Score; mean (SD)	1.7 (1.5)	1.0 (1.2)
Primary Motor Cortex Neurofibrillary Tangle Count	0	0

[a]No values were significantly different for PD+Myoclonus versus PD; two-sample *t*-test or Fisher exact test.
LRP=Lewy Related Pathology Stage as defined by McKeith et al. (DLB-III) (13). Neocortical LRP Classification refers to the assignment of "Neocortical" for the Lewy Related Pathology classification as per McKeith et al. (DLB-III) (13). Neocortical LRP Stage Total=Frontal+Temporal +Parietal Stages. AD=Alzheimer's disease. AD pathology refers to the presence of pathological criteria for AD (4).

Table 3

Biochemical results

	PD+Myoclonus Mean (SD)	PD Group Mean (SD)	p*a*	Control Mean (SD)	p*b*
Total α-Synuclein; ng/mg	212 (58)	156 (23)	.02	162 (24)	.02
Parvalbumin; ng/mg	360 (120)	390 (110)	.65	373 (78)	.86
Synaptophysin; ng/mg	390 (170)	340 (120)	.55	330 (170)	.53
GFAP; µg/mg	54 (17)	67 (30)	.24	41 (12)	.16
$A\beta_{42}$; pg/mg	16 (19)	10 (17)	.50	0.7 (1.7)	.03
pSyn:tSyn Ratio	0.155 (0.081)	0.155 (0.093)	>.99	0.071 (0.039)	.02
ApoE ε4 Carrier; n/N (%)	4/11 (36%)	2/8 (25%)	>.99	1/9 (11%)	.09

P^a is for PD+Myoclonus Group versus PD Group,

P^b is for PD+Myoclonus Group versus Control Group; two-sample *t*-test.

Table 4

Correlation for combined PD+Myoclonus and PD Groups of primary motor cortex total α-synuclein with demographic, clinical, pathological, and Apo-ε4 findings. Total UPDRS and H&Y scores were not assessed for two PD+Myoclonus subjects. Unavailable samples from certain areas caused N values to be <19 for "Neocortical" LRP Classification (N=18), LRP Stage Total (N=17), and Neocortical LRP Stage Total (N=17). The correlations are listed in the order of increasing P value for the correlation.

Total α-Synuclein versus	**N**	***r***	**95% CI**	***P***
Cortical Myoclonus presence	19	.53	.10 to .79	.02
Duration of PD	19	.40	−.07 to .72	.09
"Neocortical" LRP Classification	18	.33	−.16 to .69	.19
Female	19	.26	−22 to .64	.28
Primary Motor Cortex LRP Stage	19	−.25	−.63 to .23	.30
AD Pathology Criteria	19	−.25	−.63 to .23	.31
MMSE	19	.27	−.26 to .68	.32
LRP Stage Total (all areas)	17	.23	−.30 to .65	.38
AVLT-LTM	19	.24	−.31 to .67	.38
Dementia	19	−.19	−.59 to .29	.43
UPDRS III	17	.22	−.38 to .69	.47
Neocortical LRP Stage Total	17	.18	−.33 to .61	.48
ApoE ε4	19	.17	−.31 to .58	.48
Trails B	19	.21	−.39 to .68	.49
JLO	19	.26	−.49 to .79	.50
Wais-III Digit Span	19	−.18	−.65 to .39	.54
Hoehn & Yahr Stage	17	.10	−.42 to .57	.71
COWAT	19	.09	−.44 to .58	.74
Stroop Interference	19	.08	−.45 to .57	.78
Post-Mortem Interval	19	−.05	−.49 to .41	.85
Age	19	−.03	−.48 to .43	.91
Braak Score	19	.02	−.48 to .51	.95
Unified LB Stage	18	.01	−.46 to .47	.98
Primary Motor Cortex Senile Plaques score	19	.00	−.45 to .45	.98
Levodopa Dose Equivalents	19	.00	−.45 to .45	.99

LRP=Lewy-Related Pathology Stage as defined by McKeith et al. (DLB-III) (13). Neocortical LRP Classification refers to the assignment of "Neocortical" for the Lewy-Related Pathology classification as per McKeith et al. (DLB-III) (13). Neocortical LRP Stage Total=Frontal+Temporal +Parietal Stages.