

Neuropathological evidence of body-first vs. brain-first Lewy body disease

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ABSTRACT

Aggregation of alpha-synuclein into inclusion bodies, termed Lewy pathology, is a defining feature of Parkinson's disease (PD) and Dementia with Lewy bodies (DLB). In the majority of post mortem cases, the distribution of Lewy pathology seems to follow two overarching patterns: a caudo-rostral pattern with relatively more pathology in the brainstem than in the telencephalon, and an amygdala-centered pattern with the most abundant pathology in the "center of the brain", including the amygdala, entorhinal cortex, and substantia nigra, and relatively less pathology in the lower brainstem and spinal autonomic nuclei. The recent *body-first* versus *brain-first* model of Lewy Body Disorders proposes that the initial pathogenic alpha-synuclein in some patients originates in the enteric nervous system with secondary spreading to the brain; and in other patients originates inside the CNS with secondary spreading to the lower brainstem and peripheral autonomic nervous system. Here, we use two existing post mortem datasets to explore the possibility that clinical body-first and brain-first subtypes are equivalent to the caudo-rostral and amygdala-centered patterns of Lewy pathology seen at post mortem.

1. Introduction

Parkinson's disease (PD) and Dementia with Lewy bodies (DLB) are referred to as Lewy Body Disorders (LBDs). Aggregation of alpha-synuclein into inclusion bodies, termed Lewy pathology, is a defining feature of the LBDs (Spillantini et al., 1997). There is growing evidence that alpha-synuclein behaves in a prion-like manner including neuron-to-neuron propagation of the pathological process (Brundin and Melki, 2017; Goedert et al., 2017; Uchihara and Giasson, 2016). However, the site of origin for the first Lewy pathology in LBDs is a matter of continuous debate. Also, neuronal degeneration in LBDs is not solely the consequence of aggregated alpha-synuclein, but is modified by selective neuronal vulnerability factors (Parkkinen et al., 2011; Surmeier et al., 2017).

The Braak staging system is based on the idea that Lewy pathology starts in the olfactory bulb and enteric nervous system, and invades the brain via the vagus nerve (Braak et al., 2003a; Braak et al., 2003b; Hawkes et al., 2007). Alternative hypotheses propose that Lewy pathology does not start in the gut but inside the central nervous system (CNS), or possibly via an isolated olfactory entry route followed by intra-

cerebral propagation (Adler and Beach, 2016; Beach et al., 2009; Beach et al., 2010; Rey et al., 2013).

We recently proposed a hypothetical *body-first* versus *brain-first* model of LBD, which is essentially a combination of the two above stated hypotheses (Borghammer, 2021; Borghammer and Van Den Berge, 2019; Horsager et al., 2020). The differences between the body- and brain-first subtypes with regard to clinical and imaging findings are summarized in Table 1. This model suggests that the enteric or peripheral autonomic nervous system is a high-probability location for the appearance of the first alpha-synuclein pathology. Subsequent propagation to the CNS via the vagus and, importantly, also via a sympathetic spreading route to the sympathetic trunk and heart, then produces a body-first subtype. This concept is equivalent to Braak's hypothesis. However, in other patients, the first pathology instead appears in structures inside the CNS without prior involvement of the autonomic nervous system. It is suggested that the most probable "brain-first" locations are the amygdala, followed by the substantia nigra (SN), and locus coeruleus (LC). In some patients, these structures may be affected secondary to a primary "olfactory bulb" site of origin.

Here, we discuss human post mortem neuropathological data in

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Table 1
Differences between the hypothesized body-first and brain-first subtypes of Lewy body disorders.

	Body-first subtype	Brain-first subtype
Autonomic symptoms	Appears in prodromal phase	Appears after diagnosis
Sympathetic denervation	Occurs in prodromal phase	Occurs after diagnosis
Parasympathetic denervation	Occurs in prodromal phase	Occurs after diagnosis
Locus coeruleus degeneration	Occurs in prodromal phase	Occurs after diagnosis
REM sleep behavior disorder	Appears in prodromal phase	Appears after diagnosis
Hyposmia at time of diagnosis	Very frequent	Somewhat less frequent
Progression rate to dementia	Faster	Slower
Motor symptom progression	Faster	Slower
Motor symptoms at diagnosis	More symmetrical	More asymmetrical
Dopaminergic denervation at diagnosis	More symmetrical	More asymmetrical

Summary of clinical, imaging, and other findings in the hypothesized body- and brain-first subtypes, as previously reviewed (Borghammer, 2021; Borghammer and Van Den Berge, 2019; Horsager et al., 2020). Note that the table specifies group-averages, and that some individual patients may show variations.

relation to the brain-first versus body-first hypothesis of LBDs. Using two existing post mortem datasets (Raunio et al., 2019; Tanei et al., 2021), we argue that the distribution of Lewy pathology in the large majority of post mortem cases seem to follow two overarching patterns, a caudo-rostral pattern and an amygdala-centered pattern. The implications of these patterns for our fundamental understanding of LBDs is discussed, with special emphasis on where the initial Lewy pathology may originate.

2. Current Lewy pathology staging systems

The Braak staging system assigns cases to six stages based on the presence of Lewy pathology in the medulla oblongata (I), pons (II), mesencephalon (III), amygdala and other limbic structures (IV), association neocortex (V), and first order sensory association neocortex (VI) (Braak et al., 2003a). The system assumes that cross-sectional neuropathological data can indicate a temporal sequence of events. In other words that cases with early incidental Lewy body disease (ILBD) would have progressed to higher Braak stages and possibly symptomatic disease, had they lived longer. This assumption is supported by the observation that ILBD patients show decreased tyrosine hydroxylase intensity in the striatum and epicardial nerves, and reduced neuron counts in the dorsal motor nucleus of the vagus (DMV), LC, and SN (Dickson et al., 2008).

In the Braak system, it was originally argued that the crucial *intracerebral* starting point is the DMV, whereas early pathology simultaneously present in the olfactory bulb was thought to be of less importance (Braak et al., 2003a). It is also suggested that progressive Lewy pathology leads to the appearance of *gradients* of pathology with most extensive pathology in early stage structures and progressively less pathology in the higher stage structures. Thus, when a later stage structure (e.g. SN) shows initial mild pathology, the earlier stage structures (e.g. DMV and LC) shows more pathology.

Only a few years after its publication, studies showed that the Braak staging system was not valid for all patients. For example, some early ILBD patients did not show pathology in the DMV, whereas they had clear pathology in other regions, including in the LC, SN, or the amygdala (Jellinger, 2019; Kalaitzakis et al., 2008; Parkkinen et al., 2008). By definition, such cases cannot be staged by the Braak staging system.

Another commonly applied system for Lewy pathology is the DLB consortium staging protocol (McKeith et al., 2017; McKeith et al., 2005). In the latest iteration of this system, cases are categorized into olfactory

bulb-only, amygdala-predominant, brainstem-predominant, limbic transitional, and diffuse neocortical stages.

A potential problem with both the Braak and DLB consortium staging systems is that relatively little emphasis is put on the magnitude of Lewy pathology in a given region. Assignment of a case to a specific category or stage is based mainly on the spatial distribution of pathology. Thus, two patients who display Lewy pathology in the same anatomical regions, but who have quite different levels of pathology in those regions, will nevertheless be labeled with the same Braak or DLB consortium stage. Fig. 1 shows two such cases with prototypical profiles of caudo-rostral and amygdala-centered Lewy pathology (Raunio et al., 2019). Such cases will commonly be assigned identical Braak or DLB consortium stages despite having quite different levels of pathology in the affected regions. Thus, important information may be lost when using these staging systems.

A third system, the Unified staging system for LBDs, is similar to the DLB consortium system, but applies a more refined semiquantitative grading to assign cases to a brainstem-predominant, limbic-predominant, and combined brainstem/limbic category (Beach et al., 2009). This system is better able to assign cases to categories characterized by predominant brainstem or limbic pathology, respectively.

3. Caudo-rostral and amygdala-centered patterns

A recent paper analyzed 124 Lewy pathology-positive post mortem cases in a population-based sample of subjects aged over 85 years (Raunio et al., 2019). The authors showed that approximately two-thirds of the cases conformed to a caudo-rostral pattern with relatively more pathology in the brainstem than in the telencephalon. The remaining one-third showed an amygdala-centered pattern with a peak of pathology in the “center of the brain”, including the amygdala, entorhinal cortex, and SN, and then relatively less pathology in the lower brainstem, spinal intermediolateral column (IML), and also in neocortical regions.

Here, we take a closer look at this dataset to emphasize some important aspects. Importantly, the authors of this study staged the severity of Lewy pathology on a 4-point scale (1 = mild pathology, 4 = very severe) across all 12 anatomical regions: sacral spinal cord (Sp-Sa), thoracic spinal cord (IML), DMV, LC, SN, amygdala (Amy), hippocampus CA2, transentorhinal cortex (Tox), cingulum (Cin), and temporal (Tem), frontal (Fro), and parietal (Par) neocortex.

First, we calculated a *global burden score* (GBS) for each of the 124 patients by summing the Lewy pathology scores from the 12 regions. Thus, patients had GBS scores from 1 to 48. We then divided patients into 4 categories based on their GBS, i.e. mild global pathology (GBS 1–10, *n* = 37), moderate global pathology (GBS 11–20, *n* = 27), severe global pathology (GBS 21–30, *n* = 26), and very severe global pathology (GBS > 30, *n* = 34). Second, for each patient we calculated a simple metric to determine whether the magnitude of pathology was greatest in the autonomic structures or in the center of the brain. If the averaged pathology in the thoracic IML and DMV was greater than the averaged amygdala and transentorhinal cortex scores, the subject was designated as *caudo-rostral* (*n* = 60). Conversely, *amygdala-centered* cases were defined by having more pathology in the amygdala and transentorhinal cortex than in the autonomic structures (*n* = 45). Finally, 19 cases were not classifiable by this simple metric since their average pathology in the two locations was equal.

The average profiles in the caudo-rostral and amygdala-centered groups are shown in Fig. 2. Importantly, two different geometric shapes are clearly discernible at all four levels of global pathology: a left-skewed wedge shape in the caudo-rostral groups, and a more symmetrical, triangular peak shape in the amygdala-centered groups. Despite the obvious differences in profiles, most cases in the severe and very severe GBS categories would be designated limbic or diffuse neocortical cases in the DLB staging system, or as stage 5–6 in the Braak system. The different profiles of pathology are therefore not captured by these two

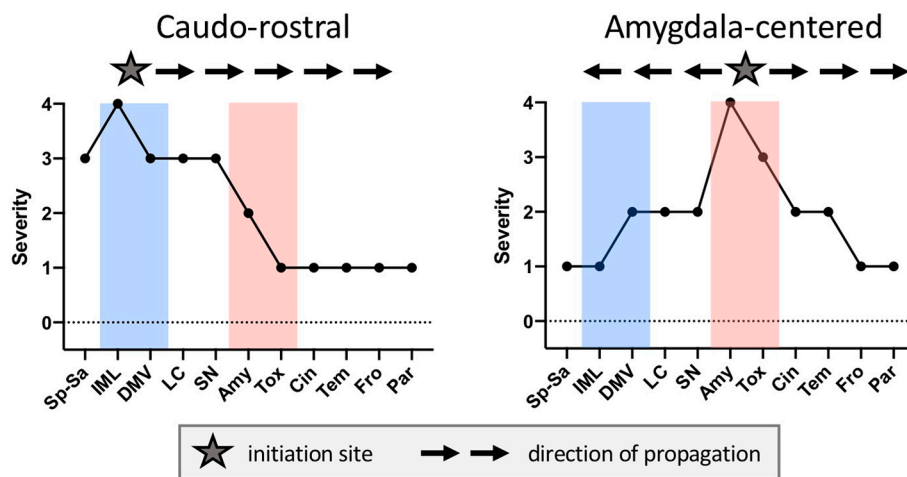


Fig. 1. Two examples of Lewy pathology positive patients from the population-based Vantaa85+ study (Raunio et al., 2019). The x-axis lists anatomical regions, and the y-axis shows the magnitude of Lewy pathology (1 = mild, 4 = very severe). Despite the substantially different profiles of the magnitude of Lewy pathology, such patients are often labeled with identical Braak and DLB consortium stages. Blue squares designate autonomic nuclei and red squares the “amygdala-centered” structures. Hypothetical Lewy pathology initiation sites are indicated by stars and the propagation direction by arrows. [Sp-Sa = sacral cord, IML = intermediolateral column, DMV = dorsal motor nucleus of vagus, LC = locus coeruleus, SN = substantia nigra, Amy = amygdala, Tox = transentorhinal cortex, Cin = cingulum, Tem = temporal cortex, Fro = frontal cortex, Par = parietal cortex]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

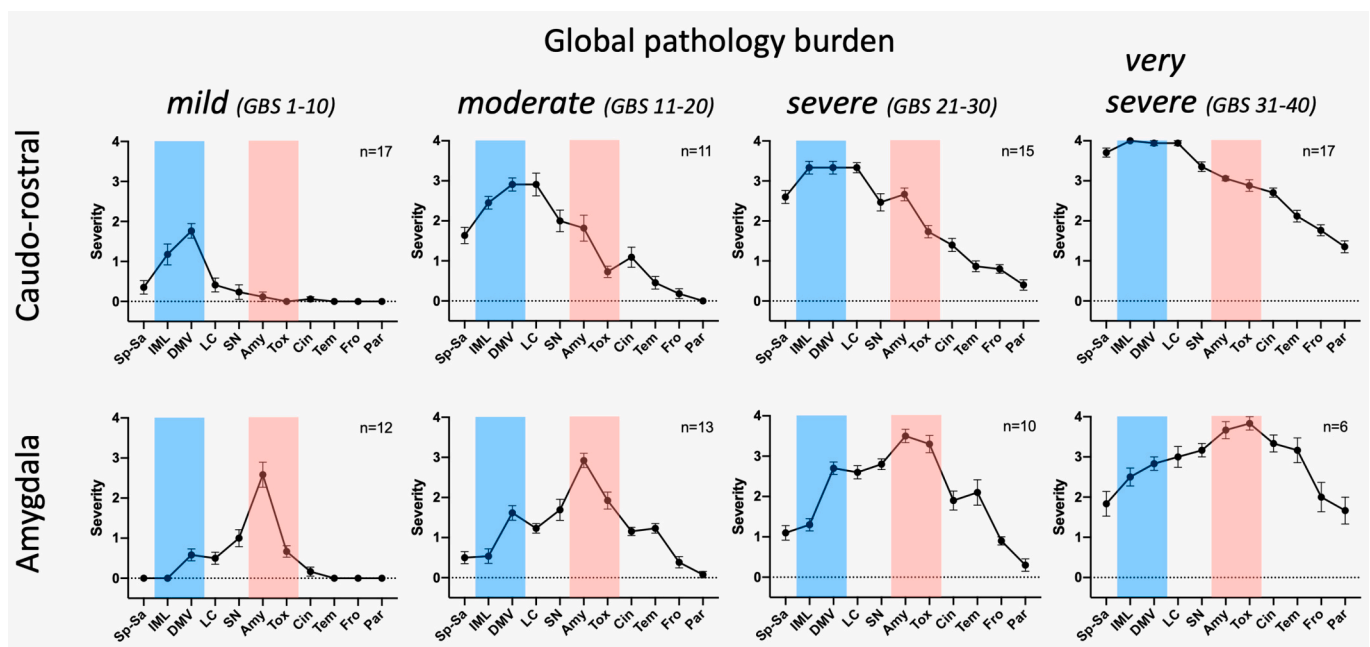


Fig. 2. Profiles of the severity of Lewy pathology (0 = negative, 4 = very severe) in groups of patients divided into four categories based on their Global pathology Burden Score (GBS). The top row shows patients with a caudo-rostral profile and the bottom row patients with amygdala-centered profiles. Note that in this dataset the two profiles are well defined across all levels of global pathology. [data from Raunio et al., 2019, abbreviations in Fig. 1 legend].

staging systems. This could have important implications for clinical and basic research in which tissue is used from deceased patients with LBDs.

It could be reasoned that the two profiles shown in Fig. 2 are artefacts - or a self-fulfilling prophecy - caused by the simple algorithm we used to categorize patients into the two categories. However, an unsupervised K-means cluster analysis clearly reproduced the same two profile patterns (Raunio et al., 2019). Fig. 3A shows nine clusters produced by the K-means cluster analysis of 123 subjects (one patient was excluded). Most of the clusters show either a caudo-rostral profile or an amygdala-centered profile. Note that the profiles in Figs. 2 and 3 are very similar but not identical. Fig. 3B shows the two dimensions of the K-means cluster analysis with the highest degree of explanatory power. Dimension 1 (x-axis) clearly reflects the global Lewy body burden. Dimension 2 (y-axis) seems to reflect a spectrum going from a caudo-rostral profile (positive weights) to an amygdala-centered profile (negative weights).

3.1. Basic principles

It is tempting to speculate that the profiles depicted in Figs. 2 and 3 represent evolving Lewy pathology over time in accordance with three basic principles:

- (1) The first pathogenic alpha-synuclein arises stochastically and amplifies inside a single neuron (*neuron zero*).
- (2) The pathology spreads to connected neurons, which are permissible to the formation of alpha-synuclein aggregates. Some neurons have little or no propensity to form Lewy pathology.
- (3) The burden of Lewy pathology in a given anatomical structure increases over time for at least two reasons. First, it has been speculated that formation of fully mature inclusion bodies takes several years (Fares et al., 2021). Second, once a nucleus is affected by Lewy pathology, local spreading to neighboring neurons inside that nucleus is probably relatively rapid. Both

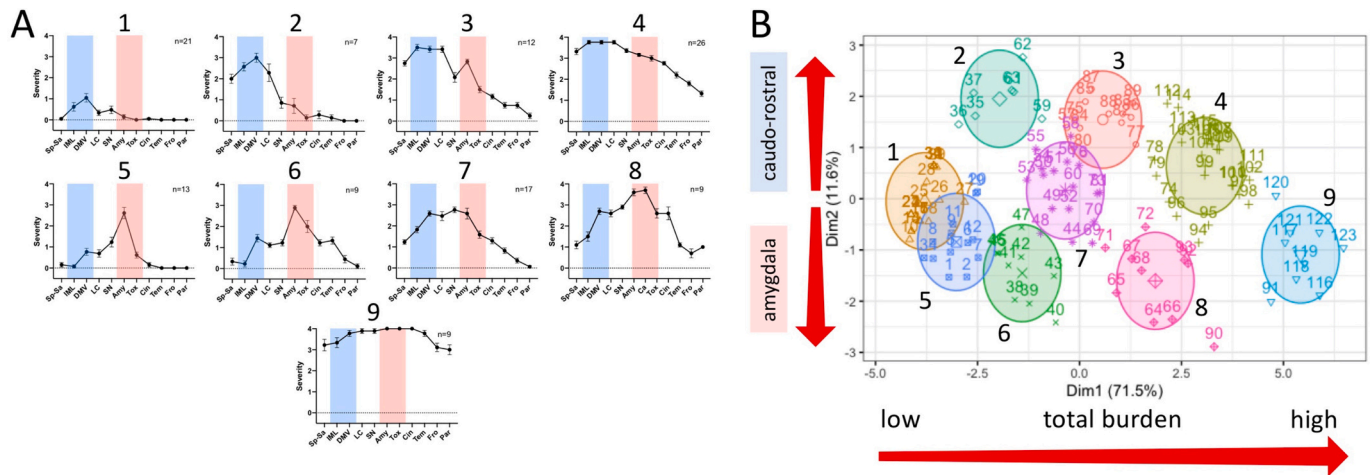


Fig. 3. A. Nine clusters derived from a K-means cluster analyses of the 123 subjects (1 subject was excluded since all regions had a Lewy pathology score of 4). Clusters 1, 2, 3, and 4 show a caudo-rostral profile, and clusters 5, 6, and 8 show clear amygdala-centered profile. Cluster 7 seems to be a mix of both types, and cluster 9 comprise subjects with very severe pathology, which nevertheless seem to resemble more the amygdala-centered profile. B. The plot displays the two dimensions with the highest degree of explanatory power. It is evident that dimension 1 (x-axis) reflects the global Lewy body burden. Dimension 2 (y-axis) seems to reflect a spectrum going from a caudo-rostral profile (positive weights) to an amygdala-centered profile (negative weights). Note that the “mixed cluster 7” is centered in the middle of the plot with an average dimension 2 weight of approximately zero.

phenomena would translate into progressively higher Lewy pathology scores in a nucleus over time.

Of note, a large body of evidence from experimental animal models strongly support that alpha-synuclein pathology behaves in this fashion (Ayers et al., 2017; Breid et al., 2016; Holmqvist et al., 2014; Kim et al., 2019; Van Den Berge et al., 2019; Van Den Berge et al., 2021). In addition, although a specific alpha-synuclein PET tracer has not yet been developed, it is interesting to reflect on evidence from the Alzheimer's disease imaging field. Here, it has been demonstrated in longitudinal PET studies that both beta-amyloid and pathological tau protein shows predictable progressive accumulation in patterns, which closely mirror the cross-sectional neuropathological data and staging systems (Collij et al., 2020; Franzmeier et al., 2020; Ismail et al., 2020; Jagust et al., 2021; Jelistratova et al., 2020; Zammit et al., 2021). Therefore, it seems probable that Lewy pathology would also behave in this fashion providing support to the idea that ILBD cases with relatively little Lewy pathology are in the early, prodromal phase of the disease.

If the three principles mentioned above are correct, it leads to some interesting implications.

First, it seems probable that regions showing the most severe pathology were also the earliest affected regions in that particular patient. This would point to the autonomic nuclei (DMV and IML) as the earliest affected CNS locations in patients with the caudo-rostral profiles. Moreover, since the nerve terminals of these structures are located in the peripheral nervous system, including the gut, this also supports the dual-hit hypothesis. In short, it suggests that the first pathogenic alpha-synuclein is formed outside the brain in patients with caudo-rostral profiles (Hawkes et al., 2007).

In patients with an amygdala-centered profile, it is probable that the earliest affected intra-cerebral structures are the amygdala, transentorhinal cortex, or closely connected structures. In rarer cases, the SN or LC may be the first affected structures. This interpretation is supported by examining data from ILBD patients, in whom only one single structure contained Lewy pathology. Here, the most common sites of single-location Lewy pathology are the olfactory bulb, amygdala, DMV, sympathetic ganglia, thoracic IML, SN, and LC (Adler and Beach, 2016; Beach et al., 2009; Beach et al., 2010; Miki et al., 2009; Parkkinen et al., 2008; Raunio et al., 2019; Saito et al., 2004; Sumikura et al., 2015; Tanei et al., 2021).

It has been suggested that isolated Lewy pathology can arise in the

olfactory bulb from where it then spreads to the limbic system including the amygdala (Beach et al., 2009; Saito et al., 2016; Sengoku et al., 2008). It is possible that this spreading route may account for some or perhaps even the majority of amygdala-centered cases. In support, data from the Vantaa85+ cohort recently showed that Lewy pathology in the olfactory bulbs/peduncles is strongly associated with Lewy pathology elsewhere in the brain, and that olfactory bulb Lewy pathology seem to exhibit two divergent patterns, consistent with the *body-first* versus *brain-first model* (Kok et al., 2021). The reader is referred to a recently published detailed discussion on the importance of the olfactory bulb in the context of LBDs (Borghammer, 2021).

3.2. Gradients

Based on the three basic principles, one would also expect to see stereotypical gradients of pathology and, indeed, most patients do show graded Lewy pathology. If a region, such as the DMV, shows severe pathology, then the LC also tends to contain marked pathology, whereas more distant regions show poorer correlation with the lower brainstem (Kingsbury et al., 2010). The presence of gradients supports that neuron-to-neuron spreading of alpha-synuclein is an important pathogenic mechanism in LBDs. Alternative theories, such as the threshold hypothesis or theories proposing that LBDs are caused by pathogenic factors crossing the blood-brain barrier, seem rather more difficult to reconcile with the presence of gradients in most patients (Engelender and Isacson, 2017).

To further test the hypothesis that strongly connected regions tend to correlate more closely, we compared the magnitude of Lewy pathology in regions, which were not used in the categorization itself. We compared the magnitude of sacral pathology to the average pathology of the cingulum and temporal cortex. In other words, we compared regions located at opposite ends of the neuro-axis. Fig. 4 demonstrates that most caudo-rostral patients had more pathology in the sacral spinal cord than they had in the cingulum and temporal cortex. In contrast, most amygdala-centered patients had more pathology in the cingulum and temporal cortex than in the sacral spinal cord. If Lewy pathology did not spread primarily to neighboring (i.e. most connected) neurons, this finding would not be expected.

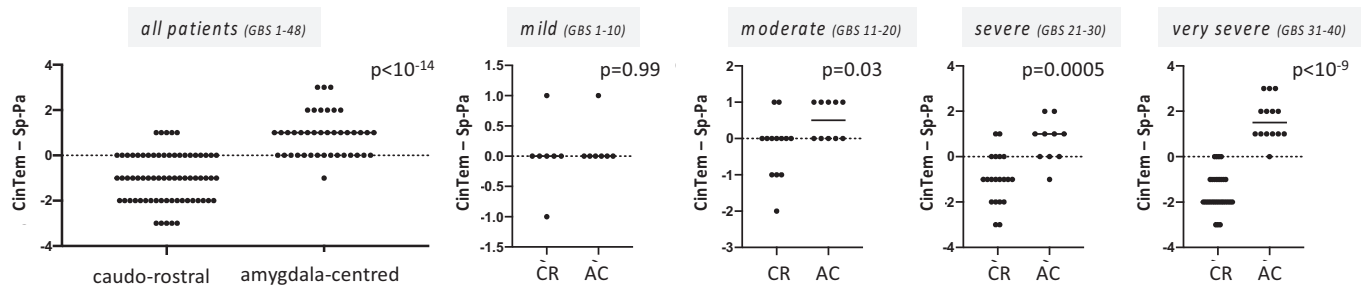


Fig. 4. The difference between average cingulum and temporal (CinTem) pathology and sacral spinal pathology (Sp-Pa) pathology was compared in caudo-rostral (CR) vs. amygdala-centered (AC) patients of the population-based Vantaa85+ study (Raunio et al., 2019). The majority of caudo-rostral patients had more pathology in the sacral spinal cord, whereas most amygdala-centered patients had more pathology in the cingulum and temporal cortex. Note that most patients in the GBS mild and moderate categories had pathology in neither structure, explaining the many patients with a difference value of zero. However, once pathology reaches these structures (in the severe and very severe GBS categories) the pattern is extremely robust. [p-values from Mann-Whitney U tests].

3.3. Unclassifiable cases

As mentioned above, 19 cases were not classifiable by the simple categorization. These cases comprised three types (Supplementary Fig. S1). One non-classifiable group comprised very late stage cases, where nearly all structures had been saturated with pathology. A small group of three cases were intermediate. Finally, a group of 8 cases had very little pathology overall, and this pathology was present primarily in the SN and/or LC. These cases suggest that the LC and SN can infrequently be sites of origin of brain-first Lewy pathology as discussed above.

Alternatively, Lewy pathology could sometimes be transient in the DMV. In other words, pathology could have reached the LC and SN via the DMV, but pathology in the DMV itself might have disappeared again. This phenomenon has been observed in some animal models of PD (Challis et al., 2020; Kim et al., 2019; Manfredsson et al., 2018; Patterson et al., 2019; Uemura et al., 2018; Van Den Berge et al., 2021). However, such transient DMV pathology has only been seen in a minority of animal models, so additional research is needed to clarify potential implications for human LBDs.

4. Possible relation to in vivo imaging data

Using in vivo multi-modal imaging, we recently showed that patients with prodromal and de novo PD can be categorized into two groups, and we hypothesized that these groups reflect brain-first and body-first sites of origin for Lewy pathology (Horsager et al., 2020). Isolated REM sleep behavior disorder (iRBD) is a prominent prodromal feature in nearly half of LBD cases (Postuma et al., 2019; Zhang et al., 2017), and is believed to be caused by damage to pontine nuclei, i.e. Braak stage II structures below the dopamine system (Boeve, 2013). Therefore, RBD should theoretically emerge before parkinsonism in body-first patients, in whom the pons is affected prior to the mesencephalon. In contrast, brain-first patients in whom the amygdala may often be the first affected region, RBD should appear after parkinsonism, if at all (Borghammer, 2021).

Fig. 5 shows imaging data of the dopamine system assessed by [18 F] FDOPA PET and of the cardiac sympathetic innervation measured with [123 I]MIBG scintigraphy in patients with iRBD, and de novo PD patients with and without RBD. It can be seen that PD patients without RBD (red data) commonly show damage to the dopamine system prior to sympathetic cardiac denervation. In contrast, patients who developed RBD in the prodromal stage (blue data) shows sympathetic cardiac denervation before the dopamine system is damaged. This is compatible with brain- and body-first trajectories of disease progression. Fig. 5 illustrates how the distribution of progressively accumulating Lewy pathology might theoretically relate to the in vivo imaging data. In short, patients with a caudo-rostral profile show earlier and more marked pathology in the sympathetic IML and ganglia compared to the SN - compatible with the in vivo evidence of cardiac sympathetic denervation before

dopamine loss in some patients. The opposite pattern is seen in amygdala-centered patients, which is compatible with a brain-first etiology and with the observed dopaminergic denervation prior to cardiac denervation in other patients.

5. Variable assessment of Lewy pathology

The rating of Lewy pathology at post mortem is most commonly based on 3- or 4-point semiquantitative assessment into categories of mild, moderate, severe, and very severe pathology, as suggested by the Braak PD and DLB staging systems (Braak et al., 2003a; McKeith et al., 2005). However, there is no clear consensus on exact criteria for these categories across staging systems, and variations occur in different studies (Beach et al., 2009; Braak et al., 2003b; Parkkinen et al., 2008; Raunio et al., 2019; Sumikura et al., 2015; Tanei et al., 2021; Toledo et al., 2016). The DLB consortium system, used by many studies, employs a 0–3 rating in the brainstem but a 0–4 rating scale in limbic and cortical structures (McKeith et al., 2005). Thus, brainstem structures have a maximum Lewy pathology score of 3, whereas the amygdala and transentorhinal cortex have a maximum score of 4. It is common to see amygdala scores of 4 in cases with only a moderate global burden of Lewy pathology and amygdala scores of 4 is given in the vast majority of cases with a high global burden (Parkkinen et al., 2008; Tanei et al., 2021). In addition, progressive neuron loss may lead to a ceiling effect in some vulnerable structures (e.g. SN, LC, and sympathetic structures), since the absolute amount of Lewy pathology is constrained by the density of remaining neurons and terminals (Braak and Del Tredici, 2008; Orimo et al., 2008). For these reasons, a simple categorization based on comparison of autonomic nuclei and the amygdala might in some cohorts only work for cases with mild-to-moderate global burdens of pathology.

5.1. A second post mortem data set

To exemplify this problem, we examined another data set in which the individual Lewy pathology scores for 178 patients were published in full (Tanei et al., 2021). In this study, the authors utilized the DLB consortium staging criteria for scoring Lewy pathology (brainstem: 0–3, limbic: 0–4). We applied the same method of categorization into caudo-rostral and amygdala-centered profiles, except that the average autonomic pathology was based on the DMV and sympathetic ganglia, since the IML was not assessed in this dataset. Ninety cases (51%) were categorized as amygdala-centered, 55 (31%) cases as caudo-rostral, and 33 cases (18%) were indeterminate, as they had identical average pathology scores in the autonomic structures and the amygdala and transentorhinal cortex (Fig. 6). Since the amygdala and transentorhinal cortex can have a maximum score of 4, whereas brainstem structures have a maximum score of 3, our simple categorization system will be biased in cases with high GBS scores and favor the amygdala-centered

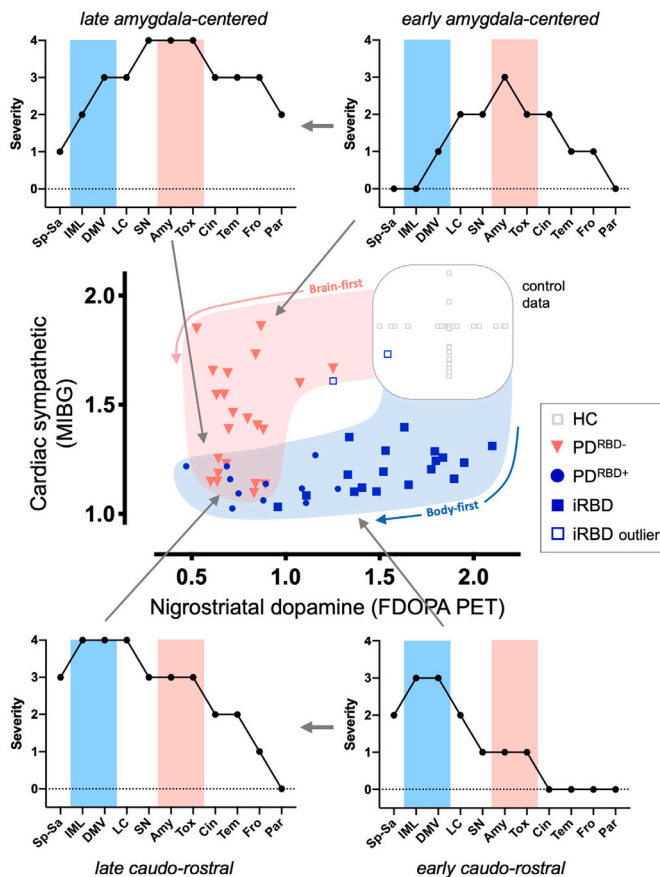


Fig. 5. The central plot depicts imaging data of the nigrostriatal dopamine system (FDOPA PET) and the cardiac sympathetic innervation (MIBG scintigraphy) in patients with iRBD, de novo PD with RBD (PD^{+RBD}) and without RBD (PD^{-RBD}), and healthy controls (HC) (from (Horsager et al., 2020)). PD patients without RBD (red triangles) seem to follow a *brain-first* trajectory characterized by dopamine loss prior to sympathetic denervation. Patients with RBD (blue squares and circles) follow a *body-first* trajectory with initial sympathetic denervation before dopamine loss. The figure illustrates how caudo-rostral and amygdala-centered profiles of Lewy pathology may theoretically reflect these in vivo imaging data. The caudo-rostral patients show earlier and more severe pathology in the sympathetic IML and ganglia compared to the SN. This would explain why the cardiac sympathetic scans become pathological before the dopamine brain scans. The opposite pattern is seen in amygdala-centered patients. Note that longitudinal imaging studies have shown that all PD patients eventually develop severely pathological FDOPA and MIBG scans, emphasizing that all patients converge on a homogeneous end-stage condition. This is compatible with the concept that the entire CNS and autonomic nervous system eventually become saturated with pathology as suggested by Fig. 2 (very severe GBS stage). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

category. Indeed, only a single case was designated as caudo-rostral in the very severe GBS category, since all those cases had amygdala scores of 4. But importantly, in the mild and moderate GBS categories the caudo-rostral and amygdala-centered profiles were still clearly discernible.

These strikingly different profiles in the mild and moderate GBS categories support that early, prodromal stages of Lewy pathology mainly fall into these two categories - irrespective of variations in how different staging systems or neuropathologists rate the Lewy pathology. By extension, this suggests that later-stage cases, where the profiles were less clear in this second dataset, have probably developed from either of these two initial starting conditions. This is again compatible with the idea that LBDs can start either in the peripheral autonomic nervous system or in the amygdala and/or olfactory bulb, but that both routes

converge on a more homogeneous late-stage condition.

The final insight from this second dataset relates to Lewy pathology in the esophagus, which was specifically studied by the authors of that study. Fig. 7 shows the number of cases displaying Lewy pathology in the esophagus in each of the eight groups depicted in Fig. 6. Esophagus pathology was much less common in cases with the amygdala-centered profile. Not a single amygdala-centered patient in the mild and moderate GBS groups had any detectable pathology in the esophagus. By contrast, in the caudo-rostral group, 14% of cases in the mild GBS group and 69% in the moderate GBS group displayed esophageal Lewy pathology. Importantly, this marked overrepresentation of esophagus pathology in the caudo-rostral groups was seen despite the fact that the caudo-rostral groups had a similar or lower average Braak stage than those of the amygdala-centered groups - see table in Fig. 7.

Note that this esophageal pathology is probably caused mainly by *anterograde* spreading from the DMV, once this nucleus becomes sufficiently affected (Arotcarena et al., 2020; Ulusoy et al., 2017; Van Den Berge et al., 2019; Van Den Berge et al., 2021). The lower esophagus and stomach receives the highest density of DMV motor efferents (Berthoud et al., 1991). Thus, esophageal pathology will therefore also develop in amygdala-centered cases once the bottom of the brainstem including the DMV is reached by the top-down spreading of alpha-synuclein aggregates. Finally, the esophagus data provides another confirmation that pathology in closely connected regions correlate more closely as was illustrated in Fig. 4.

6. Can Lewy pathology originate in the peripheral nervous system?

The presence of Lewy pathology in the peripheral autonomic nervous system was first reported in 1928, only a few years after Friedrich Lewy had made the original discovery (Wohllwill, 1928). However, not much attention was paid to this peripheral pathology throughout the 20th century. It is now known that aggregates of pathological alpha-synuclein can probably be detected in all organs receiving autonomic innervation (Beach et al., 2010; den and Bethlem den Hartog and Bethlem, 1960; Donadio et al., 2016; Vilas et al., 2016; Wakabayashi et al., 1988).

However, despite the clear evidence that Lewy pathology is widespread in autonomic nerves, it has not been definitively proven that the pathology actually starts there. Supportive evidence comes from the observation that full truncal vagotomy seems to protect against PD (Liu et al., 2017; Svensson et al., 2015). Also, animal studies have provided mechanistic evidence by showing that injections of alpha-synuclein seeds into the gut wall is followed by gut-to-brain propagation of pathology via the parasympathetic and sympathetic nervous system (Ayers et al., 2017; Breid et al., 2016; Holmqvist et al., 2014; Kim et al., 2019; Van Den Berge et al., 2019). Importantly, we recently showed that robust gut-to-brain spreading of alpha-synuclein was only seen in old wildtype rats but not in young rats (Van Den Berge et al., 2021). This observation resonates well with the fact that LBDs mainly afflict elderly people and points to the importance of senescence factors. It also suggests that alpha-synuclein seeding studies, which showed mostly negative results in young animals, might have shown positive results in aged animals (Manfredsson et al., 2018; Uemura et al., 2018).

Importantly, there is currently no consensus on how to define pathological alpha-synuclein and this is especially true for the peripheral nervous system. Most histology studies have applied immunohistochemistry and used antibodies targeting total or hyperphosphorylated alpha-synuclein. These methods are generally validated with respect to fully mature pathology, i.e. Lewy bodies and neurites found in the brainstem. However, it seems probable that the very first pathogenic alpha-synuclein species would consist of oligomeric immature aggregates with fewer post-translational modifications including less hyperphosphorylation. Such aggregates might be invisible to immunohistochemistry protocols validated on mature brainstem pathology. Indeed, studies using alternative methodologies including real-time quaking-

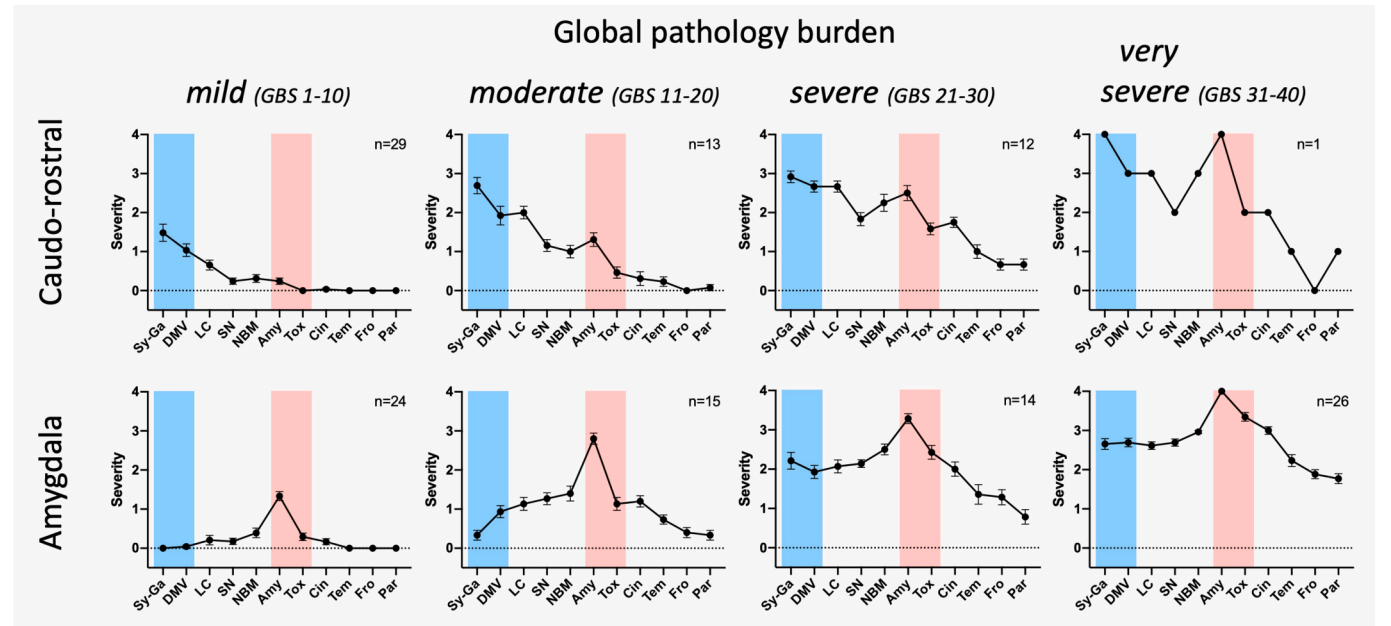
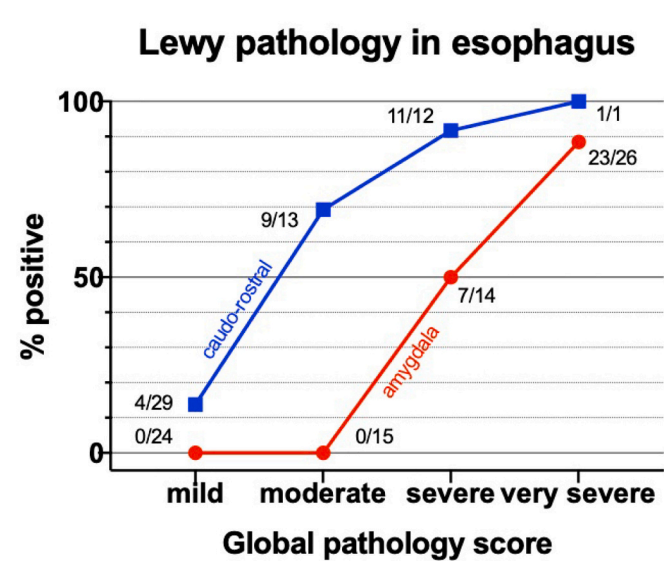


Fig. 6. Caudo-rostral and amygdala-centered profiles in patients from a Japanese cohort of Lewy pathology positive cases (Tanei et al., 2021). The caudo-rostral and amygdala-centered profiles are well defined in the mild and moderate global pathology burden groups, but tend to converge on a more common profile in the severe global pathology group. This convergence is caused by different thresholds for defining very severe pathology. No cases in this dataset had a score of 4 in brainstem nuclei, whereas most cases in the severe category and all cases in the very severe category had a score of 4 in the amygdala.



	mild	moderate	severe	very severe
Caudo-rostral	1.6	2.9	3.6	5
Amygdala	1.6	3.1	4.5	5.5

Average Braak stage

Fig. 7. Esophagus Lewy pathology in cases with caudo-rostral (blue) and amygdala-centered (red) profiles sorted according to their global Lewy pathology scores. The fraction of positive-to-total cases is provided for each group. The table shows the average Braak stage in each of the eight groups. Note that the caudo-rostral groups had much more frequent esophagus Lewy pathology, despite those groups having a similar or lower average Braak stage. [Data from (Tanei et al., 2021)]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

induced conversion (RT-QuIC) or protein misfolding cyclic amplification (PMCA) have clearly demonstrated that brain regions, which are negative on classical immunohistochemistry assessments nevertheless contain alpha-synuclein seeds, which can be amplified into pathogenic species (Fenyl et al., 2021). Therefore, studies reporting a lack of classical alpha-synuclein inclusions in the CNS or peripheral nervous system cannot automatically be taken as evidence that such cases would have negative seeding capacity. The formation of pathogenic alpha-synuclein might, during the earliest stages, require more sensitive methods of detection than classical immunohistochemistry.

There are some studies, which have reported the presence of pathological alpha-synuclein in the colon and appendix of otherwise healthy adults, and in PD patients up to 20 years before diagnosis (Bottner et al., 2012; Hilton et al., 2014; Killinger et al., 2018; Ruffmann and Parkkinen, 2016; Shannon et al., 2012; Stockholm et al., 2016). Moreover, reversible aggregates of alpha-synuclein are seen in the gut of children with norovirus infections (Stolzenberg et al., 2017). Several studies have reported cases in whom only the sympathetic ganglia contain Lewy pathology, while the entire CNS was negative (Miki et al., 2009; Sumikura et al., 2015; Tanei et al., 2021). This would suggest that enteric pathology sometimes spreads initially via the sympathetic system before the DMV is affected. We recently showed that this sympathetic spreading route propagates alpha-synuclein pathology very efficiently in rats injected with alpha-synuclein seeds in the duodenal wall (Van Den Berge et al., 2019). Taken together, all of this data provide support for the existence of “gut-first” LBD.

In contrast, observations from the Arizona PD consortium seem to provide evidence against a gut-start in the LBDs (Adler and Beach, 2016; Beach et al., 2015), as they found not a single case with “gut-only” pathology in more than 600 whole-body autopsies. This observation has to our knowledge only been reported in review papers (Adler and Beach, 2016), and the full methodology and data has yet to be published. It is important to carefully consider what can and cannot be concluded from this impressive dataset.

The negative findings in this large, well-characterized cohort can be taken as strong evidence that PD patients do not develop *widespread, mature Lewy pathology* in large parts of the gastrointestinal tract before

the appearance of the first CNS pathology. On the other, the study in no way rules out that the first pathology nevertheless starts in the gut. There are at least three important arguments for this claim.

The first argument concerns methodology and detection sensitivity. The Arizona cohort is examined with immunohistochemical methods optimized to detect mature brainstem pathology (Beach et al., 2015). As mentioned above, the earliest alpha-synuclein aggregates may consist of immature oligomers and protofibrils which could be invisible to immunohistochemistry methods, which prioritize specificity over sensitivity.

The second argument is temporal in nature, i.e. the time window to identify a gut-only case may be very short or even non-existent. It is conceivable that once the first micro-aggregates are formed in synapses of the enteric nervous system, the retrograde propagation to the DMV and sympathetic ganglia starts immediately. Indeed, it has been shown that alpha-synuclein oligomers transport more readily through the vagus nerve than more mature aggregates (Holmqvist et al., 2014; Peelaerts et al., 2015; Rey et al., 2013; Reyes et al., 2014). Thus, it is possible that more mature Lewy inclusions amenable to detection by immunohistochemistry will start forming at approximately the same time in the nerve terminals of the gut, in the DMV, and the sympathetic ganglia. It is therefore possible that gut-only cases simply cannot be found using classical immunohistochemistry. Alternatively, if a gut-only stage actually does exist, it is possible that the duration of this stage is very brief – perhaps only a few weeks.

Importantly, the difficulties of identifying gut-only cases has also been reported in the prion literature. In cattle, sheep, and hamsters orally challenged with bovine spongiform encephalopathy or scrapie prions, it is almost impossible to identify a gut-only stage (Hoffmann et al., 2007; Kruger et al., 2009; Kaatz et al., 2012; McBride et al., 2001; van Keulen et al., 2008). In these animal models, the prion pathology is initiated in the gastrointestinal tract and travels via the vagus and sympathetic connectome to the CNS. However, at the time point when the enteric nervous system becomes immunohistochemically positive, prions are also detectable in the DMV and sympathetic ganglia. Thus, in realistic models of true prion disorders where the disease process is initiated in the gut, and has been shown to spread through the autonomic connectome to the DMV and IML, it is almost impossible to define a gut-only stage using immunohistochemistry. It would therefore not be surprising, if the same holds true for human gut-first PD.

The third argument is spatial in nature and concerns the geometry of the gastro-intestinal tract. The GI tract in humans is 8–10 m long at post mortem with a geometric surface area of more than 7000 cm². It seems likely that the initial patch of alpha-synuclein pathology is small, especially if it starts stochastically inside a single neuron (*neuron zero*). Of note, the terminal end field in the gut of a parasympathetic motor neuron probably covers only a few cm², which is roughly the size of a coin (Holst et al., 1997). The probability of locating a coin-sized patch in a 7000 cm² search space using standard microscopy slide tissue sections is probably less than 0.1% per slide. Thus, hundreds or even thousands of tissue samples are required from each patient. The published studies from the Arizona cohort report that the entire GI tract was screened with nine samples (Beach et al., 2010). This translates into an approximate probability of 1% (i.e. 1–0.999⁹) of locating a coin-sized patch of pathology in a single patient. Thus, if the Arizona cohort contains 10 gut-only cases, which is probably an overestimation given the arguments above, it is still 90% probable (i.e. 0.99¹⁰) that all 10 cases would be missed and designated as (false) negatives.

In summary, the lack of gut-only cases in the Arizona cohort does not rule out the gut-start hypothesis in LBDs. But it does provide strong evidence that a gut-first stage in LBDs is *not* characterized by widespread enteric mature alpha-synuclein inclusions, which persist for a long time before spreading to the CNS. Moreover, and as noted above, other investigators have reported Lewy pathology restricted to the sympathetic ganglia. Such cases might well represent positive evidence for the existence of gut-only or “periphery-only” LBD (Miki et al., 2009; Sumikura

et al., 2015; Tanei et al., 2021).

7. DLB versus PD

It has been suggested that a brainstem-predominant profile of Lewy pathology is suggestive of PD, whereas a limbic-predominant profile is more associated with early cognitive decline and DLB (Toledo et al., 2016). At first thought, it might seem reasonable to assume that a brain-first type of LBD would progress more rapidly to dementia. However, an abundance of clinical data does not support this view, as recently reviewed in detail (Borghammer, 2021). In short, a high risk of dementia in the LBDs is associated with constipation, orthostatic hypotension, and RBD, which are all markers of the body-first subtype of LBD (Fereshtehnejad et al., 2015; Kong et al., 2020; Merola et al., 2020; Oka et al., 2020; Pilotto et al., 2019; Tanaka et al., 2020). By extension, this suggests that patients with a caudo-rostral distribution of Lewy pathology are actually at an elevated risk of dementia, which exceeds the risk of patients with the amygdala-centered profile.

In contrast, brain-first PD patients, who are RBD-negative and display dopamine degeneration before sympathetic denervation, probably have an underlying amygdala-centered distribution of pathology. Such patients show a slower progression towards dementia. This suggests that an amygdala-centered (limbic-predominant) profile can frequently progress to clinical PD rather than DLB.

8. The SOC model

We recently proposed the *alpha-synuclein origin and connectome* (SOC) model as a unifying theory to explain how variable patterns and rates of non-motor symptoms, motor asymmetry, and cognitive decline may be explained by where the first alpha-synuclein pathology originates (the origin site) and how it then spreads through the connectome (Borghammer, 2021). In short, the model proposes that enteric pathology in body-first patients spreads *bilaterally* through both vagus nerves and sympathetic connections due to the left-right overlapping innervation patterns of these autonomic systems. The propagating alpha-synuclein inside the CNS is therefore more symmetric from the onset, leading to symmetric dopamine loss and more symmetric parkinsonism. We recently confirmed that body-first PD patients display more symmetric dopamine loss at diagnosis (Knudsen et al., 2021). According to the SOC model, the early, marked and symmetric affection of the modulatory brainstem nuclei leads to a more rapid dissemination of *bilateral* Lewy pathology to the telencephalon. In this framework, early dementia in the body-first type is caused, in part by more severe subcortical damage, but also by a higher global burden of Lewy pathology, since the telencephalic pathology is more bilateral.

In contrast, the SOC model proposes that brain-first PD starts in a single location inside the brain, which by necessity will be in either the left or right hemisphere. Due to the highly lateralized connections (i.e. only ~1% of axons project to the contralateral hemisphere), the alpha-synuclein pathology will initially disseminate mostly in the ipsilateral hemisphere, leading to asymmetric dopaminergic loss and asymmetric parkinsonism. Measured from the time of diagnosis, the full-blown dissemination of Lewy pathology to both the brainstem nuclei and the contralateral hemisphere takes longer time in brain-first patients compared to newly diagnosed body-first patients, which may explain why patients with a brain-first clinical phenotype display slower progression to dementia.

9. Summary

In summary, caudo-rostral and amygdala-centered profiles of Lewy pathology seem to be the two most common patterns seen in post mortem studies. In cases with a low global burden of Lewy pathology, thought to be early prodromal disease, the patterns are particularly clear. Therefore, it seems likely that patients with Lewy body disorders

most commonly evolve from one of these two initial conditions.

Using in vivo, multi-modal imaging, it was recently shown that patients with prodromal and de novo PD can be divided into two clusters compatible with brain-first and body-first etiology. In this paper we have argued that body-first patients, in whom the sympathetic system degenerates before the dopamine system, are equivalent to post mortem cases with a caudo-rostral distribution of Lewy pathology. In early caudo-rostral cases, the autonomic structures display more pathology than the substantia nigra and limbic structures. In contrast, brain-first patients show dopaminergic degeneration before sympathetic denervation. We argue that these patients are equivalent to post mortem cases with an amygdala-centered distribution of Lewy pathology, where limbic structures and the nigra show more pathology than do the autonomic structures. This hypothesis could be tested in future studies, especially once a PET tracer targeting alpha-synuclein becomes available.

This line of reasoning suggests that the gut-first hypothesis posed by Braak and colleagues is only valid for that part of LBD patients, which we refer to as the body-first subtype. Importantly, if the Lewy pathology in the remaining part of patients truly originates inside the brain or olfactory bulb and generally shows a top-down direction of propagation, it will be much more difficult to identify brain-first LBD at the prodromal stage. These patients will generally not develop the most recognizable prodromal symptoms such as RBD and autonomic dysfunction until after parkinsonism or dementia have manifested. If correct, this further emphasizes the importance of developing robust biomarkers in body fluids to detect brain-first LBD at an early stage, where neuroprotective treatments will be most effective.

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