Neuropathological studies of serotonergic and noradrenergic systems in Lewy body disease patients with delusion or depression

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Aim: The association between psychiatric symptoms in Lewy body disease (LBD) and the noradrenergic and serotonergic systems is still controversial. This study investigated the quantitative relationships of depression and delusion with these systems.

Methods: We studied 24 postmortem tissues from individuals with a pathological diagnosis of LBD with sufficient clinical history. The numbers of neurons and Lewy bodies (LBs) in the locus coeruleus (LC) and dorsal raphe nucleus (DRN) were counted, and the density of neurons in the DRN was analyzed. In addition, the densities of tryptophan hydroxylase-positive neurites and norepinephrine transporter-positive neurites in the amygdala and dorsal prefrontal cortex were measured. Finally, we divided the cases into two groups: with or without depressive mood, and with or without delusion. Quantitative histological data were compared between the groups.

Results: The group with depressive mood had a significantly smaller number of neurons in the LC compared with the group without depressive mood. The group with delusion had a significantly larger number of LBs in the DRN compared with the group without delusion. The density of norepinephrine transporter-positive neurites in the dorsal prefrontal cortex was significantly correlated with the number of neurons in the LC.

Conclusions: The accumulation of LBs in the DRN of individuals with LBD was associated with delusion, whereas a decrease in the number of neurons in the LC was associated with depressive mood. These neurodegenerative changes involved the serotonergic and noradrenergic systems and may be associated with the formation of delusion and depression, respectively, in LBD.

Keywords: delusion, depression, Lewy body disease, neuropathology, raphe nuclei.

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Parkinson’s disease (PD), PD with dementia, and dementia with Lewy bodies (DLB) are neurodegenerative disorders in which Lewy bodies (LBs) and Lewy neurites are thought to play important roles in pathogenesis and clinical phenotype. They are therefore grouped under the pathological classification of Lewy body disease (LBD).

In addition to cognitive dysfunction, psychiatric symptoms are frequently seen in cases with LBD. These symptoms include depressive mood, appetite loss, insomnia, and psychotic symptoms such as visual hallucination and delusion. However, the biological pathogenesis of these symptoms is not completely understood. For example, the relationship between the serotonergic system and depressive symptoms is still controversial, although selective serotonin reuptake inhibitors and serotonin and norepinephrine reuptake inhibitors are reported to be effective for the treatment of depression. Other studies that investigated tissues from postmortem brains obtained from individuals with depression and without cognitive impairment reported no obvious relationships between them.

The noradrenergic system, of which the locus coeruleus (LC) is a major component, is impaired in LBD. Previous qualitative studies suggested a relationship between depression and degenerative changes in the LC, which implies that dysfunction of the noradrenergic system may be the cause of depression in LBD, however, this has not yet been confirmed in quantitative studies. Although the involvement of frontal lobe dysfunction has been suggested, no pathological mechanism supporting this possibility has been reported to date.

Similar to the LC, the critical center of the noradrenergic system, raphe nuclei contain the highest number of serotonergic neurons in the brain. The raphe nuclei are distributed predominantly in the median part of the brainstem and are divided into subgroups. The dorsal raphe nucleus (DRN) is in the tegmentum of the caudal part of the midbrain and the rostral part of the pons. Serotonergic neurons of these nuclei project to a broad area, including the cerebrum, brainstem, and cerebellum. Moreover, because the DRN has more projection fibers to the cerebral cortex than other raphe nuclei, a relationship between these nuclei and psychiatric symptoms has been suggested, although there is still limited evidence to support this connection. A study by Frisina et al. investigated the potential relationship between depression and neuronal loss or gliosis in DRN; however, the DRN was not visualized by immunohistochemistry because its small size and complex structure make identification difficult.
Because LBD can be classified into brainstem, limbic, and neocortical subtypes, it is important to investigate the areas associated with depressive symptoms and those located in the limbic system and neocortex. In the limbic system, the amygdala, which is thought to be related to memorizing emotional events, and in the neocortex, the prefrontal cortex, which has been studied in relation to depression and delusions, are important.

Here, we investigated the relationships between depressive symptoms and degenerative changes in the LC, DRN, and the serotonergic and noradrenergic projections into the limbic system and neocortex, as well as the relationships between psychotic symptoms and these circuits in a quantitative study using postmortem tissues with LBD.

Methods

Clinical data

We identified 32 consecutive autopsied cases with a pathological diagnosis of LBD. We obtained the clinical history of individuals from their attending physician before or immediately after the autopsy and checked whether they had psychiatric symptoms, such as depressive mood or delusion. In this process, our definition of delusion excluded beliefs that arose from visual hallucinations, such as “black bugs are walking around” or other beliefs that should be interpreted as visual hallucinations. One case with an absence of information about clinical history was excluded from the study.

Tissues

We used postmortem brainstem tissues obtained from 31 cases of LBD. Autopsies for all cases were performed at the National Center of Neurology and Psychiatry. Brainstem tissues were split along the median plane, and the half with more severe markers of disease was fixed in 10% formalin. If the tissues had no laterality, the left tissue was taken and fixed in formalin. We dissected tissues into 5-mm slices in axial planes, which were routinely processed into liquid paraffin. En bloc specimens were cut at 5 μm on a microtome. Hematoxylin and eosin (H&E) staining was performed for each slice including the lower midbrain and pons.

From these cases, we cut tissues containing the amygdala and dorsal prefrontal cortex (DPC) into 5-mm slices in the coronal plane and picked up a pair of representative tissues per case. For the DPC, we cut the tissue to a depth of 25 mm from the top of the superior frontal gyrus. Tissues containing the amygdala and DPC were processed in the same way as the LC and DRN.

Histochemistry

Phosphorylated alpha-synuclein immunohistochemistry (015–25191, Fujifilm, Japan, monoclonal, 1:15 000, formic acid) was performed for each slice including the lower midbrain and pons, and tryptophan hydroxylase (TPH) (T0678, Sigma, monoclonal, 1:4000, heat) immunohistochemistry was performed only for slices that included the lower midbrain. Immunostaining was performed as follows: The slides were dewaxed in xylene, and rehydrated in descending alcohol series. An epitope retrieval method was used to unmask the antigens. The sections were blocked in pH6 buffer and incubated at 37°C with primary antibodies for 60 min. The sections were incubated for 16 min at 37°C with universal anti mouse HRP secondary antibody. Immunocomplexes were visualized with DAB+ Chromogen, and then the sections were counterstained with hematoxylin and cover-slipped. These steps were automatically performed by an immunostaining platform (Ventana XT DISCOVERY; Ventana, Tucson, AZ, USA). Of the 31 tissues, seven cases lacked the median part of the DRN because of a slight shift in the cutting plane. The remaining 24 tissues, including the interfascicular nucleus, ventromedial nucleus, and ventrolateral nucleus composing the DRN, were used for analyses. We performed TPH and norepinephrine transporter (NET) (AMAB91116, Atlas, monoclonal, 1:200, formic acid) immunohistochemistry for slices from the 24 cases that included the amygdala and DPC using the method described above.

Measurements

We used the method described by Mai et al. to identify the location of the DRN and LC. We manually counted the number of nucleoli of TPH-positive cells, measured the area, and calculated the density of neuronal cells in the DRN from every case (Fig. 1). Next, we counted the number of LBs and determined their prevalence in the DRN using H&E staining and α-synuclein staining, respectively. We counted the number of nucleoli of neural cells in the DRN in the LC by H&E staining (Fig. 2). We did not measure the area of the LC in any cases because severe neuronal cell loss made it difficult to recognize the LC border in most cases.

We manually counted TPH-positive neurites and NET-positive neurites in the amygdala and DPC specimens that had a length greater than approximately 20 μm. We then measured the area of the specimen, and calculated the densities of TPH-positive neurites, and NET-positive neurites in the amygdala and DPC (Fig. 3).

Statistics

We analyzed the data using two methods. First, we divided the 24 cases into two groups based on whether they had depressive mood, and compared them on the basis of pathological data. Second, we divided the same 24 cases into two groups on the basis of whether they had delusions, and conducted the same comparisons.

For the DRN, we examined the pathological data including the numbers of neuronal cells and LBs, the prevalence of LBs, the cross-sectional area of nuclei, and the densities of neuronal cells and LBs. For the LC, we counted the numbers of neuronal cells and LBs, and the prevalence of LBs. For the DPC, we determined the densities of TPH-positive neurites and NET-positive neurites, but we examined only the densities of NET-positive neurites in the amygdala because TPH-positive neurites were poorly stained.

Additionally, we examined correlations between neuronal cells in the LC and the density of NET-positive neurites in the noradrenergic system, and we investigated correlations between the number of density of neuronal cells in DRN and the density of TPH-positive neurites in the serotonergic system, independent of comorbid depression and delusions.

Statistical analysis was performed using EZR on R commander Version 1.41 for Windows 64 bit (Japan). Because of the non-normal distribution of data, we applied non-parametric correlation analyses (Mann–Whitney U-test) to check the correlation between the pathological indicators and psychiatric symptoms. We calculated Spearman’s rank correlation coefficient to check the correlation between the pathological indicators. We used Fisher’s test to analyze demographic information when the data were binary. If the data were not binary, we used the Mann–Whitney U-test. The chi-square test was used for DLB consensus guidelines. To draw some of the statistical graphs, we used the beeswarm package for R, Version 0.2.0 along with EZR. P-values <0.05 were considered statistically significant.

Results

Clinical and pathological demographics

The clinical demographics of the 24 individuals are summarized in Table 1. The mean age of disease onset was 65.1 years old, the mean age of death was 79.0 years old, and the mean disease duration was 13.9 years. The mean post-mortal interval to autopsy was 15 h. In accordance with the 3rd consensus guidelines for defining DLB, 12 samples were categorized as neocortical type, 11 as transitional limbic type, and one as brainstem type. According to Braak’s staging, four patients were at stage III, eight were at stage IV, eight were at Stage V, and four were at stage VI. Seven of the patients had a history of depressive mood, and eight had a history of depressive mood.
of delusion. Two of these patients had both history of depressive mood and delusion. Eighteen patients had visual hallucination or dementia. All 24 patients had Parkinsonism. Regarding past treatments, four patients had been treated with antidepressants, 12 with antipsychotics, and 12 with benzodiazepines. Except for psychiatric consultation, there were no significant differences in the basic content related to autopsy and clinical background between those with and without depressive mood or delusion.

Number of LBs in the LC and DRN

The numbers of LBs in the LC and DRN counted in specimens stained with H&E were significantly correlated with the numbers of LBs in the LC and DRN counted in immunostained specimens, respectively (Fig. 4a). We used the numbers counted on the specimens stained with H&E for all subsequent analyses because of the difficulty distinguishing between α-synuclein-positive structures in axons and LBs in neuronal cell bodies on α-synuclein-stained specimens. The numbers of LBs counted in the LC and DRN were not significantly different among the three subtypes defined by the 3rd consensus guidelines (Fig. 4b).

Depressive mood and neuropathological measurements

The group with depressive mood had significantly fewer neuronal cells in the LC compared with the group without depressive mood (Fig. 5). The number of LBs in the LC, the area, number of neural cells, neural cell density, and number of LBs in the DRN were not significantly different between the two groups.

There was no significant difference in the density of NET-positive neurites in the amygdala, or the densities of NET-positive neurites and TPH-positive neurites in the DPC between the two groups.
Delusion and neuropathological measurements

The group with delusion had a significantly larger number of LBs and a higher prevalence, reflecting the number of LBs/number of neuronal cells, of LBs in the DRN (Fig. 6). The cross-sectional area, number of neuronal cells, and neuronal cell density of the DRN, and the number of neural cells and LBs in the LC were not significantly different between the two groups.

There was no significant difference in the density of NET-positive neurites in the amygdala, or the densities of NET-positive neurites and TPH-positive neurites in the DPC between the two groups.

Pathological correlation between the brainstem, amygdala, and DPC

The density of NET-positive neurites in the DPC was significantly correlated with the number of neurons in the LC ($P = 0.007$) and the density of NET-positive neurites in the amygdala ($P < 0.001$) (Fig. 7).
density of TPH-positive neurites in the DPC was not significantly correlated with any of the other pathological indicators examined.

We summarized the correlations between the pathological features and α-synuclein burden in the LC, DRN, amygdala, and DPC in Table S1.

Discussion

In this study, we quantitatively examined pathological differences in the LC, DRN, amygdala, and DPC in tissues from LBD patients with or without depressive mood and delusion. Our results show that depressive mood is related to neuronal loss in the LC.

As mentioned above, researchers studying depression have suspected the presence of abnormalities in the serotonergic system. However, LBD researchers have suggested that depression occurring in LBD is associated with degeneration of the LC. Although there have been no quantitative immunohistochemical studies of the DRN, our results indicated that depression in LBD is more likely to be associated with abnormalities in the noradrenergic system than in the serotonergic system. A previous study reported that the accumulation of α-synuclein in the LC was not associated with depression; however, the subjects did not have dementia, suggesting that they might have had relatively low levels of α-synuclein. Our study revealed that the development of depression was related to the severity of LC pathology in subjects with high levels of α-synuclein.

The density of NET-positive neurites in the limbic system or neocortex, which can project from the LC, was not significantly decreased in the group with depression. A previous study suggested that a reduction in blood flow in the frontal lobe was related to depressive and psychotic symptoms; however, the pathogenesis of depression might be more related to damage to the LC itself rather than to the area the LC projects into. Alternatively, other structures receiving projections from the LC may be more important in the pathogenesis of depression.

Our study also provides a new method for studying the DRN, which has been notoriously challenging for researchers. Because the DRN is a small structure, it is difficult to evaluate its function without specific radiological methods such as high-resolution positron emission tomography–magnetic resonance imaging fusion. In addition, pathological research of the DRN is also difficult because it is located on the narrow median part of the brainstem. With a very slight deviation of the split plane, the bulk of the DRN, especially the interfascicular part, may be included within the tissue of the side to be frozen, with the smaller remaining part being included within the tissue on the side to be formalin-fixed. In contrast, if the bulk of the DRN is included within the tissue on the side to be formalin-fixed, the amount of DRN in the frozen tissue may be insufficient for subsequent molecular studies.

Our results show that delusion is associated with an accumulation of LBs in the DRN. α-Synuclein accumulation in LBD has been...
The trauma may be the cause of depression and delusion. Although the pathogenesis of delusion is still unclear, another potential cause of delusion is the dysfunction of unknown neurotransmitters or other neurotransmitter systems. For example, based on the hypothesis that LB is delivered via axonal transport, some neuronal organs to which the DRN projects may be the primary cause of delusion. Our results raise questions regarding why the number of neurons in the DRN of patients with delusion was not decreased and why the prevalence of LBs in the LC of patients with depressive mood was not increased. There was a significant decrease in neural cells in the LC in patients with depressive mood, whereas there was a significant difference in the prevalence of LBs in the DRN of those with delusion. The first finding can be explained by the complexity of the structure of the DRN. Whereas the LC has a linear structure in the pons, the DRN is composed of several small subnuclei, and their cross-sectional shapes can easily be changed by a small difference in the height of the cutting plane. This technical problem in the DRN may have a greater effect on the number of counted neuronal cells than the degenerative changes induced by LBs. The second finding can be explained by the high accumulation rate of neurodegenerative proteins in the LC. This was observed in the LBD and other neurodegenerative diseases, such as Alzheimer’s disease, in which neurodegenerative proteins are found in the LC, even in early stages. Indeed, even in cases without a diagnosis of neurological or psychiatric disease, it is common to find some LBs in the LC. This suggests that noradrenergic function is not impaired in the LBD and other neurodegenerative diseases, such as Alzheimer’s disease, in which neurodegenerative proteins are found in the LC, even in early stages. Indeed, even in cases without a diagnosis of neurological or psychiatric disease, it is common to find some LBs in the LC. This suggests that noradrenergic function is not impaired even in early stages.

Statistical analysis was carried out between the cases with and without depression or delusion. *P < 0.05. PMI, post-mortem interval; DLB, dementia with Lewy body; SD, standard deviation; m/f, male/female; NFT, neurofibrillary tangle. Statistical analysis was carried out between the cases with and without depression or delusion.

Table 1. Comparison between individuals with and without depressive mood or delusion

| Demographic information on all subjects (n = 24) | With depressive mood (n = 7) | Without depressive mood (n = 17) | P value | With delusion* (n = 8) | Without delusion (n = 16) | P value |
|-----------------------------------------------|-----------------------------|---------------------------------|---------|------------------------|--------------------------|---------|
| Sex (m/f)                                      | 11/13                       | 4/3                             | 0.388   | 3/5                    | 8/8                      | 0.581   |
| Age at onset (years), mean (SD)                | 65.1 (11.0)                 | 68.0 (6.3)                      | 0.187   | 63.2                   | 66.0 (10.7)              | 0.569   |
| Age at death (years), mean (SD)                | 79.0 (8.3)                  | 80.9 (5.2)                      | 0.71    | 77.0 (8.1)             | 80.0 (8.3)               | 0.291   |
| Duration (years), mean (SD)                    | 13.9 (7.6)                  | 12.9 (8.0)                      | 0.576   | 13.8 (8.8)             | 14.0 (6.9)               | 1       |
| PMI (h), mean (SD)                             | 15.0 (14.3)                 | 19.6 (17.8)                     | 0.534   | 8.8 (5.4)              | 18.2 (16.2)              | 0.172   |
| Brain weight (g), mean (SD)                    | 1237 (142)                  | 1267 (143)                      | 0.576   | 1170                   | 1271 (130)               | 0.081   |
| Number of cases with psychiatric consultation   | 16                          | 7                               | 0.004   | 5                      | 11                       | 0.057   |
| Number of cases with visual hallucination      | 18                          | 5                               | I       | 7                      | 11                       | 0.621   |
| Number of cases with dementia                  | 12                          | 7                               | 0.629   | 5                      | 13                       | 0.001   |
| Number of cases with benzodiazepine prescription| 4                           | 4                               | 0.25    | 1                      | 3                        | 0.344   |
| Number of cases with antidepressants prescription| 12                         | 6                               | 0.125   | 5                      | 7                        | 0.344   |
| 3rd consensus guidelines for defining DLB (neocortical/limbic/brainstem) | 12 / 11 / 1 | 2 / 5 / 0 | 10 / 6 / 1 | 0.251 | 3 / 5 / 0 | 9 / 6 / 1 | 0.448 |
| Braak’s NFT stage (0/1/2/3/4/5/6)              | 2 / 8 / 13 / 0 / 1 / 0 / 0   | 1 / 7 / 8 / 0 / 1 / 1 / 5 / 0 / 0 | 0.572 | 1 / 2 / 4 / 1 / 6 / 9 / 0 | 0.562 |
| CERAD stage (0/A/B/C)                          | 6 / 4 / 6 / 8               | 3 / 2 / 1 / 1 / 3 / 2 / 5 / 7   | 0.335   | 1 / 1 / 3 / 5 / 3 / 3 / 5 | 0.706 |
| Thal phase (0/1/2/3/4/5)                       | 6 / 5 / 1 / 3 / 5 / 2        | 3 / 2 / 0 / 0 / 3 / 3 / 1 / 3 / 4 | 0.724 | 1 / 1 / 0 / 5 / 4 / 1 / 2 | 0.42 |

*Four cases showed persecutory delusion. The others include cases with Cotard syndrome, Capgras syndrome, and unspecified delusion.
loss and accumulation of alpha-synuclein in the substantia nigra and ventral tegmentum in a group of DLB patients with depressive symptoms. Wilson et al. reported the accumulation of α-synuclein in the ventral tegmentum of subjects with depressive symptoms. Hypofunction or dysfunction of the dopaminergic system is one hypothesized mechanism for the development of psychotic symptoms. The non-normal distribution of data related to the small number of samples makes it difficult to compare the effect size of neuronal loss and α-synuclein burden in LC, DRN, and substantia nigra.

Our study involved some limitations. First, we analyzed a relatively small number of tissues. For instance, our results showed that one group with delusion tended to have a higher prevalence of diffuse neocortical LBD, but confounders were not excluded in this study for that reason. In another instance, we were unable to analyze the pathological features of a subgroup with both depressive mood and delusion. Second, this study was retrospective, and we could not use quantitative clinical data for statistical analyses. The representative scales for depressive symptoms or psychotic symptoms such as the Hamilton depression rating scale.

Fig. 4 (a) The number of Lewy bodies counted in specimens stained with H&E correlates significantly with the number of Lewy bodies counted in immunostained specimens, in the LC and DRN. (b) The number of LBs in the LC, the number of LBs in the DRN, and the density of LBs in the DRN were not significantly correlated among the three patterns of accumulation of Lewy bodies.

Fig. 5 The number of neurons in the locus coeruleus (LC) was significantly different between the groups with depressive mood and without depressive mood, whereas no difference was observed in the number of LBs or the ratio of LBs to neurons.
Fig. 6 The number of LBs, the ratio of LBs to neurons, and the density of LBs in the DRN were significantly different between the groups with delusion and without delusion.

Fig. 7 The density of norepinephrine transporter-positive neurites in the dorsal prefrontal cortex is significantly correlated with the number of neurons in the LC and the density of norepinephrine transporter-positive neurites in the amygdala. The density of tryptophan hydroxylase-positive neurites in the DPC was not significantly correlated with the number and density of neurons in the DRN.
Zung self-rating depression scale, 36 Beck depression inventory, 37 and positive and negative syndrome scale 38 were not sufficiently examined in our cases. Third, we did not assess other psychiatric symptoms such as appetite loss, insomnia, and hallucination. Fourth, we did not examine the other neurotransmitter systems such as the cholinergic or dopaminergic systems. Finally, we only divided the cases on the basis of symptoms, and not on other diagnostic categories.

Conclusions

We demonstrated that a decrease in neuronal cells in the LC of individuals with LBD can induce the development of depressive mood, whereas the accumulation of LBs in the DRN can induce the development of delusion. Dysfunction of the noradrenergic system and serotonergic system may be associated with the formation of depression and delusion, respectively.

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Disclosure statement

None. All authors meet the ICMJE authorship criteria.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Table S1. Correlations between pathological features and α-synuclein burden.