Brainstem Aminergic Nuclei and Late-Life Depressive Symptoms

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**IMPORTANCE** The neurobiologic basis of late-life depressive symptoms is not well understood.

**OBJECTIVE** To test the hypothesis that neurodegeneration and neuronal density in brainstem aminergic nuclei are related to late-life depressive symptoms.

**DESIGN, SETTING, PARTICIPANTS, AND EXPOSURE** Longitudinal clinicopathological cohort study at residences of participants in the Chicago, Illinois, metropolitan area. Participants included 124 older persons without dementia in the Rush Memory and Aging Project who had annual evaluations for a mean (SD) of 5.7 (2.8) years, died, and underwent a postmortem neuropathological examination that provided estimates of the densities of Lewy bodies, neurofibrillary tangles, and aminergic neurons in the locus ceruleus, dorsal raphe nucleus, substantia nigra, and ventral tegmental area.

**MAIN OUTCOMES AND MEASURES** The number of depressive symptoms (mean [SD], 1.61 [1.48]; range, 0–6; skewness, 0.94) on the Center for Epidemiological Studies Depression Scale averaged across annual evaluations.

**RESULTS** Brainstem Lewy bodies were associated with depressive symptoms, and the association was attenuated in those taking antidepressant medication. Brainstem tangles were associated with more depressive symptoms in those without cognitive impairment but with fewer symptoms in those with mild cognitive impairment. Lower density of tyrosine hydroxylase–immunoreactive neurons in the ventral tegmental area was robustly associated with a higher level of depressive symptoms (mean [SE] estimate, −0.014 [0.003]; P < .001; 16.3% increase in adjusted R²). The association was not modified by medication use or cognitive impairment. Neither tyrosine hydroxylase–immunoreactive neurons in the locus ceruleus nor tryptophan hydroxylase–immunoreactive neurons in the dorsal raphe nucleus were related to depressive symptoms.

**CONCLUSIONS AND RELEVANCE** The results suggest that the mesolimbic dopamine system, especially the ventral tegmental area, has an important role in late-life depressive symptoms.
Late-life depressive symptoms are common and are associated with increased risk of disability, dementia, and death. Because brainstem aminergic nuclei have an important role in the pathogenesis of depression and are early sites of age-related neurodegeneration, it has long been hypothesized that late-life depressive symptoms are partly due to neurodegenerative changes in these nuclei. Support for the hypothesis has been inconsistent, likely because of several factors. Most studies have had more than 50 participants or used continuous measures of depressive symptoms, limiting statistical power. Also, much research has been conducted among individuals with dementia. However, the presence of dementia introduces substantial error into the measurement of depressive symptoms, in addition to extensive pathological conditions elsewhere in the brain, and the neurodegenerative changes associated with dementia are robustly related to cognitive, sensory, and motor symptoms years before dementia onset, suggesting that associations with depressive symptoms might be easier to identify during this period. Finally, despite evidence that brainstem dopamine neurons have an important role in regulating depression-related behavior, previous investigations have primarily focused on the locus ceruleus and dorsal raphe nuclei.

The present study examines the association of neurodegenerative lesions and aminergic neurons in 4 brainstem nuclei with the level of depressive symptoms in late-life. At annual intervals for a mean of 5.7 years, older persons without dementia had structured evaluations that included a standard self-report measure of depressive symptoms. At death, a uniform neuropathological examination was performed that yielded estimates of the densities of Lewy bodies, neurofibrillary tangles, and aminergic neurons in the locus ceruleus, dorsal raphe nucleus, substantia nigra, and ventral tegmental area. We tested the hypothesis that higher pathological burden and lower neuronal density are associated with more depressive symptoms.

Methods

Participants

The Rush Memory and Aging Project is an ongoing clinico-pathological study that began September 1, 1997, and includes annual clinical evaluations and brain autopsy at death. Persons were recruited from social service agencies, subsidized housing facilities, retirement communities, and churches in the Chicago, Illinois, metropolitan region. After a presentation on the project, interested individuals met for further discussions with staff, who obtained written informed consent. The project was approved by the institutional review board of Rush University Medical Center, Chicago, Illinois.

There had been 556 deaths (among 1533 participants) at the time of these analyses. A brain autopsy had been performed in 439 (79.8%), and a uniform neuropathological examination had been completed in the first consecutive 417 individuals. From these, 170 individuals were selected to provide a wide range of cognitive, motor, and affective functioning proximate to death for a clinico-pathological substudy of brainstem aminergic nuclei as described elsewhere. We excluded 45 individuals with a diagnosis of dementia (see the Clinical Evaluation subsection), and 1 person was missing data about depressive symptoms. Analyses are based on the remaining 124 individuals, who had a mean (SD) age at death of 87.7 (5.7) years. They had completed a mean (SD) of 14.3 (2.5) years of education, 69.4% were women, and 43.5% had mild cognitive impairment.

Assessment of Depressive Symptoms

A 10-item version of the Center for Epidemiological Studies Depression Scale was used to assess depressive symptoms at each annual clinical evaluation. For each item, the interviewer read a brief statement (eg, “I felt depressed”), and the participant indicated whether or not he or she had felt that way much of the past week. The score is the number of symptoms reported (range, 0-10). This 10-item scale is less burdensome than the original 20-item scale but has similar psychometric properties, and its measures have been associated with adverse health outcomes, including dementia and death. Supported by prior factor analyses, subscales were created for negative affect (3 items, including “I felt depressed,” “I felt lonely,” and “I felt sad”), positive affect (2 items, including “I was happy” and “I enjoyed life”), somatic symptoms (3 items, including “I felt like everything I did was an effort,” “My sleep was restless,” and “I could not get going”), and interpersonal problems (2 items, including “People were unfriendly” and “I felt that people disliked me”), as previously described.

Clinical Evaluation

The annual evaluations included a structured medical history, neuropsychological testing, and neurological examination. Prescription and over-the-counter medications were inspected, identified, and coded using the Medi-Span Master Drug Database system. The neurological examination included a modified version of the motor portion of the Unified Parkinson’s Disease Rating Scale, as previously described. After each evaluation, an experienced clinician diagnosed dementia and mild cognitive impairment. Classification of dementia was based on the criteria of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association, which require a history of cognitive decline and impairment in at least 2 cognitive domains. The diagnosis of mild cognitive impairment required impairment in 1 or more cognitive domains in the absence of dementia. Upon death, all clinical data were reviewed by a board-certified neurologist blinded to all pathological data, and final diagnoses of mild cognitive impairment and dementia were made.

Neuropathological Examination

The brain was removed a median of 6.0 hours (interquartile range, 4.8-8.6 hours) after death, which occurred a median of 9.3 months (interquartile range, 5.5-12.9 months) after the last complete clinical evaluation. Examiners blinded to all clinical data followed a standard protocol for tissue preservation, tissue sectioning, and quantification of pathological findings.
To examine the nuclei of interest, consecutive transverse blocks of fixed tissue were obtained at 4 brainstem levels, as previously described. The first block included the substantia nigra and paranigral nucleus of the ventral tegmental area (Figure 1A); the second block included the rostral level of the dorsal raphe nucleus, the trochlear nucleus, and the decussation of the superior cerebellar peduncles; the third block included the caudal level of the dorsal raphe nucleus, rostral levels of the locus ceruleus nuclei, and the mesencephalic nucleus of the trigeminal nerve; and the fourth block included the main body of the locus ceruleus. Following routine processing, blocks were embedded in paraffin, and 6-μm sections stained with hematoxylin-eosin were used to survey the regions of interest. Blocks without anatomically matching levels of the regions of interest were excluded. Immunohistochemistry was performed using an indirect immunoenzyme horseradish peroxidase method with 3,3′diaminobenzidine as the chromogen in an autostainer (Leica Bond Max; Leica Microsystems) using a kit (Bond Polymer Refine Detection Kit; Leica Microsystems). Tyrosine hydroxylase–positive and tyrosine hydroxylase–negative neurons in the substantia nigra, ventral tegmental area, and locus ceruleus were identified using a monoclonal anti–tyrosine hydroxylase antibody (1:750; ImmunoStar). Nickel chloride (5%) added to the 3,3′diaminobenzidine substrate (Vector Laboratories) enhanced the signal of the tyrosine hydroxylase–positive neurons and their processes to a brown-black color. The count of ventral tegmental area neurons was based on the paranigral nucleus. The figures show examples of high and low densities of tyrosine hydroxylase–positive neurons in the paranigral nucleus of the ventral tegmental area (Figure 1B and C), substantia nigra (Figure 2A and B), and locus ceruleus (Figure 2C and D). Serotonergic neurons in the dorsal raphe nucleus were identified using an anti–tryptophan hydroxylase monoclonal antibody (1:1000; Sigma Chemical Company). Examples of high and low densities of tryptophan hydroxylase–positive neurons are shown in Figure 2E and Figure 2F, respectively.

Neuronal density, neurofibrillary tangles, and Lewy bodies were quantitated at 1 level of the substantia nigra and the ventral tegmental area, at 2 levels of the dorsal raphe nucleus, and at 2 levels of the right and left locus ceruleus. The immunohistochemical preparations were used to outline the regions of interest using software (Stereo Investigator Program; MBF Biosciences) attached to a microscope (BX60; Olympus) with a motorized stage. These outlines were superimposed on slides used to determine the neuron, neurofibrillary tangle, and Lewy body counts in the locus ceruleus, dorsal raphe nucleus, and ventral tegmental area. The counting frame and motorized stage facilitated counting in contiguous fields in the outlined regions. In these fields, unstained and immunostained neurons with nuclei were manually selected at a magnification of ×400. Densities of neurons, tangles, and Lewy bodies in the 4 quadrants of the substantia nigra were determined, as described previously.

Analyses were based on the density per millimeter squared of immunostained neurons. The interrater reliability of these neuronal density measures, estimated among a subset of 12 par-
participants, was high for the locus ceruleus ($r = 0.97$), dorsal raphe nucleus ($r = 0.96$), substantia nigra ($r = 0.97$), and ventral tegmental area ($r = 0.99$) ($P < .001$ for all). Their discriminant validity is supported by previous research linking neuronal density in the substantia nigra to subclinical parkinsonism and linking neuronal density in the locus ceruleus to cognitive decline.

Neurofibrillary tangles were identified using a monoclonal anti–paired helical filament tau phosphorylated antibody (clone AT8, 1:2000; Thermo Scientific), and Lewy bodies were identified using a monoclonal phosphorylated antibody to α-synuclein (1:20 000; Wako Chemical USA Inc). The total numbers of neurofibrillary tangles and Lewy bodies in each brainstem nucleus within the outlined area referred to above were counted manually in the regions of interest, and results were expressed as density per millimeter squared.

To assess pH, 250 to 300 mg of frozen cerebellum was homogenized (Polytron PT10-35GT; Kinematica) in 5 volumes of nuclease-free water adjusted to pH 7.2. The pH of each homogenate was measured twice at room temperature (pHOny SB70 pH meter; VWR). Analyses are based on the mean of the 2 readings, which were highly correlated ($r = 0.998$, $P < .001$).

**Statistical Analysis**

All analyses included terms to adjust for the effects of sex, age at death, and education. A mixed-effects model was used to determine whether depressive symptoms changed over time. The mean depressive symptom score was regressed on postmortem variables in a series of linear regression models. The initial model had a term for the composite measure of brainstem Lewy bodies. The model was repeated using the following variables: controlling for parkinsonism; with depression subscores as outcomes; separately analyzing Lewy bodies within each nucleus; with an indicator for antidepressant use and its interaction with Lewy bodies; with an indicator for mild cognitive impairment and its interaction with Lewy bodies; and controlling for pH, postmortem interval, and time from the last clinical evaluation to death. Similar analyses were conducted with the measures of brainstem tangle density. The 4 neuronal density scores were modeled separately, ventral tegmental area neurons and nigral neurons were modeled together, and ventral tegmental area neurons were modeled controlling for pH, postmortem interval, and time from the last clinical evaluation to death. We also assessed whether ventral tegmental area neurons were related to depression subscores, accounted for the association of Lewy bodies with depressive...
symptoms, or interacted with antidepressant medication use or mild cognitive impairment.

Results

At the initial evaluation, depressive symptom scores ranged from 0 to 5 (mean [SD], 1.48 [1.66]; skewness, 1.04). During a mean (SD) of 5.7 (2.8) annual follow-up years, depressive symptoms did not seem to change (eFigure 1 in the Supplement). In a mixed-effects model adjusted for age, sex, and education, there was no evidence that the number of antidepressant medications used during the observation period (eTable 1 in the Supplement) or mild cognitive impairment (43.5%) was obscuring the association of Lewy bodies with depressive symptoms. There was no interaction with mild cognitive impairment (mean [SE] estimate, 0.859 [0.911]; P = .39), but an interaction was observed with medication use (mean [SE] estimate, −2.476 [0.913]; P = .008): the association of Lewy bodies with depressive symptoms was attenuated in those taking antidepressant medication compared with those not taking antidepressant medication.

Table. Information on the Density of Tangles and Lewy Bodies in Brainstem Aminergic Nuclei

<table>
<thead>
<tr>
<th>Pathological Density Measure</th>
<th>No. of Individuals</th>
<th>Mean (SD)</th>
<th>Skewness</th>
<th>Rotated Factor Loadings*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locus ceruleus tangle density</td>
<td>118</td>
<td>1.82 (2.01)</td>
<td>2.50</td>
<td>0.82 0.02</td>
</tr>
<tr>
<td>Dorsal raphe nucleus tangle density</td>
<td>117</td>
<td>3.41 (3.19)</td>
<td>1.81</td>
<td>0.71 0.15</td>
</tr>
<tr>
<td>Substantia nigra tangle density</td>
<td>106</td>
<td>0.30 (0.45)</td>
<td>2.36</td>
<td>0.84 −0.10</td>
</tr>
<tr>
<td>Ventral tegmental area tangle density</td>
<td>109</td>
<td>0.91 (1.67)</td>
<td>2.46</td>
<td>0.75 −0.09</td>
</tr>
<tr>
<td>Locus ceruleus Lewy body density</td>
<td>117</td>
<td>0.22 (0.79)</td>
<td>4.25</td>
<td>−0.09 0.87</td>
</tr>
<tr>
<td>Dorsal raphe nucleus Lewy body density</td>
<td>117</td>
<td>0.01 (0.07)</td>
<td>5.92</td>
<td>−0.03 0.62</td>
</tr>
<tr>
<td>Substantia nigra Lewy body density</td>
<td>111</td>
<td>0.05 (0.23)</td>
<td>5.72</td>
<td>0.00 0.82</td>
</tr>
<tr>
<td>Ventral tegmental area Lewy body density</td>
<td>109</td>
<td>0.08 (0.40)</td>
<td>6.93</td>
<td>0.07 0.52</td>
</tr>
</tbody>
</table>

* Factor 1 shows the cluster of individual measures of tangle density in 4 nuclei, and factor 2 shows the cluster of individual measures of Lewy body density in 4 nuclei. This result justifies the use of 2 composite measures of tangle and Lewy body densities in the analysis.

Tangles and Depressive Symptoms

The composite measure of brainstem tangle density was not related to depressive symptoms (mean [SE] estimate, −0.038 [0.090]; P = .68); adjustment for pH, postmortem interval, and time from the last clinical evaluation to death yielded comparable results (mean [SE] estimate, 0.087 [0.093]; P = .35), and the results of a subsequent analysis with a quadratic term for tangles (mean [SE] estimate, −0.061 [0.039]; P = .12) did not suggest a nonlinear association. No association with tangles was present in the individual nuclei, with mean [SE] estimates of 0.031 (0.071) for locus ceruleus (P = .66), −0.007 (0.045) for dorsal raphe nucleus (P = .88), 0.482 (0.332) for substantia nigra (P = .15), and −0.105 (0.087) for ventral tegmental area (P = .23).

Lewy Bodies and Depressive Symptoms

In a linear regression model adjusted for sex, age at death, and education, higher density of brainstem Lewy bodies was associated with a higher level of depressive symptoms (mean [SE] estimate, 1.133 [0.443]; P = .01; 4.0% increase in adjusted R²). Results were similar after controlling for pH, postmortem interval, and time from the last clinical evaluation to death (mean [SE] estimate, 1.159 [0.437]; P = .009) or parkinsonism proximate to death (mean [SE] estimate, 1.168 [0.450]; P = .01). The association was observed in 3 of 4 nuclei, with mean (SE) estimates of 0.363 (0.175) for locus ceruleus (P = .04), 5.302 (2.000) for dorsal raphe nucleus (P = .009), 1.301 (0.609) for substantia nigra (P = .04), and 0.420 (0.368) for ventral tegmental area (P = .26). In analyses of subscores, higher Lewy body density was marginally related to negative affect (mean [SE] estimate, 0.495 [0.237]; P = .04), robustly related to somatic symptoms (mean [SE] estimate, 0.634 [0.204]; P = .002), and unrelated to positive affect (mean [SE] estimate, 0.007 [0.107]; P = .95) or interpersonal problems (mean [SE] estimate, −0.005 [0.033]; P = .88).

We conducted additional analyses to determine whether antidepressant medication use (32.3% during at least part of the observation period) (eTable 1 in the Supplement) or mild cognitive impairment (43.5%) was obscuring the association of Lewy bodies with depressive symptoms. There was no interaction with mild cognitive impairment (mean [SE] estimate, 0.859 [0.911]; P = .39), but an interaction was observed with medication use (mean [SE] estimate, −2.476 [0.913]; P = .008): the association of Lewy bodies with depressive symptoms was attenuated in those taking antidepressant medication compared with those not taking antidepressant medication.
Aminergic Neuronal Density and Depressive Symptoms

Because of missing or insufficient tissue, 6 to 13 participants per nucleus had missing neuronal data. eFigure 3 in the Supplement shows the distributions of aminergic neuronal density in the locus ceruleus (n = 118; mean [SD], 44.1 [17.8]; skewness, 0.39), dorsal raphe nucleus (n = 117; mean [SD], 105.4 [28.3]; skewness, 0.54), substantia nigra (n = 113; mean [SD], 31.1 [11.9]; skewness, 0.01), and ventral tegmental area (n = 111; mean [SD], 82.8 [47.9]; skewness, 0.33). None of the density measures were related to sex, age at death, or education; their crude correlations with pH, postmortem interval, time from the last clinical evaluation to death, and brainstem pathological conditions are summarized in eTable 2 in the Supplement.

We constructed a series of linear regression models adjusted for sex, age at death, and education to assess the hypothesized association between brainstem aminergic neurons and depressive symptoms. Lower density of tyrosine hydroxylase-immunoreactive neurons in the ventral tegmental area was related to higher level of depressive symptoms in the ventral tegmental area (Figure 3) (mean [SE] estimate, −0.014 [0.003]; P < .001; 16.3% increase in adjusted R²) and substantia nigra (mean [SE], estimate, −0.025 [0.012]; P = .03; 1.5% increase in adjusted R²) but not the locus ceruleus (mean [SE] estimate, −0.011 [0.008]; P = .18). There was no association for tryptophan hydroxylase-immunoreactive neurons in the dorsal raphe nucleus (mean [SE] estimate, 0.000 [0.005]; P > .99). When the ventral tegmental area and substantia nigra were included in the same model, only neurons in the ventral tegmental area were related to depressive symptoms (mean [SE] estimate, −0.012 [0.003]; P < .001). Adjustment for pH, postmortem interval, and time from the last clinical evaluation to death did not affect the association of ventral tegmental area neurons with depressive symptoms.

To further investigate how ventral tegmental area neurons were related to depressive symptoms, we analyzed different symptom domains. Lower density of tyrosine hydroxylase-immunoreactive neurons in the ventral tegmental area was associated with negative affect (mean [SE] estimate, −0.007 [0.002]; P < .001), lack of positive affect (mean [SE] estimate, −0.002 [0.001]; P = .003), and somatic symptoms (mean [SE] estimate, −0.004 [0.001]; P = .007) but not with interpersonal problems (mean [SE] estimate, −0.0003 [0.0001]; P = .17).

Discussion

During a mean of approximately 5 years of observation, depressive symptoms were assessed annually in 124 older persons who subsequently died and had a postmortem neuropathological examination. Lower density of tyrosine hydroxylase-immunoreactive neurons in the ventral tegmental area was associated with higher level of depressive symptoms, with 16.3% of the variability in symptoms explained by individual differences in density. The results suggest that the...
mesolimbic dopamine system has an important role in late-life depressive symptoms.

Animal models\textsuperscript{21-23} and human neuroimaging studies\textsuperscript{49-51} have highlighted the potential importance of mesolimbic dopaminergic mechanisms in depression, particularly for aspects of apathy and anhedonia. However, we are unaware of previous clinicopathological studies of ventral tegmental area neuronal density and depressive symptoms. Higher neuronal ratings in the substantia nigra were associated with lower likelihood of depressive symptoms in a study\textsuperscript{14} of older persons without dementia. The association in the present study between the density of nigral dopaminergic neurons and depressive symptoms is consistent with this finding and extends it by more precisely quantifying nigral neurons and by showing that the association is largely confounded by the density of dopaminergic neurons in the ventral tegmental area. We did not find the density of noradrenergic neurons in the locus ceruleus or serotonergic neurons in the dorsal raphe nucleus to be related to depressive symptoms. These null results agree with some studies\textsuperscript{42-43} but not another study\textsuperscript{44} of the locus ceruleus and are consistent with the weight of the evidence from studies of dorsal raphe nucleus neurons in depression, which have reported levels to be lower,\textsuperscript{11,45} no different,\textsuperscript{42,46} and higher\textsuperscript{47} than controls.

The factors responsible for the observed individual differences in the density of dopaminergic neurons in the ventral tegmental area are unknown, and because the present study did not include persons who died before old age, it is uncertain whether the association between dopaminergic neurons in the ventral tegmental area and depressive symptoms is specific to late life. That the association was observed after excluding individuals with dementia and controlling for postmortem markers linked to dementia suggests that some of the variability in dopamine neuron density was long-standing. Therefore, it is possible that a less populated ventral tegmental area due to neurodevelopmental or other causes occurring earlier in adulthood somehow increases vulnerability to depressive symptoms. However, a substantial subgroup had few dopamine neurons in the ventral tegmental area (eFigure 3 in the Supplement), and older age had an almost significant correlation with lower neuronal density (eTable 2 in the Supplement), suggesting that age-related pathological processes are also involved. Because Parkinson disease also involves a loss of brainstem dopaminergic neurons for uncertain reasons, it provides a useful point of comparison. Depressive symptoms are common in the disease\textsuperscript{48} and are reduced by pramipexole hydrochloride use,\textsuperscript{49,50} a dopamine agonist, suggesting that late-life depressive symptoms in some persons without Parkinson disease might also be alleviated by pharmacologic enhancement of dopamine transmission with agonists or with reuptake inhibitors such as bupropion hydrochloride\textsuperscript{53} or nomifensine maleate.\textsuperscript{52}

Neurodegenerative lesions in brainstem amnergic nuclei were related to depressive symptoms, but the associations were weak and conditional on other factors. Therefore, the presence of Lewy bodies in brainstem amnergic nuclei was related to depressive symptoms, consistent with a previous clinicopathological study\textsuperscript{47} and the prominent place of depressive symptoms in synucleinopathies, such as Parkinson disease and Lewy body disease. However, the association was only seen in those not taking antidepressants, and controlling for ventral tegmental area neuronal density eliminated it.

There was no overall association between brainstem tangles and depressive symptoms, consistent with previous research in persons without dementia\textsuperscript{48,64} and with some studies\textsuperscript{9,44} but not another study\textsuperscript{13} of persons with dementia. In contrast with previous research, the present study included clinical classification of mild cognitive impairment, and higher brainstem tangle density was associated with a higher level of depressive symptoms in persons without cognitive impairment but with a lower level of depressive symptoms in those with mild cognitive impairment. This suggests that tangles make a modest contribution to depressive symptoms before the onset of cognitive impairment but that, as cognitive problems develop, depressive symptoms may be less consistently reported, possibly because they are harder to remember or acknowledge.\textsuperscript{16,17} In addition, incipient cognitive decline may disrupt the continuity of mood states and their ability to regulate behavior. These observations agree with longitudinal data that suggest little increase in depressive symptoms during the development of mild cognitive impairment and dementia.\textsuperscript{31,53,54}

Study strengths and limitations should be noted. There was a high rate of participation in the annual clinical evaluations and brain autopsy, minimizing bias due to selective attrition. Uniform annual evaluations and the application of widely used diagnostic criteria allowed us to reliably classify mild cognitive impairment and dementia. The measure of depressive symptoms was based on a standard scale administered at regular intervals for several years, which probably enhanced assessment of the enduring tendency to be depressed while reducing measurement error and the influence of episodic fluctuations in mood. An important limitation is that the cohort was selected, so replication of these results will be important. In addition, the findings for the ventral tegmental area are based on the paranigral nucleus, and it is uncertain whether they will generalize to other subpopulations of ventral tegmental area dopaminergic neurons.\textsuperscript{55}
Brainstem Aminergic Nuclei

Original Investigation Research

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