

Exploring Brainstem Structural Abnormalities: Potential Biomarkers for Panic Disorder

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Panic disorder (PD), characterized by recurrent and intense panic attacks, presents a complex interplay between psychological and neurobiological factors. Although the amygdala and hippocampus have been studied extensively in the context of PD, the brainstem's involvement remains relatively underexplored. This study aims to address this gap by examining structural abnormalities within specific brainstem regions, including the medulla, pons, and midbrain. The study sample population comprised twenty-one adult patients diagnosed with PD and an age-gender-education-matched control group. Utilizing rigorous inclusion and exclusion criteria, confounding factors related to comorbid psychiatric conditions and brain structure abnormalities were minimized. Our findings revealed a significant reduction in medulla volume among PD patients, a finding that persisted even after correcting for individual differences in total intracranial volume. The medulla's role in cardiovascular regulation and autonomic function, coupled with its involvement in fear responses, underscores its potential significance in the pathophysiology of PD. This study elucidates the medulla's structural abnormalities as a potential biomarker for PD. Understanding the role of the brainstem in PD could pave the way for more targeted and effective interventions for this condition.

Key words: Autonomic nervous system, Brain stem, Medulla oblongata, Panic disorder

INTRODUCTION

Panic disorder (PD) is characterized by recurrent, unexpected panic attacks manifesting as sudden and intense fear or discomfort. These episodes often involve autonomic symptoms and cognitive fear-related symptoms [1]. Although several brain regions are believed to be involved in the development of PD, the brainstem plays a critical role [2]. Gorman's fear network model proposes that the amygdala, hypothalamus, and brainstem collectively contribute to fear responses, forming a network through their connections [3]. Conventionally, the central amygdala's efferent projections are

known to mediate the behavioral and autonomic aspects of conditioned fear [4]. However, when it comes to the generation of panic attacks, the brainstem takes on significant importance. Studies in monkeys conducted using autoradiographic methods have suggested multiple projection pathways from the amygdala to several brainstem nuclei associated with autonomic function [5]. The brainstem's role as an intermediary between the hypothalamus and autonomic neurons underscores its involvement in the pathophysiology of PD, potentially linked to disturbances in the autonomic nervous system [6]. In response to threats, a two-dimensional defense system is categorized into defensive avoidance and defensive approach. Proximal threats activate the periaqueductal gray (PAG) in the brainstem, creating a panic-freeze response, whereas distal threats engage the limbic system, which is associated with phobia-related responses. Serotonin, norepinephrine, and gamma-aminobutyric acid, among other neurotransmitter systems, are thought to play a role in the pathogenesis of panic and anxiety. In particular, serotonin is pivotal in the pathophysiology, with drugs

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that enhance serotonergic activity showing promise in alleviating panic symptoms by desensitizing fear networks between the amygdala and brainstem [7, 8]. Research indicates that PD is associated with diminished serotonergic activity in the raphe nuclei, indicating the role of the brainstem's serotonergic system [9, 10]. Brain imaging techniques have also shown correlations between PD and decreased 5-HT_{1A} receptor availability in the raphe and 5-HT transporter binding within the midbrain raphe [11, 12]. These findings suggest an imbalance in the serotonin system in individuals with PD, with a negative correlation between symptom severity and midbrain 5-HT transporter binding. Similarly, neuroimaging studies have reported associated abnormalities in the brainstem. Neumeister et al. [13] used positron emission tomography (PET) on patients with PD and found a pronounced decrease in 5-HT_{1A} receptor binding within the midbrain raphe in patients, implying a connection between PD and the brainstem. Other research has revealed increased midbrain activity among patients with PD, both before and after exposure to substances such as pentagastrin [14]. A study by Sakai and colleagues showed that cerebral glucose metabolism was characterized by elevated glucose utilization in the midbrain, caudal pons, and medulla of individuals with PD [15]. While research has identified structural anomalies in various brain regions within the fear network in PD, the brainstem's specific role has not been thoroughly investigated. This study aimed to explore structural abnormalities in specific brainstem regions, including the midbrain, pons, and medulla, using magnetic resonance imaging (MRI), with a focus on their contribution to the pathophysiology of PD while considering potential confounding variables.

MATERIALS AND METHODS

Subjects and procedures

The study sample consisted of twenty-one adult patients with PD, aged 18 to 65, who were recruited from the Department of Psychiatry at the Korea University Anam Hospital. All participants were right-handed, as assessed using Edinburgh Handedness Test [16]. Patients who met the criteria for the diagnosis of PD, as defined by the DSM-IV (Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition), were recruited for participation [17]. For diagnosing psychiatric disorders, the Korean version of the MINI (Mini-International Neuropsychiatric Interview) tool was administered by board-certified psychiatric professionals [18]. The exclusion criteria for patients with PD were as follows: (1) any past or current comorbid psychiatric disorders, such as mood disorders, psychotic disorders, anxiety disorders, substance-related disorders, or personality disorders; (2) intelligence quotient under 80; (3) clinically significant general and/or neurological disease

that may influence brain structure on imaging; (4) pregnancy or breastfeeding; and (5) any contraindication to undergoing MRI, such as the presence of a pacemaker. Twenty-one healthy controls matched for age ($p=0.487$), gender (same numbers), and education level ($p=0.283$) were recruited via community advertisements. All controls were screened to exclude a history of major psychiatric disorders, ensuring no participant in the healthy control group had psychiatric disorders. Following a thorough explanation of the study, all participants provided written informed consent. The study procedures were approved by the Institutional Review Board of the Korea University Anam Hospital.

Assessment for psychopathology

The intensity of participants' panic symptoms was assessed using the Panic Disorder Severity Scale (PDSS) [19]. Three different scales were used to assess anxiety symptoms: the Hamilton Anxiety Rating Scale (HAM-A), the Anxiety Sensitivity Index-Revised (ASI-R), and the State-Trait Anxiety Inventory (STAI), comprising both State (STAI-S) and Trait (STAI-T) versions [20-22].

Image acquisition

All imaging was performed using the 3-Tesla Siemens Magnetom Trio Tim System (Siemens Medical Solutions, Inc., Iselin, NJ, USA) at the Korea University Brain Imaging Center. The structural brain images were taken with a high-resolution T1-weighted MP-RAGE (magnetization prepared rapid acquisition gradient echo) sequence (1900 ms repetition time, 2.6 ms echo time, 220 mm field of view, 256×256 matrix size, 176 coronal slices without gap, 1×1×1 mm³ voxels, 16° flip angle, number of excitations=1). A radiologist reviewed T1 and fluid-attenuated inversion-recovery (FLAIR) scans to exclude overt structural abnormalities in the brain from further analysis.

Brain parcellation

Automated brain parcellation was conducted using FreeSurfer (software version 7.4.1, <http://surfer.nmr.mgh.harvard.edu>). FreeSurfer was adopted in the current study because of its well-established accuracy and reliability for automated structural brain image analysis [23, 24] and higher reproducibility compared with other methods [25]. The automated pipeline comprised motion artifact correction, removal of non-brain tissue, Talairach transformation, tissue segmentation, tessellation of the gray matter-white matter boundary, correction for topological defects, intensity normalization, surface deformation, and parcellation into anatomical regions. Visual inspection for brainstem segmentation accuracy was also performed. Total intracranial volume (TIV) was calculated with FreeSurfer and used as a covariate to correct for inter-

Table 1. Demographic data and psychological scales

	Controls (n=21)	Patients (n=21)	df	T	p-value
Age ^{a)}	41.71 (12.14)	39.29 (10.22)	40.0	0.701	0.487
Sex (male/female) ^{b)}	12/9	12/9	1	-	1.000
Education (years) ^{a)}	14.0 (2.67)	13.1 (3.25)	40.0	1.089	0.283
TIV ^{a)}	1358 (159)	1452 (126)	40.0	2.130	0.039*
HAM-A		17.67 (10.19)	-	-	-
PDSS		9.62 (4.61)	-	-	-
ASI-R		54.01 (41.83)	-	-	-
STAI-S		44.05 (7.28)	-	-	-
STAI-T		42.10 (9.44)	-	-	-

ASI-R, Anxiety Sensitivity Index-Revised; PDSS, Panic Disorder Severity Scale; HAM-A, Hamilton Anxiety Rating Scale; STAI-S, State-Trait Anxiety Inventory-State; STAI-T, State-Trait Anxiety Inventory-Trait; TIV, Total Intracranial Volume. Data are presented as means (SD). ^{a)} independent samples t-test, ^{b)} Chi-square test * p<0.05.

individual differences in brain size. Following the guidelines used by FreeSurfer, all participant data were processed on a single Linux workstation (Ubuntu 18.04, dual E5-2687W 3.10 GHz processor, 128 GB memory) to prevent unexpected data irregularities.

Brainstem volume analysis

A robust Bayesian inference algorithm was adopted to detect local variation within the brainstem subfield to segment and calculate the volumes of each subarea. The Bayesian segmentation approach based on a probabilistic atlas allows for the volumes of the medulla, pons, midbrain, and superior cerebellar peduncle to be calculated automatically [26].

Statistical analysis

Statistical analyses were performed using R software, version 4.1.2 (<https://www.r-project.org>). First, independent t-tests were performed to examine the age, education and TIV differences between groups. Second, an independent samples t-test was conducted to assess the between-group differences in the four substructures of the brainstem. A multivariate analysis of covariance (MANCOVA) was performed using the R jmv package (version 2.4.11) to assess volumetric differences while controlling for TIV. Bonferroni correction was applied to correct for multiple comparisons of four subregions [27]. The analysis included group (panic, control) as the between-subject independent factor, the volume of the regions of interest in four brainstem structures (medulla, pons, midbrain, and superior cerebellar peduncle volumes) as the dependent variable, and TIV as the covariate.

RESULTS

Demographic and clinical variables

A total of twenty-one PD patients and age-gender-education-matched healthy controls were recruited to participate in this

study. There were no significant differences in age, gender, or education between the control group and the PD group (Table 1). In the PD group, the mean PDSS score was 9.01 ± 4.74 , with a cutoff score of 8 serving as an indicator for identifying patients with PD [19]. The mean HAM-A score was 15.62 ± 10.39 , with scores within the range of 15 to 23 indicating moderate anxiety [28]. The mean STAI-S and STAI-T scores were 44.33 ± 7.02 and 42.90 ± 9.65 , respectively. For these scales, designed to assess current anxiety symptoms and propensity toward anxiety, respectively, a score of 40 is the established cutoff point [29-31]. The mean ASI-R score was 51.10 ± 17.95 . In a study of 267 patients diagnosed with PD, the mean ASI-R score was 47.41, demonstrating a notable association with functional impairment [32].

Brainstem structure

When compared with the control group, individuals diagnosed with PD were found to have significantly smaller medulla oblongata volumes (Fig. 1, 2). However, no statistically significant differences were observed in other brainstem structures (Table 2). A MANCOVA was conducted to further control for the effect of TIV. In this analysis, the medulla oblongata was still found to have a significantly lower volume in individuals diagnosed with PD (Table 3) after Bonferroni correction ($p < 0.011$, Bonferroni-adjusted threshold of $0.05 = 0.0125$).

DISCUSSION

The major finding of our study was that individuals with PD had significantly smaller average volumes of the medulla compared to healthy participants. This finding remained significant after controlling for variations in TIV. The medulla is associated with cardiovascular regulation and functions as an autonomic center. Sympathetic premotor neurons in the medulla influence sympathetic outflows via the descending efferent pathway. The

C1 adrenergic neurons in the medulla regulate sympathetic vasomotor activity and function as a sympathoexcitatory response to stressors [6, 33-35]. The medulla receives signals from various regions, including the midbrain and the dorsal raphe nucleus, and plays a role in mediating automatic fear responses and receiving projections from PAG [36-40]. When activated, neurons in the PAG influence the 5-HT_{1A} receptors in the rostral ventrolateral medulla (RVLM), which reduces sympathetic activity and lowers blood pressure. A study in cats demonstrated that injection of the 5-HT_{1A} receptor activator 8-OH-DPAT into the RVLM resulted

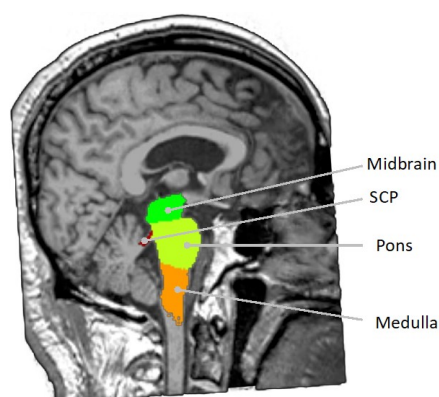


Fig. 1. Substructures of brainstem segmentation and the comparison of averaged volumes of each group.

in a reduced sympathetic response. Neurons from the dorsal raphe nucleus, which are serotonergic, innervate the C1 adrenergic neurons, and influence sympathetic activity via 5-HT_{1A} receptors. Accordingly, the RVLM can suppress stress-induced sympathetic responses by releasing 5-HT [34, 41-43]. Lactate infusion induces

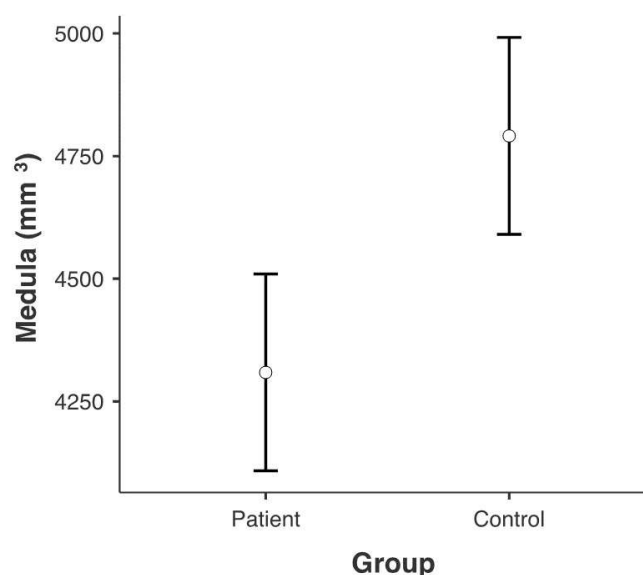


Fig. 2. Plot representing estimated marginal means (n=42, 95% confidence interval).

Table 2. The result of independent samples t-test of brainstem structures volume between PD patients and control group

	Controls (n=21)	Patients (n=21)	df	T	p-value
Medulla	4733.06 (467.29)	4367.22 (474.73)	40.0	2.517	0.016*
SCP	274.55 (40.39)	289.02 (59.83)	40.0	0.919	0.364
Midbrain	6248.38 (633.08)	6172.21 (464.26)	40.0	0.445	0.659
Pons	14504.48 (1666.58)	14337.99 (1407.40)	40.0	0.350	0.728
Whole brainstem	25760.47 (2676.07)	25166.45 (2193.95)	40.0	0.787	0.436

SCP, superior cerebellar peduncle. Data are presented as means (SD). * p<0.05.

Table 3. Summary of the multivariate analysis of covariance (MANCOVA) results

	Dependent variable	Sum of squares	df	Mean square	F	p-value
Group	Medulla	1410000	1	1410000	7.187	0.011*
	Pons	291057	1	291057	0.200	0.657
	SCP	2200	1	2200	0.850	0.362
	Midbrain	60921	1	60921	0.380	0.541
TIV	Medulla	1250000	1	1250000	6.385	0.016
	Pons	38500000	1	38500000	26.454	<0.001
	SCP	3265	1	3265	1.261	0.268
	Midbrain	6070000	1	6070000	37.796	<0.001
Residuals	Medulla	7630000	39	195540		
	Pons	56700000	39	1450000		
	SCP	100951	39	2588		
	Midbrain	6260000	39	160509		

SCP, superior cerebellar peduncle; TIV, total intracranial volume. * p<0.05.

a panic response in the forebrain, limbic structures, and brainstem, activated via the dorsomedial hypothalamus/perifornical efferent pathway. The accompanying cardiovascular response can be attributed to activation in several brainstem areas. C1 and C2 adrenergic neurons, associated with autonomic regulation, respond to lactate. Similarly, C1 neurons, particularly in the medulla, can influence cardiovascular responses, and the nucleus of the solitary tract influences the baroreflex. Tachycardia occurs because lactate stimulates the parabrachial nucleus in the brainstem, which is responsible for respiratory control [44]. Furthermore, Yoshida et al. [45] found that, in the context of conditioned fear, neurons in the RVLM that synthesize prolactin-releasing peptides influence neuroendocrine responses in animals. The medulla is an area where 5-HT can function to prevent panic-associated cardiovascular responses, and dysfunction in the RVLM can lead to exaggerated panic symptoms [44, 46].

Few neuroimaging studies have focused on specific subregional volume alterations to brainstem structures in PD; however, alterations to these structures have been observed [47]. Fujiwara et al. [48] reported an increased dorsal midbrain volume, including the PAG, in patients compared to healthy controls, and these volume changes might reflect the severity. Gray matter volume has been measured using voxel-based morphometry, with increases noted in the midbrain and pons [49]. Another study demonstrated increased gray matter volume in the midbrain and rostral pons [50]. However, to our knowledge, no studies to date have investigated morphological changes in the medulla in the PD context. The current study showed that individuals with PD had significantly lower brain volume in the medulla relative to the control group. These results align with our understanding of the neurobiology related to panic attacks.

The present study has several limitations that should be acknowledged. First, the sample size is small, mainly because of the strict inclusion and exclusion criteria. Patients with PD often have comorbid psychiatric disorders; however, comorbid psychiatric conditions are known to be associated with structural abnormalities in the brain. We believe that the absence of a comorbid anxiety disorders may enhance the validity of this study by minimizing potential confounding factors that could have affected the structural data. Nevertheless, the generalizability of this study's findings is inherently constrained by the limited sample size, which may not provide a comprehensive representation of the larger population. Second, the limited number of samples available made it impossible to analyze volume differences according to symptom severity. Finally, this study was limited by its cross-sectional design based on retrospective reports, which rendered temporal relationships and causative conclusions indiscernible. Therefore, subse-

quent investigations must engage in prospective study modalities to substantiate and expand upon the preliminary insights derived from this study. Future systematic research with a larger sample size is needed. In conclusion, structural abnormalities in the medulla may play a central role in the underlying pathophysiology of PD. The limited sample size in this study made it difficult to conduct further analysis to identify relationships between symptom severity and structure volume. Structural abnormalities in the amygdala and hippocampus have been suggested as potential biomarkers for PD; however, despite some limitations, our findings suggest the possibility of structural abnormalities in the medulla as another potential biomarker. Future studies could benefit from combining structural MRI with functional imaging techniques and neurotransmitter research [51].

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