PARKINSON'S DISEASE AND THE HABENULA

An Ultra-High Field MRI Study Beyond the Basal Ganglia



Bedia Samanci

Propositions

1. Parkinson's disease cannot be fully understood within the boundaries of the nigrostriatal dopaminergic system. (This thesis).

2. Habenular volume alterations in early Parkinson's disease are not explained by depressive symptom severity. (This thesis).

3. Discordant lateralization across imaging modalities does not necessarily indicate methodological inconsistency; different modalities may capture distinct aspects of the same disease process. (This thesis).

4. Network-level alterations in Parkinson's disease may be detectable even when conventional diffusion metrics remain unchanged. (This thesis).

5. Understanding Parkinson's disease beyond the basal ganglia may ultimately improve how patients are diagnosed, stratified, and treated. (Impact)

6. The future of neurodegenerative disease prevention lies in combining disease-modifying therapies with effective management of modifiable risk factors.

7. The rigid distinction between "motor" and "non-motor" or "neurological" and "psychiatric" manifestations in movement disorders is a historical artifact that hinders comprehensive patient care.

8. Disease-modifying therapies will have limited societal impact unless advances in treatment are matched by advances in early detection and equitable access to care.

9. "Scientific observation is always a polemic against the obvious." – *Gaston Bachelard*

10. "The map is not the territory." – *Alfred Korzybski*

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SUMMARY

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by motor symptoms such as bradykinesia, rigidity, and tremor, resulting from dopaminergic neuronal loss in the substantia nigra pars compacta. However, PD extends beyond the dopaminergic system, involving multiple neurotransmitter pathways and brain regions that contribute to its broad range of non-motor symptoms, including depression, anxiety, and cognitive impairment. Recent research has drawn attention to the habenula, a small epithalamic nucleus that plays a crucial role in mood regulation, reward processing, and motivation. Its unique anatomical position between the limbic system, basal ganglia, and brainstem makes it an intriguing structure for understanding the complex pathophysiology of PD.

This thesis provides a comprehensive investigation of the habenula's structural and functional alterations in PD, using advanced neuroimaging techniques at ultra-high field strength (7 T MRI). By integrating volumetric, diffusion, and resting-state functional analyses, this work explores the habenula's potential contribution to both motor and non-motor symptoms and evaluates its value as a possible biomarker of early-stage PD.

Chapter 2 presents a narrative review of the anatomy, function, and clinical relevance of the habenula, with a particular focus on its role in mood disorders and PD. The chapter summarizes the current understanding of habenular neurocircuitry, emphasizing its connections with monoaminergic nuclei and the basal ganglia, and discusses how its dysregulation may contribute to depressive and motivational symptoms in PD. Despite growing evidence for habenular involvement in affective disorders, the review highlights the paucity of imaging studies investigating this structure in PD, thereby defining a clear gap that the following chapters address.

Chapter 3 examines habenular volume alterations in PD using high-resolution 7 T structural MRI. In a cohort of 104 PD patients and 44 healthy controls from the TRACK-PD study, manual segmentation revealed significantly larger mean and right habenular volumes in PD, independent of depressive symptom severity. These results suggest that habenular changes occur even in PD patients without clinical depression, implying a role beyond mood regulation, possibly linked to motor or compensatory mechanisms. This study provides the first high-field in vivo evidence of habenular volumetric alterations in PD.

Chapter 4 explores the structural connectivity changes of the habenula using diffusion MRI. In 84 PD patients and 38 controls, diffusion tensor imaging and probabilistic tractography revealed enhanced structural connectivity between the habenula and regions such as the hippocampus, fusiform gyrus, pallidum, and nucleus accumbens. Moreover, increased connectivity between the right habenula and the left cingulate isthmus correlated with depressive symptom scores. These findings suggest that early-stage PD is associated with altered

habenular network integration, potentially reflecting compensatory reorganization or early neurodegenerative changes within fronto-limbic and basal ganglia circuits.

Chapter 5 investigates functional connectivity alterations of the habenula and the whole brain in early PD using resting-state fMRI at 7 T. Compared with 45 healthy controls, 104 early-stage PD patients showed increased connectivity in motor and cognitive networks involving the paracentral lobule, supplementary motor area, middle cingulate gyrus, and cerebellum. The left habenula exhibited increased connectivity with the right middle temporal and angular gyri, while dopaminergic medication dose correlated positively with connectivity between the postcentral gyrus and cerebellum. These findings highlight early motor and cognitive network disruptions and suggest these regions may serve as potential markers of PD-related neurobiological changes.

Together, the studies in this thesis provide converging evidence that the habenula undergoes distinct volumetric and connectivity alterations in PD, detectable even in the early disease stages and independent of overt depression. These changes may represent early markers of non-dopaminergic involvement, offering new insights into the network-level mechanisms underlying PD's motor and non-motor symptoms.

In conclusion, by combining ultra-high-field MRI, advanced diffusion imaging, and functional connectivity analyses, this thesis advances our understanding of the habenula's role in PD. The results emphasize that PD is not merely a disorder of dopaminergic degeneration but a network disease involving intricate interactions among monoaminergic, limbic, and cortical systems. The habenula emerges as a key node within this network, representing a potential biomarker for addressing the complex clinical manifestations of PD.

SAMENVATTING

De ziekte van Parkinson (Parkinson's disease, PD) is een progressieve neurodegeneratieve aandoening die voornamelijk wordt gekenmerkt door motorische symptomen zoals bradykinesie, rigiditeit en tremor, als gevolg van verlies van dopaminerge neuronen in de substantia nigra pars compacta. PD beperkt zich echter niet tot het dopaminerge systeem, maar omvat meerdere neurotransmittersystemen en hersengebieden die bijdragen aan het brede spectrum van niet-motorische symptomen, waaronder depressie, angst en cognitieve stoornissen. Recent onderzoek heeft de aandacht gevestigd op de habenula, een kleine epithalamische kern die een cruciale rol speelt bij stemmingsregulatie, beloningsverwerking en motivatie. Haar unieke anatomische positie tussen het limbische systeem, de basale ganglia en de hersenstam maakt de habenula tot een intrigerende structuur voor het begrijpen van de complexe pathofysiologie van PD.

Dit proefschrift biedt een uitgebreid onderzoek naar structurele en functionele veranderingen van de habenula bij PD, met behulp van geavanceerde neuroimagingtechnieken bij ultrahoge veldsterkte (7 T MRI). Door volumetrische, diffusie- en rusttoestandfunctionele analyses te integreren, onderzoekt dit werk de mogelijke bijdrage van de habenula aan zowel motorische als niet-motorische symptomen en evalueert het de waarde ervan als mogelijke biomarker voor PD in een vroeg stadium.

Hoofdstuk 2 presenteert een narratief overzicht van de anatomie, functie en klinische relevantie van de habenula, met bijzondere aandacht voor haar rol bij stemmingsstoornissen en PD. Het hoofdstuk vat de huidige kennis over de neurocircuits van de habenula samen, waarbij de nadruk ligt op haar verbindingen met monoaminerge kernen en de basale ganglia, en bespreekt hoe ontregeling ervan kan bijdragen aan depressieve en motivationele symptomen bij PD. Ondanks het toenemende bewijs voor betrokkenheid van de habenula bij affectieve stoornissen, benadrukt het overzicht het geringe aantal beeldvormingsstudies waarin deze structuur bij PD is onderzocht, waarmee een duidelijke kennislacune wordt gedefinieerd die in de daaropvolgende hoofdstukken wordt behandeld.

Hoofdstuk 3 onderzoekt veranderingen in het volume van de habenula bij PD met behulp van structurele MRI met hoge resolutie bij 7 T. In een cohort van 104 patiënten met PD en 44 gezonde controles uit de TRACK-PD-studie liet handmatige segmentatie significant grotere gemiddelde en rechtszijdige habenulavolumes zien bij PD, onafhankelijk van de ernst van depressieve symptomen. Deze resultaten suggereren dat veranderingen van de habenula ook optreden bij patiënten met PD zonder klinische depressie, wat wijst op een rol die verder reikt dan stemmingsregulatie en mogelijk verband houdt met motorische of compensatoire mechanismen. Deze studie levert het eerste in-vivo bewijs bij hoge veldsterkte voor volumetrische veranderingen van de habenula bij PD.

Hoofdstuk 4 onderzoekt veranderingen in de structurele connectiviteit van de habenula met behulp van diffusie-MRI. Bij 84 patiënten met PD en 38 controles toonden diffusion tensor imaging en probabilistische tractografie een versterkte structurele connectiviteit aan tussen de habenula en gebieden zoals de hippocampus, gyrus fusiformis, het pallidum en de nucleus accumbens. Bovendien correleerde een toegenomen connectiviteit tussen de rechter habenula en de linker isthmus van de gyrus cinguli met scores voor depressieve symptomen. Deze bevindingen suggereren dat PD in een vroeg stadium gepaard gaat met een veranderde integratie van de habenula binnen hersennetwerken, mogelijk als weerspiegeling van compensatoire reorganisatie of vroege neurodegeneratieve veranderingen binnen frontolimbische circuits en circuits van de basale ganglia.

Hoofdstuk 5 onderzoekt veranderingen in de functionele connectiviteit van de habenula en de gehele hersenen bij vroege PD met behulp van resting-state fMRI bij 7 T. Vergeleken met 45 gezonde controles vertoonden 104 patiënten met PD in een vroeg stadium een verhoogde connectiviteit in motorische en cognitieve netwerken waarbij de lobulus paracentralis, de supplementaire motorische schors, de middelste gyrus cinguli en het cerebellum betrokken waren. De linker habenula vertoonde een verhoogde connectiviteit met de rechter middelste temporale en angulaire gyri, terwijl de dosis dopaminerge medicatie positief correleerde met de connectiviteit tussen de gyrus postcentralis en het cerebellum. Deze bevindingen benadrukken vroege verstoringen van motorische en cognitieve netwerken en suggereren dat deze gebieden mogelijk kunnen dienen als markers van PD-gerelateerde neurobiologische veranderingen.

Gezamenlijk leveren de studies in dit proefschrift convergerend bewijs dat de habenula bij PD duidelijke volumetrische en connectiviteitsveranderingen ondergaat, die zelfs in vroege stadia van de ziekte en onafhankelijk van manifeste depressie detecteerbaar zijn. Deze veranderingen kunnen vroege markers vormen van betrokkenheid van niet-dopaminerge systemen en bieden nieuwe inzichten in de netwerkmechanismen die ten grondslag liggen aan de motorische en niet-motorische symptomen van PD.

Concluderend draagt dit proefschrift, door ultrahoge-veld-MRI, geavanceerde diffusiebeeldvorming en analyses van functionele connectiviteit te combineren, bij aan een beter begrip van de rol van de habenula bij PD. De resultaten benadrukken dat PD niet slechts een aandoening van dopaminerge degeneratie is, maar een netwerkziekte waarbij complexe interacties tussen monoaminerge, limbische en corticale systemen betrokken zijn. De habenula komt naar voren als een belangrijk knooppunt binnen dit netwerk en vertegenwoordigt een potentiële biomarker voor het benaderen van de complexe klinische manifestaties van PD.



CHAPTER 1

GENERAL INTRODUCTION

GENERAL OVERVIEW OF PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive neurodegenerative disorder primarily affecting the motor system due to the degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta, leading to a reduction of dopamine levels in the striatum. This dysfunction within the basal ganglia network results in hallmark motor symptoms of PD, which include bradykinesia, rigidity, resting tremor, and postural instability. However, the clinical presentation of PD extends beyond motor impairments, encompassing a broad spectrum of non-motor symptoms such as mood disorders, cognitive deficits, sleep disturbances, and autonomic dysfunction. These symptoms often precede the onset of motor impairments, indicating that PD pathology extends beyond the basal ganglia and involves widespread brain regions [1] (Figure 1).

While the traditional understanding of PD has focused on the dopaminergic system, increasing evidence suggests that other neurotransmitter systems, such as the serotonergic and noradrenergic pathways, also contribute to the disease pathophysiology [1] (Figure 1). Emerging research has pointed to the habenula, a small structure involved in regulating reward processing, mood, and aversion, as a key player in modulating these neurotransmitter systems and influencing non-motor aspects of PD such as mood disorders and altered reward processing [2 3 4] Despite its importance, the habenula's contribution to PD pathophysiology remains underexplored, particularly regarding its structural and functional connectivity changes.

The Role of the Habenula in Brain Function

The habenula, located in the dorsal diencephalon, is a critical structure that connects the limbic system, basal ganglia, and brainstem monoaminergic nuclei. In normal brain function, the habenula acts as a negative reward signal, inhibiting dopaminergic activity when an expected reward is not received. This mechanism plays a crucial role in motivational learning, stress response, and emotion regulation [5]. Research on habenular anatomy has highlighted its unique connectivity patterns, which facilitate its role as an interface between limbic, motor, and autonomic systems. This small structure has gained significant attention in recent years due to its role especially in mood disorders, particularly depression, addiction, and neurodegenerative diseases [6 7 8 9 2] . Research efforts have increased, focusing on how this small but functionally crucial brain region becomes dysregulated in neuropsychiatric disorders.

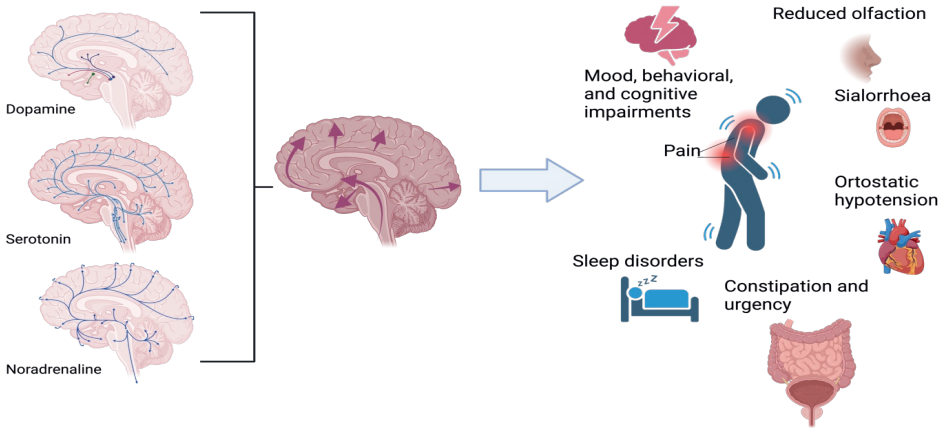

Figure 1. Impairment of key neurotransmitter pathways, including dopamine, serotonin, and noradrenaline, is observed in Parkinson's disease. The disease progresses from the lower brainstem to widespread cortical regions and causes neurodegeneration beyond the basal ganglia. In every stage, motor symptoms (bradykinesia, rigidity, tremor, and postural instability) and non-motor symptoms significantly impact quality of life of the patients.

Anatomically, it is divided into the medial habenula (MHb) and lateral habenula (LHb), each with distinct functions. The LHb primarily consists of glutamatergic neurons that receive inputs from the basal ganglia and limbic forebrain and project onto GABAergic interneurons in the ventral tegmental area (VTA) and rostromedial tegmental nucleus. This pathway inhibits dopaminergic (VTA) and serotonergic (dorsal raphe nucleus) activity, making the LHb a critical hub in modulating monoamine transmission. Functionally, the LHb, also known as "disappointment center", plays a key role in motivation, stress adaptation, sleep, encoding aversive stimuli and negative reward prediction errors, meaning it is activated when an expected reward is not received [10 11 5 3]. This negative reward signaling is essential for adaptive behavior, allowing individuals to modify their actions based on negative experiences. However, hyperactivity of the LHb has been implicated in depression, anhedonia, and cognitive impairment, highlighting its role in mood regulation and cognition.

The MHb, composed of glutamatergic, cholinergic, and substance P neurons, projects to the interpeduncular nucleus (IPN) and influences the dopaminergic and serotonergic systems via IPN-VTA and IPN-dorsal raphe pathways. It plays a role in fear, anxiety, and nicotine addiction and has been implicated in depression. However, research on the MHb is limited due to its small size and unclear clinical significance [10].

These findings highlight the need for further clinical research to understand its involvement in mood disorders, cognition, motor involvement, and potential therapeutic implications.

Habenula Dysfunction in Mood Disorders and PD

Neuroimaging studies have revealed volumetric asymmetries and functional connectivity patterns in the habenula that differ between healthy individuals and those with mood disorders. These differences are hypothesized to reflect the habenula's involvement in the pathogenesis of depression and anxiety [6 12 13]. Postmortem analyses have also shown reduced habenular volume in individuals with mood disorders, providing further evidence of its role in affective processing [14].

Depression is one of the most common non-motor symptoms of PD, affecting up to 50% of patients. The relationship between habenular dysfunction and PD-associated mood disorders has recently gained attention. Recent studies suggest that habenula dysfunction contributes to the pathology of PD, particularly in the development of non-motor symptoms such as mood disorders and impulse control disorders [15 4 2]. The hyperactivity of the LHb has been linked to PD-related depression due to its excessive inhibition of dopaminergic and serotonergic neurons. Research suggests that excessive inhibition of dopamine and serotonin release via an overactive LHb contributes to depressive symptoms in PD [3].

Moreover, changes in habenular neurotransmission are thought to exacerbate motor dysfunction in PD. Studies in animal models have shown that GABAergic and glutamatergic dysregulation in the LHb influences both dopamine and serotonin release, affecting movement initiation and emotional processing [16]. Additionally, cognitive impairment in PD has been linked to alterations in habenular connectivity with the prefrontal cortex and limbic system. Lesions of the LHb in rat models have been shown to improve working memory and reduce depressive-like behaviors in PD [17].

While the habenula has traditionally been overlooked in PD research, recent advances in neuroimaging have allowed for more detailed investigations of its structural and functional changes in the disease. These findings suggest that the habenula's alterations are closely linked to the diverse clinical manifestations of PD and highlight the need for larger neuroimaging studies focusing on the role of habenula in PD to better understand this structure's role in the disease.

AIMS AND SCOPE OF THE THESIS

While the degeneration of dopaminergic neurons in the SN pars compacta has been well established as the hallmark pathology of PD, increasing evidence suggests that non-dopaminergic systems, including the habenula, may play a significant role in early diagnosis and disease progression. Despite its critical role, the involvement of the habenula in PD remains underexplored.

This thesis aims to provide a comprehensive investigation of habenular alterations in PD using high-resolution MRI techniques. Specifically, the thesis focuses on quantifying habenular volume changes, assessing white matter integrity using diffusion MRI, and examining functional connectivity through resting-state fMRI. By integrating these neuroimaging approaches, the thesis seeks to provide a comprehensive understanding of how the habenula contributes to the complex clinical spectrum of PD and aims to identify potential neuroimaging biomarkers of early-stage PD.

The overarching objective of this thesis is to elucidate the role of the habenula in the pathophysiology of PD, particularly in relation to its structural and functional alterations during early disease stages. Given the habenula's regulatory influence on monoaminergic systems and its involvement in mood and cognitive processing, we hypothesize that habenular abnormalities may contribute to both motor and non-motor symptoms of PD. Utilizing an advanced neuroimaging approach, including high-resolution structural MRI, DTI, and rs-fMRI, this thesis investigates whether specific imaging biomarkers related to habenular integrity and connectivity can differentiate PD patients from HCs and correlate with clinical symptoms.

The objectives of this work were:

Objective 1: To evaluate structural volume changes of the habenula in early-stage PD using high-resolution 7T MRI.

The hypothesis was PD patients exhibit reduced habenular volume, which correlates with motor and non-motor symptom severity.

Objective 2: To assess microstructural integrity and structural connectivity of the habenula using DTI.

The hypothesis was early-stage PD is associated with altered diffusion metrics such as fractional anisotropy, mean diffusivity and structural connectivity in habenular tracts, reflecting early neurodegenerative processes.

Objective 3: To examine functional connectivity between the habenula and key brain networks using resting-state fMRI.

The hypothesis was functional dysconnectivity between the habenula and cortical-subcortical regions is present in early PD and is associated with motor and non-motor symptoms.

OUTLINE OF THIS THESIS

- **Chapter 2** provides a comprehensive review of habenular anatomy, function, and its implications in mood disorders and PD. It discusses the habenula's anatomical and functional properties, its connections with the basal ganglia, limbic system, and monoaminergic brainstem nuclei, and its potential role in modulating motor, cognitive, and affective symptoms in PD. Furthermore, the chapter examines the current state of neuroimaging studies on the habenula in both human and animal models of PD, identifying gaps in knowledge that this thesis aims to address.
- **Chapter 3** investigates the volume changes of the habenula in early-stage PD patients compared to healthy controls using high-resolution 7T MRI and the relationship with symptomatology.
- **Chapter 4** explores the habenular microstructural changes and structural connectivity alterations in early-stage PD patients using diffusion tensor imaging. It aims to understand the early structural changes and their relationship with motor and non-motor symptoms in PD.
- **Chapter 5** focuses on the habenular and whole-brain functional connectivity changes in early-stage PD using resting-state fMRI.
- **Chapter 6** provides a summary of the main findings of all chapters and a general discussion.
- **Chapter 7** discusses the broader impact of the findings, exploring potential clinical applications and directions for future investigations.

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CHAPTER 2

The habenula in Parkinson's disease: Anatomy, function, and implications for mood disorders - A narrative review

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ABSTRACT

Parkinson's disease (PD), a widespread neurodegenerative disorder, often coexists with mood disorders. Degeneration of serotonergic neurons in brainstem raphe nuclei have been linked to depression and anxiety. Additionally, the locus coeruleus and its noradrenergic neurons are among the first areas to degenerate in PD and contribute to stress, emotional memory, motor, sensory, and autonomic symptoms. Another brain region of interest is habenula, which is especially related to anti-reward processing, and its function has recently been linked to PD and to mood-related symptoms. There are several neuroimaging studies that investigated role of the habenula in mood disorders. Differences in habenular size and hemispheric symmetry were found in healthy controls compared to individuals with mood disorders. The lateral habenula, as a link between the dopaminergic and serotonergic systems, is thought to contribute to depressive symptoms in PD. However, there is only one imaging study about role of habenula in mood disorders in PD, although the relationship between PD and mood disorders is known. There is little known about habenula pathology in PD but given these observations, the question arises whether habenular dysfunction could play a role in PD and the development of PD-related mood disorders. In this review, we evaluate neuroimaging techniques and studies that investigated the habenula in the context of PD and mood disorders. Future studies are important to understand habenula's role in PD patients with mood disorders. Thus, new potential diagnostic and treatment opportunities would be found for mood disorders in PD.

1. INTRODUCTION

Parkinson's disease (PD) is a common degenerative neurological condition with an increasing incidence rate among adults 50 years and older [1]. One of the key neuropathological hallmarks is the progressive dopaminergic neuron loss within the substantia nigra pars compacta (SNc), which causes dopamine deficiency in the basal ganglia [2] and the emergence of typical PD motor symptoms, including bradykinesia and rigidity.

PD is often accompanied by non-motor mood disorders, such as depression, anxiety, apathy, and impulse control disorders [3-9]. The average estimated prevalence of depression and anxiety ranged from 2.7–90% and 6–55% in PD, respectively [10,11]. Degeneration of SNc dopaminergic neurons is accompanied by the loss of other monoaminergic neurons. Degeneration of serotonergic (5-hydroxytryptamine; 5-HT) neurons in the brainstem raphe nuclei has been linked to depression and anxiety. The locus coeruleus and its noradrenergic neurons are among the first areas to degenerate in PD and contribute to stress, emotional memory, motor, sensory, and autonomic symptoms. Another brain region of interest is the habenula, which is an epithalamic structure especially related to anti-reward processing, and its function has recently been linked to PD and in particular to mood-related symptoms.

There are several neuroimaging studies that investigated the role of the habenula in mood disorders. Differences in habenular size and hemispheric symmetry were found in healthy controls when compared to individuals with a history of depression [12-14]. A postmortem study also found decreased habenular volume in individuals with depression [15].

There is relatively little known about habenula pathology in PD, but given these observations, the question arises whether habenular dysfunction might have a role in PD and the development of PD-related mood disorders. This manuscript presents a narrative review wherein we selectively examine and synthesize existing literature to provide a comprehensive overview of the role of the habenula in PD, with a particular focus on its association with mood disorders. Our literature search strategy involved a thorough examination of relevant studies and reviews published in major databases, including PubMed, Scopus, and Web of Science. Specifically, the literature search was conducted on January 1, 2023, using the key terms "habenula," "Parkinson's Disease," "mood disorders," "neuroimaging," and "neuropathology" to ensure a focused yet comprehensive coverage of the subject. The selection of literature was guided by the relevance to the habenula's anatomical and functional aspects, its role in PD, and its implications in mood disorders as observed in PD patients. Our review primarily encompasses neuroimaging studies, both in human subjects and animal models, to elucidate the habenula's involvement in PD-related mood disorders, alongside referencing key pathological and clinical studies that provide context to these imaging findings. The intent of this review is to collate and discuss the current understanding of the habenula in the context of PD, highlighting the gaps in knowledge and suggesting directions for future research.

2. ANATOMY AND CIRCUITRY OF THE HABENULA

The habenula is a bilateral epithalamic structure with boundaries formed anteriorly by the stria medullaris of the thalamus, posteroventrally by the posterior commissure, medially by the third ventricle, and dorsolateral by the thalamus (Figure 1). The habenular nuclei consist of medial (MHb) and lateral (LHb) subdivisions, which are connected via the habenular commissure. They are components of the dorsal diencephalic conduction system, which links the forebrain and brainstem. Radiological and histological investigations have identified both the LHb and MHb, with the LHb being considerably larger than the MHb [16,17]. The habenular nuclei receive inputs from the forebrain via the stria medullaris. The fasciculus retroflexus contains the habenular efferent projections to the midbrain.

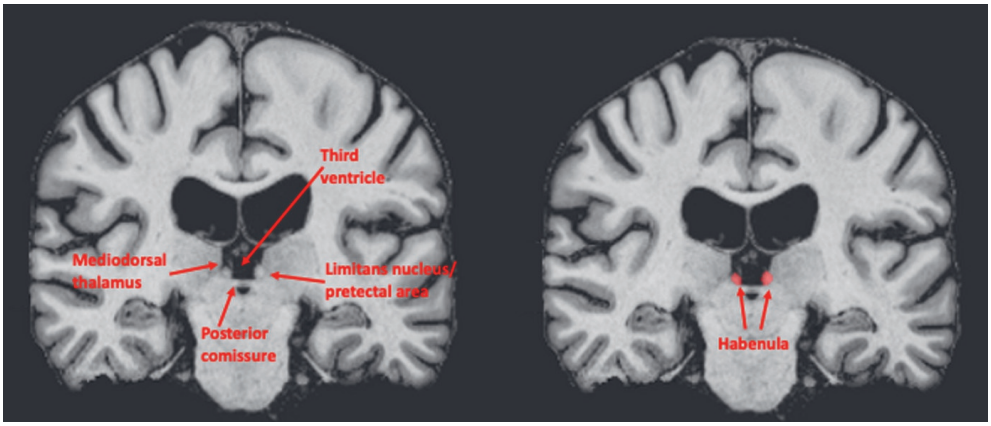

Figure 1. Coronal MRI sections showing the habenula and the local anatomical landmarks for manual segmentation on 7T MRI from TRACK-PD Study.

The medial septum and limbic areas provide inputs to the MHb, and the diagonal band of Broca projects to the interpeduncular nucleus, which in turn targets the midbrain raphe nuclei, including the dorsal and median raphe and the dorsal tegmental region [18] (Figure 2). The frontal cortices, thalamus, hypothalamus, and globus pallidus internus (GPi) are the primary sources of projections to the LHb [19] (Figure 2), and LHb can be further divided into a lateral and medial subdivision. The lateral LHb (LHb-L) receives excitatory inputs from the border zone of the globus pallidus and projects downward to the rostromedial tegmental nucleus (RMTg). These neurons are mainly glutamatergic and regulate DA neuronal activity predominantly by activating GABAergic neurons in the ventral tegmental area (VTA) and SNc. Both LHb-L and RMTg also project to the dorsal and median raphe nuclei. The medial LHb (LHb-M) receives inputs from the prefrontal cortex, ventral pallidum, lateral preoptic area, lateral hypothalamic area, lateral septum, and diagonal band nuclei, and primarily targets 5-HT neurons of the dorsal and median raphe nuclei [20].

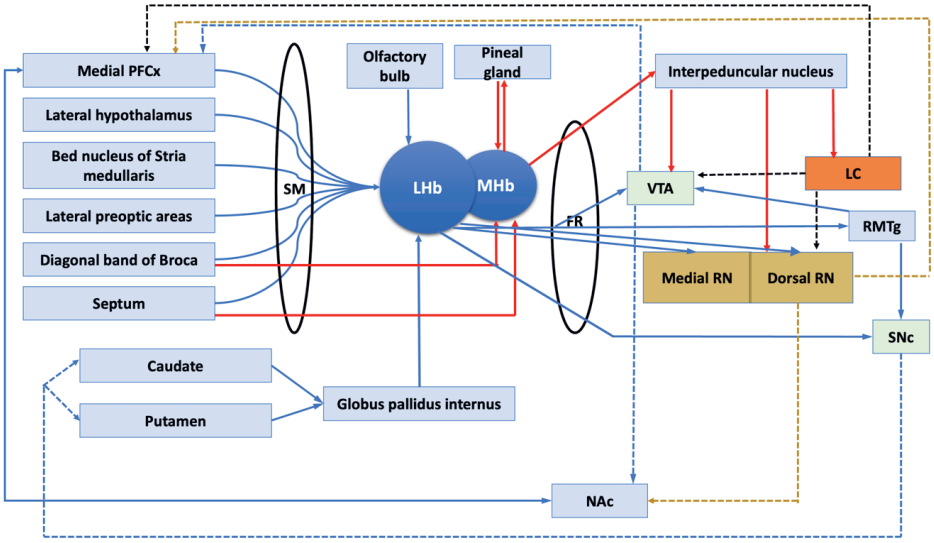

Figure 2. Figure demonstrating inputs to and from the lateral and medial habenula. Blue arrows show the inputs and outputs of the lateral habenula; red arrows are the inputs and outputs of the medial habenula; light green boxes are the dopaminergic system; yellow boxes show the serotoninergic system, and orange box is the norepinephrine system. Dotted blue lines are the dopaminergic projections, dotted yellow lines are serotoninergic projections, and dotted black lines are norepinephrine projections.

Abbreviations: PFCx: Prefrontal cortex, SM: Stria medullaris, mHb: Medial habenula, lHb: Lateral habenula, FR: Fasciculus retroflexus, RMTg: Rostro-medial tegmental nucleus, SNc: Subtantia nigra pars compacta, VTA: Ventral tegmental area, LC: Locus coeruleus, RN: Raphe nucleus, NAc: Nucleus accumbens.

Many of the neural circuits, known to regulate dopamine and serotonin release and to be associated with depression, anxiety, and other neuropsychiatric disorders, are linked to the striatum, a key component in mood regulation and motor control [21]. In rodents, it has been determined that subgroups of striosomal neurons project directly to the SNc [22] and indirectly to the LHb via the pallidum [23,24]. Hong et al. [25] recently demonstrated a functional circuit between striosomes and LHb neurons in macaques that excites and inhibits LHb neurons. This circuit originates from dispersed striosomes in the caudate nucleus and putamen, with output converging on a relatively constrained region, the LHb.

Electrical stimulation of the LHb reduces the firing activity of the dopaminergic neurons [26]. LHb lesions are associated with dopaminergic neuronal disinhibition in the SNc [27]. Moreover, dopamine neurotransmission can modulate LHb activity, particularly via D4 receptor activation. It was hypothesized that D4 receptor-mediated excitation of (RMTg-projecting) LHb neurons constitutes an indirect inhibitory feedback pathway that inhibits continued dopaminergic neuron activity [28]. In addition, Shen et al. [29] showed single-pulse and tetanic electrical stimuli of VTA and SNc to elicit suppression and increase the firing activity of LHb neurons, respectively.

Studying the habenula remains challenging because of the relatively small size (approximately 5-9 mm diameter and 31 mm^3 volume) and deep location. This is further complicated by hemispheric differences in volume [30], asymmetric functional connectivity [31], and asymmetric activation as a response to stress [32]. A postmortem volumetric analysis revealed that the human habenula is significantly larger on the left side compared to the right side in both sexes, caused by enlargement of the left LHb [30]. In line with these findings, a volumetric study utilizing high-resolution MRI revealed a tendency for increased left habenular volume in both healthy controls (19.5 mm^3 for left, 17.0 mm^3 for right) and individuals with various neuropsychiatric disorders (19.1 mm^3 for left, 15.6 mm^3 for right in patients with major depressive disorder (MDD); 19.4 mm^3 for left, 16.8 mm^3 for right in patients with medicated bipolar disorder) [12]. The human habenula appears to have an asymmetry in functional connectivity as well. The left habenula was more functionally coupled with the right parahippocampal region and the right habenula towards the SNc/VTA [31].

3. THE FUNCTION OF THE HABENULA

The human habenula is essential for (anti-)reward processing. Additionally, the habenula processes information from limbic regions and the GPi to produce goal-directed behavior. Research on non-human primates indicates that the LHb plays a crucial role in modulating dopaminergic neurons in the midbrain [33,34]. LHb neurons are activated by negative reward prediction error and inhibited positive reward prediction error, which results in inhibition and excitation of DA neurons, respectively. A comparable neuronal response profile is observed in response to the outcomes, with LHb neurons responding with strong excitation to negative outcomes and inhibition to positive outcomes [34]. The activity of LHb neurons is influenced by prediction and outcome mismatch, as shown by weaker excitatory responses when negative outcomes are entirely predictable, as compared to ambiguous outcomes. Additionally, greater negative prediction errors are correlated with a higher firing rate of LHb neurons. Therefore, the LHb responds to negative prediction errors and rewards, specifically negative motivational value, with the opposite encoding direction of dopaminergic neurons.

The challenge of detecting signal changes in small brain structures such as the habenula has restricted the use of task-based functional MRI (fMRI) in neuroimaging studies aiming to elucidate habenular function in humans. Using fMRI, Ullsperger and von Cramon [35] conducted two experiments to explore the brain activity associated with negative and positive feedback in a dynamically adaptive motion prediction task. They discovered that receiving negative feedback led to the activation of the rostral cingulate motor area, the inferior anterior insula, and the habenular complex. Shepard et al. [36] demonstrated that projections from the habenula to the midbrain could cause a temporary but almost complete suppression of dopaminergic neuron activity at the population level. This effect is comparable to what is seen in primates following an unanticipated negative outcome. Using fMRI, Salas et al. [37] found

increased activity of the human habenula during negative prediction error events. Compared to reward anticipation, analysis of the regions of interest showed that anticipation of punishment increases left hemispheric habenula activity. However, the anticipation of rewarding rather than neutral outcomes had no effect on the left habenula signal change [38]. Similar to primates, the human habenula is sensitive to probabilities. Cues indicating a high likelihood of losing points in a guessing game caused greater activation in the left-hemispheric habenula, but not in the right hemisphere [39]. Another study showed that when presented with cues indicating a high versus low probability of upcoming punishment or monetary rewards, the habenula demonstrated bilateral activation when anticipating punishment, but a decrease in activation when anticipating monetary rewards [40]. Weidacker and colleagues [41] observed reduced activity in the habenula, in both hemispheres, when participants were presented with loss avoidance outcomes, which acted as a reward in comparison to neutral and monetary loss. The authors reported greater left habenula activity during monetary loss versus loss avoidance outcomes. When participants anticipated a loss compared to neutral outcomes, greater functional connectivity was found between the right habenula, subcallosal cingulate, and hippocampus. Yoshino et al. [42] demonstrated that the bilateral habenula were active during aversive outcomes and that this activation was linked to aversive prediction errors. Finally, a study by Shelton et al. [43] mapping the activation of the habenula after a painful thermal stimulus indicated a role in pain perception. It was later speculated that since the LHb controls the raphe nuclei, it may regulate pain-associated depression. Li et al. [44] found an increase in LHb activity and β calmodulin-dependent protein kinase type II expression and a reduction in neuronal activity in the dorsal raphe nucleus and 5-hydroxy indole acetic acid (5-HIAA)/5-HT ratios in rats with chronic constriction injury of the sciatic nerve, and these changes were accompanied by depression-like behaviors. The authors concluded that increases in the LHb-dorsal raphe nucleus pathway activity were a shared neurobiological mechanism for both pain and depression, which might clarify their co-occurrence.

In different animal models of depression, it has been demonstrated that the LHb becomes hyperactive [45-48]. In both acute and congenital learned helplessness in rats, VTA-projecting habenular neurons exhibit increased synaptic activity [49]. In several human studies, habenula activity is increased and correlates positively with depression rating scales among humans with depression [50,51]. In the 6-hydroxydopamine rat model of PD, Luo et al. [23] discovered increased activity in the LHb of rats with depressive-like behaviors. Additionally, LHb lesions reduced depressive-like behavior in the forced swim test of these PD rats expressed by a reduction in the duration of immobility and an increase in the duration of climbing and caused the increased 5-HT levels in raphe nuclei. The authors concluded that LHb mediates the effects of dopaminergic neurons in the substantia nigra and serotonergic neurons in the raphe nuclei, thereby contributing to depressive-like behavior in PD rats.

The habenula is a relatively new DBS target to treat various psychiatric disorders, including depression, and the procedure is mostly experimental for now. Habenula and stria medul-

laris stimulation, which is the primary afferent of the LHb, has been shown to alleviate depressive symptoms among a small number of patients diagnosed with treatment-refractory depression or bipolar disorder (BD) [52-54]. Human studies of DBS provide perhaps the most compelling evidence linking LHb dysregulation with MDD. Four months after bilateral LHb DBS surgery, remission of depressive symptoms was observed in a single female patient with chronic unremitting depression [52]. In another case report, a 34-year-old patient with treatment-resistant depression (TRD) had significant improvement after bilateral Hb DBS surgery in mood, anxiety, sleep quality, and quality of life. Hamilton depression rating scale score was reported as 10 in the 12th week after surgery, while it was 23 preoperatively. With these case reports, Hb DBS seems to be a potentially effective therapy for individuals with TRD. Further research with a larger number of participants is necessary to establish the clinical significance and advantages of Hb-DBS

In summary, various animal and human studies suggest that the habenula acts as a negative modulator of the reward system and that increased activity in the LHb is linked to symptoms of depression. However, the model systems employed in the literature should be carefully evaluated and interpreted. Studies in both rodents [17,22,23] and humans [16] have provided insights into these structural differences, though we note that direct comparisons between species must be approached with caution due to inherent anatomical and functional variations. Animals, especially common research models like rodents, have significant anatomical and physiological differences compared to humans. These differences can be particularly pronounced in brain structures and functions. For example, while the basic structure of the habenula might be conserved, its relative size, connectivity, and function could differ significantly between species. The human brain is more complex, and this complexity is not just anatomical but also functional, involving more intricate neural networks and higher-order cognitive processes. This makes it challenging to directly translate findings related to brain structures and functions. Besides, many aspects of human behavior and cognition, including emotions and higher-order thinking, are difficult to model in animals. This limits the ability to fully understand how findings in animals, especially concerning mood and cognitive functions related to the habenula, apply to humans.

On the other hand, human studies, such as those using high-resolution MRI to assess habenular volume in individuals with neuropsychiatric disorders [12], offer more directly applicable insights into the human condition, albeit with their own methodological limitations, such as sample size and generalizability. Such studies with small sample sizes, while offering valuable insights, may have limitations in terms of potential biases and are prone to type II errors. Another issue is that neuroimaging studies that identify structural differences in the habenula [30] must be evaluated in terms of their resolution and sensitivity, especially considering the small size and deep location of the habenula. Similarly, functional MRI studies exploring habenular activation in response to negative and positive feedback [35] need

careful interpretation, as the signal changes in small brain structures like the habenula can be challenging to detect.

Finally, there are studies presenting contrasting findings on the role and function of the habenula. These discrepancies underscore the complexity of this research area and necessitate a cautious approach to interpreting results. The divergent outcomes could be attributed to a variety of factors, including differences in study methodologies, variations in sample populations, and distinctions between experimental designs. For instance, while some neuroimaging studies suggest a specific pattern of habenular activation in mood disorders, others report contradictory results. These conflicting findings might be influenced by factors such as the resolution of imaging techniques, the demographic characteristics of study participants, or even the specific parameters used in the experiments. Given these considerations, it is essential to approach the existing literature on the habenula with an appreciation for its intricacies and an understanding that our current knowledge may be shaped by these varying perspectives. Future research in this field should aim to reconcile these differences, possibly through more standardized methodologies or larger, more diverse study populations, to build a more cohesive understanding of the habenula's role in neurological and psychiatric conditions.

4. NEUROIMAGING OF THE HABENULA

Acquiring *in vivo* habenula imaging data is challenging because of its central location, small size [55], and close proximity to the third ventricle. The latter causes partial volume effects, especially in clinical MRI scannners. The availability of high-resolution MRI has aided in detailing habenular morphology, connectivity, functional activation, and reduced magnetic susceptibility [12,50,56,57].

Ultra-high field MRI improves the spatial resolution and contrast-to-noise ratio (CNR) required to capture the habenula in detail. Previously, anatomical imaging of the human habenula counted on relaxation time contrasts generated by the high fiber myelination density. Increased myelin content leads to hyperintense T1-weighted (T1W) and hypointense T2-weighted (T2W) images [58]. Schmidt et al. [59] made T1W maps and employed coronal landmarks to find the habenula but could not divide the habenula into medial and lateral parts. Another study employed T1W spin density and T2W to visualize the LHb, and reported that the habenula had lower T2W values compared to nearby regions, most likely due to the high iron content [55]. Several combinations of inversion times, flip angles, echo times, and averaging can result in improved CNR in the images, while this frequently leads to a reduction in the signal-to-noise ratio (SNR) and the cost of longer acquisition times. As a result, the CNR is not enough to differentiate and definitively characterize the subdivisions, as well as to distinguish unequivocally the activity measured by fMRI thought to be associated with the LHb. T1W maps at 300μm isotropic resolution and T2W images

at 60 μm isotropic resolution were obtained in a human postmortem study at 7T, and both MHb and LHb could be differentiated [60]. Schenck et al. [61] discovered considerable magnetic susceptibility enhancement in the habenula in multiple volunteers researching **quantitative susceptibility mapping (QSM).** Considering the effectiveness of QSM in producing prominent contrast for areas of the basal ganglia that contain high levels of iron, these findings suggest the possibility that magnetic susceptibility could similarly provide anatomically functional contrast for human habenula imaging [62]. He et al. [57] used **susceptibility-weighted imaging (SWI)** and QSM to localize the LHb and exhibited bilateral signal alterations in the posterior region of the habenula relative to the anterior region at 3T, which may imply increased putative iron concentrations in the LHb. The authors concluded that lateral component of the habenula could be identified using SWI and QSM at 3T. One study used high-resolution ($0.5 \times 0.5 \times 0.8$ mm^3) MRI of the human habenula with QSM at 3T and suggested that QSM could be an effective method for *in vivo* substructure habenula imaging [63]. **T2W diffusion-weighted imaging (DWI)** MRI has been utilized to investigate axonal and myelin structure in white matter for over 15 years. Strotmann et al. [55] described anatomical characteristics visible in high-resolution MR images (including DWI) of the human habenula, comparing *in vivo* and *ex vivo* findings and linking these to established histological maps. They concluded that high-resolution 7T imaging of the human habenula provided sufficient SNR and contrast to enable identification of the LHb and MHb, and that high-resolution DWI at 7T could differentiate LHb and MHb and detect major fiber tracts that connect the habenula with other brain regions. In general, the LHb appears to be predominantly connected with the frontal cortex and limbic system, whereas the MHb appears primarily linked with the brainstem and habenular commissure [55,64]. The few fMRI studies that have been conducted describe that the habenular nuclei are functionally related to the serotonergic system via the dorsal RN, to the dopaminergic system via the VTA and SNc, and to the noradrenergic system via the locus coeruleus [16,31,65,66]. Ely et al. [67] recently identified significant habenula associations with brainstem regions, subcortical structures, and cortical areas related to the salience network and early sensory processing.

Volumetric assessment of the habenula is most commonly performed via manual segmentation [12]. The habenula is segmented by one or more blinded researchers in coronal planes and is visibly protruding into the third ventricle along the ventromedial part of the thalamus or located ventral and medial to the stria medullaris of the thalamus [68]. The medial boundary is defined by the third ventricle, while the ventral border is defined by the white matter of the posterior commissure. The dorsal and lateral borders are determined by the white matter of the stria medullaris of the thalamus in anterior planes or by the mediodorsal thalamic nucleus, limitans nucleus, or pretectal area in posterior planes [12]. Another method is a geometric approach defined by three anatomical landmarks [69]. Firstly, the junction of the habenular medial margin and the posterior commissure is shown (Point A). Secondly, the dorsal point of the MHb border, where the curve of the medial boundary meets the medial-dorsal thalamus (Point B). Thirdly, the lateral portion of the mesopontine junction is close

to the tentorial incisure (Point C). The lateral boundary of the habenula was determined in these slices by drawing a straight line between the second and third landmarks (Line z). The ventral boundary's medial extent is formed by drawing a straight line horizontally and laterally from the first landmark (Line x). The ventrolateral apex of the habenula is defined as the intersection of the Lines 'z' and 'x'. The medial boundary is defined by the CSF of the third ventricle in all slices and connected points A and B in posterior slices (Line y) (Figure 3).

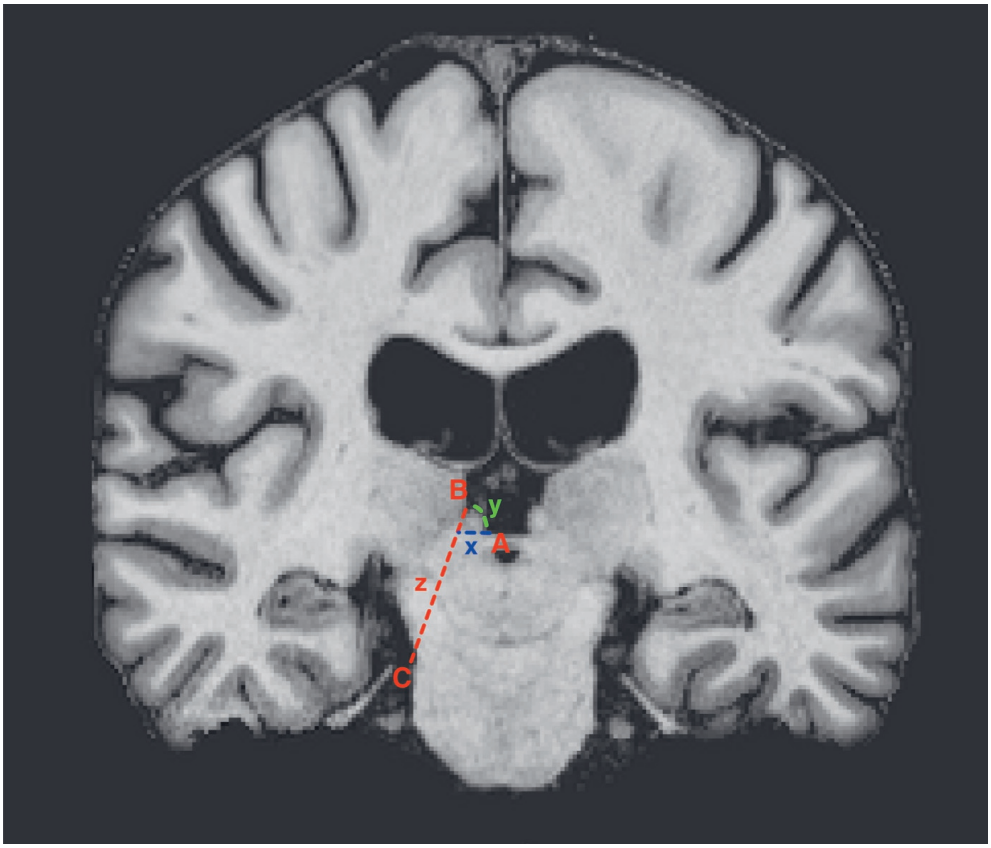

Figure 3. Detail of the geometrically defined protocol for delineating the habenula on 7T MR image from TRACK-PD Study. See text for definitions of points A, B and C, and connecting lines x, y, and z.

Kim et al. [58] proposed a semi-automated habenular segmentation approach for *in vivo* 3T T1W and T2W images, including histogram-based thresholding, region growing, geometric constraints, and partial volume estimation. This approach is based on the greater CNR of myelin-sensitive images due to the habenula's higher myelin content than the surrounding thalamus. More recently, fully automated habenular segmentation methods have been proposed, such as registration-based multi-atlas-driven and deep learning-based segmentation using T1W images [70-72]. These are potentially quick, precise, and reproducible. However, the registration-based method by Germann et al. (108) depends on the registration quality of their atlases and takes long-time process. The deep learning method by Lim et al. [71] requires 7T T1 maps and manual pre-selection of slices. Kim et al. [72] suggested using 3D deep learning instead of 2D deep learning on T1W images. The potential and limitations of these methods are reviewed in Table 1.

In addition to the volumetric imaging of habenula, several studies at conventional fMRI resolutions reported habenular activation in response to aversive outcomes in controls [35,73-77], in learning and motivation [40], in the error detection process and its ability to modify accordingly [74], as mentioned in the "Function of the Habenula" section above.

Table 1. Pros and cons of various methods used for habenula segmentation.

Method	Field strength	Pros	Cons
Manual segmentation	3T, 7T	-No need for a secondary visual check for apparent erroneous segmentation -More common and accepted method	-Subjective (rater bias) -The rater must acquire a high level of technical ability and anatomical knowledge for accurate segmentation -Laborious -Not possible to separate the habenula into medial and lateral parts -Time-consuming
Geometric segmentation	3T	- Use a subject-specific ROI approach	-Needs a training period -Laborious -Not possible to separate the habenula into medial and lateral parts -Involves manually reorienting each structural image into close alignment with the atlas
Myelin content-based segmentation	3T, 7T	-Combination of T1w and T2-weighted (T2w) anatomical images to improve the contrast-noise ratio for segmentation -Objective -Less prone to systematic signal variation	-Sometimes underestimates Hb near a less myelinated area but overestimates along highly myelinated fibers connected to the Hb (e.g., fasciculus retroflexus or stria medullaris)
Automated segmentation (registration-based multi-atlas-driven)	7T	-Large datasets of images can be studied -Reproducible -Functional MRI, magnetic resonance spectroscopy and positron emission tomography imaging could be used	-Depends on the quality of the registration to the atlas -Takes a long process time -A visual inspection of each subject's segmentation to find obvious erroneous segmentation is required
Automated segmentation (deep learning-based)	7T	-Versatile -Short processing time -Reproducible -Reducing atlas bias -Averaging registration errors -Large datasets of images can be studied -Functional MRI, magnetic resonance spectroscopy and positron emission tomography imaging could be used	-Requires manual pre-selection of slices -Requires >1000 of training data to achieve high accuracy and avoid overfitting -A visual inspection of each subject's segmentation to find obvious erroneous segmentation is required

4.1. Neuroimaging of the habenula in Mood Disorders

4.1.1. Structural MRI findings

Over recent years neuroimaging studies have focussed on structural changes of the habenula in mood disorders (Table 2). Savitz et al. [12] performed the first MRI volume examination in MDD and BD patients. The authors acquired high-resolution (0.4 mm^3) images utilizing a 3T scanner, and one rater manually segmented habenula. Seventy-four healthy controls were compared with medicated (n=15) and unmedicated depressed BD (n=22) patients, as well as unmedicated MDD patients (n = 28) and unmedicated MDD patients in remission (n=32). They found significantly smaller absolute and normalized habenula volumes in unmedicated BD patients than in controls. They also detected smaller absolute habenula volumes in currently depressed women with MDD compared to female controls. The study concluded that a decrease in volume could have functional consequences that increase the risk of developing an affective disorder. Another study by Carceller-Sindreu et al. [13] sought to determine whether habenular volume differed between healthy controls and patients in various stages of unipolar MDD. Images were obtained from 34 healthy controls and 61 MDD patients utilizing a 3T scanner. They found a higher white matter habenula volume in first-episode depressive women than in controls and chronic MDD. The authors reported that the habenular volume alterations may not be present in unipolar MDD, regardless of the disease stage, even among patients experiencing severe chronic depression. However, they also stated that the increased white matter volume seen in women with a first episode shows that habenula and its projections are involved in the early phases of recovery and the course of MDD. Johnston et al. [78] observed reduced habenular volumes in individuals with TRD compared to healthy controls. In a recent study, Cho et al. [14] used high-resolution 7T MRI data from 33 MDD patients and 36 healthy controls and stated that MDD patients had reduced right habenula volume compared to controls. In addition, the right habenula in the MDD group was smaller and had a lower T1 value than the left habenula. Contrary, Liu et al. [79] discovered increased habenula volume in MDD patients, which was linked to anhedonic symptoms. Schafer et al. [80] investigated the differences between BD and healthy controls and did not find such a longitudinal volumetric difference between the groups.

Table 2. Imaging studies investigating habenula in mood disorders.

Study	Number of participants	Type of assessment	Psychopathology	Strength of MRI	Sequences/Hb segmentation	Findings
Ranft et al., 2010 [15]	44 (13 HCs)	Postmortem	14 mood disorders 17 schizophrenia	N/A	N/A	Significantly reduced habenular volumes of the MHb and LHb in depressive patients in comparison to HC and schizophrenia patients.
Savitz et al., 2011 [12]	171 (74 HCs)	*In vivo*	37 BD 60 MDD	3T	MPRAGE / manual	The unmedicated BD patients displayed significantly smaller absolute and normalized habenula volumes than the HC subjects.
Carceller-Sindreu et al., 2015 [13]	129 (34 HCs)	*In vivo*	95 MDD	3T	3D-MPRAGE / manual	Women with a first-episode MDD had greater Hb-white matter volumes than HCs and patients with treatment-resistant/chronic MDD.
Johnston et al., 2015 [78]	41 (21 HCs)	*In vivo*	20 TRD	3T	MPRAGE	Significantly reduced grey matter volumes in regions corresponding to the habenula in TRD patients.
Liu et al., 2017 [79]	38 (17 HCs)	*In vivo*	21 unmedicated patients with major depression and 17 healthy participants.	3T	T2* weighted, echo planar single shot pulse sequence fMRI / manual	Activation in the left habenula during receipt of punishment in HCs. Attenuated left habenula activation to punishment in depressed patients. Greater left habenula activation was associated with more severe depressive symptoms and anhedonia. Greater habenula volume in patients with depression.
Schafer et al., 2018 [80]	140 (40 HCs)	*In vivo*	68 schizophrenia 32 BD	3T	3D-MPRAGE / semi-automated	Case-control differences in Hb volume did not reach statistical significance.
Cho et al., 2021 [82]	71 (37 HCs)	*In vivo*	34 MDD	3T	DTI, MPRAGE	Higher right habenula-left mediodorsal thalamus tracts in patients with MDD than in HCs.
Cho et al., 2021 [14]	69 (36 HCs)	*In vivo*	33 MDD	7T	MPRAGE and MP2RAGE / manual	A smaller right Hb volume and left-right asymmetry of Hb volume in MDD.

Table 2. Imaging studies investigating habenula in mood disorders. (*continued*)

Study	Number of participants	Type of assessment	Psychopathology	Strength of MRI	Sequences/Hb segmentation	Findings
Elias et al., 2022 [81]	32	*In vivo*	32 patients who received SCC-DBS	1.5T and 3T	3D-SPGR, GRE-EPI multiphase fMRI / automated	Increased Hb volume over time in responders, decreased Hb volume in non-responders. Active DBS was significantly associated with increased Hb connectivity to several prefrontal and corticolimbic regions.

HC: Healthy control, N/A: not available, MHb: Medial habenula, LHb: Lateral habenula, MDD: Major depressive disorder, DTI: diffusion tensor imaging, MPRAGE: magnetization-prepared rapid acquisition with gradient echo, BD: bipolar disorder, TRD: treatment-refractory depression, SCC-DBS: subcallosal cingulate area deep brain stimulation, SPGR: spoiled gradient recalled, GRE-EPI: gradient-recalled echo echo-planar imaging.

Elias et al. [81] evaluated habenular volume in 32 patients treated with DBS with the subcallosal cingulate region as neurostimulation target for treatment-resistant mood disorders. Clinical response to DBS was strongly related to longitudinal habenula volume change. Responders had a tendency to exhibit an increase in habenular volume, while non-responders tended to have a decrease in volume.

In accordance with the imaging studies, a postmortem study by Ranft et al. [15] demonstrated a decline in neuron count and density of the habenula in a mixed group of subjects who had a history of BD or MDD. In addition to the volumetric studies, Cho et al. [82] demonstrated that the ratio of the right ipsilateral tract between the thalamus and habenula was significantly higher in MDD patients compared to the left ipsilateral tract. They hypothesized that MDD is associated with the disintegration of the left thalamus-right habenula tract function, with a compensatory increase in the number of tracts.

In summary, there are different findings regarding habenula volume in mood disorders. The small number of participants and the relatively subjective manual habenula segmentation might be key methodological issues explaining inconsistencies in the volumetric analyses.

4.1.2. Functional MRI findings

fMRI studies indicate that the abnormal circuitry that contributes to TRD may involve affective, salience, auditory, visual networks, and language processing cortex [83]. Recent research has found an abnormal functional correlation (FC) in the habenular nucleus, particularly with the default mode network (DMN) in TRD patients [84,85]. A recent study sought to map the intricate neural circuitry architecture of the habenula in order to detect the neural mechanisms of TRD [86]. The authors performed FC analyses using the left and right habenula as seed regions, and whole-brain voxel-wise comparisons were used to compare the three groups (35 TRD patients, 35 patients with treatment-responsive depression, and 38 healthy controls). The TRD group showed increased connectivity in the left habenula, especially with the left precuneus and the right precentral gyrus, compared to the treatment-responsive group and the right precuneus, compared to the treatment-responsive and healthy control groups, indicating that an increased interplay between reward and DMN is associated with suicidality and may be a potential mechanism for anhedonia in TRD.

Increased habenula activation in depression has been demonstrated in an H2(15)O-PET study assessing different cognitive tasks involving either a paced word repetition task or a paced orthographic verbal fluency task alternating across the six scans in each session [51]. Furthermore, an emotional-processing task during fMRI, along with the resting-state regional blood-flow measurement using arterial spin labeling, showed habenula hyperactivation in depression [51,87]. Furman and Gotlib [39] observed that negative feedback responses activated the LHb more profoundly than positive feedback responses in MDD patients and healthy controls. A recent study investigating phasic habenular function by completing a passive (Pavlovian) conditioning task with appetitive (monetary gain) and aversive (monetary loss and electric shock) outcomes during high-resolution fMRI in MDD patients revealed decreased habenular responses within the patient group as the unconditioned stimulus became progressively more shock predicting [50]. In the TRD group, Luan et al. [85] discovered a high correlation between the right habenular nucleus and the medial superior frontal gyrus, anterior cingulate cortex (ACC), and medial orbitofrontal cortex. They reported that TRD could

be caused by dysfunction in the habenular nucleus-related circuitry for processing unpleasant emotions.

4.2. Neuroimaging of the mood disorders in Parkinson's disease

The present approach to diagnose PD relies on assessing clinical manifestations. Due to PD's heterogeneous presentation, early diagnosis can be challenging. At present, MRI is utilized in clinical settings to eliminate other possible reasons for parkinsonian symptoms, such as tumors. New MRI paradigms and ultra-high field imaging are being increasingly recognized for their potential in the diagnosis of PD in recent years [88]. Numerous studies, including cross-sectional and cohort designs, have established links between MRI features and clinical manifestations in PD [89-94]. TRACK-PD is a longitudinal observational 7T MRI study in the novo patients with PD [95]. The objective of this study was to offer significant insights into the diverse clinical presentations of PD and their corresponding MRI features. The ultimate goal of this study is to enhance the knowledge of PD and to develop novel biomarkers to track the progression of the disease, which will aid in developing new therapies. The first dataset of TRACK-PD is now complete and will be used to study habenula anatomy in PD patients compared to healthy controls.

Anxiety, depression, pain, and autonomic dysfunction are common non-motor symptoms observed in PD patients. In 2015, Wen et al. [96] conducted a systematic review of neuroimaging findings in depression (38 studies), anxiety (8 studies), and apathy (14 studies) in PD. Other than PET/SPECT studies, four studies used T1W imaging [97-99], three used DTI [100-102], and six used resting state-fMRI [103-108] in depression. There were also four studies reporting findings from structural T1W [109], DTI [110], task fMRI [111], and resting state-fMRI [112], respectively. Only one of the seven studies utilizing T1W revealed an increase in bilateral thalamic grey matter volume in PD patients with depression (dPDs) compared to PD patients without depression [112]. Three studies did not observe any differences in gray matter volume between healthy controls and dPDs [97,109,113]. On the other hand, three studies reported that dPD patients exhibited reduced gray matter volumes in areas such as the prefrontal, parietal, and insular regions, as well as the limbic system (ACC and amygdala) [97,98,111] compared to healthy controls or non-depressed PD patients. Furthermore, they discovered that the volume of the prefrontal and limbic regions had inverse correlations with depression [98,99]. A study utilizing T2-weighted imaging revealed higher hyperintensity in the midline mesencephalic structure in patients with dPD, indicating a modification in the basal limbic system [114]. Three out of four studies using DTI techniques reported reduced white matter connectivity, as indicated by decreased fractional anisotropy (FA), in various tracts such as the bilateral anterior cingulate cortex (ACC) and thalamus, as well as multiple tracts connecting the left frontal and deep temporal lobes [100-102]. This FA decrease can indicate axonal degeneration, demyelination, or more complex fiber crossings. Compared to DTI results, studies using resting-state fMRI to investigate depression in PD revealed increased and decreased resting state neural activities in dPDs, compared to non-depressed PD patients and healthy controls.

In dPDs, an increased FC between spatially distinct regions in the resting state was detected in the subcortical regions, such as the connectivity of the left amygdala with the bilateral mediodorsal thalamus. In contrast, connectivity between corticolimbic or corticocortical networks was reduced. The severity of depression was found to be inversely related to the FC between the amygdala and prefrontal and posterior cingulate cortices. Furthermore, there was a positive correlation between depression and the neural activity in the left amygdala, right cingulate and thalamus, and bilateral cerebellum prefrontal cortices. Regarding regional neural functional activity during the resting state, dPD patients demonstrated increased activity in the prefrontal, limbic, temporal, and parietal regions. These studies mostly imply that dPD patients have higher neural activity in the prefrontal regions and impaired FC between the prefrontal-limbic networks. Functional imaging studies found an inverse relationship between dopaminergic density in the caudate and putamen and the intensity of anxiety symptoms in PD, while there was no clear link between thalamic dopaminergic density and anxiety. Apathy and metabolism or activity in the striatum, amygdala, prefrontal, temporal, and parietal regions were found to have both positive and inverse associations. The authors reported no findings of the habenula, despite both nigrostriatal and extra-nigrostriatal pathways being affected in PD, particularly the frontal region and its connecting regions.

In a recent study, Liu et al. investigated the complexity of the spontaneous blood-oxygen-level-dependent (BOLD) of fMRI in dPDs. They found that the whole-brain complexity and complexity in 18 different regions of cognition and behavior were significantly lower in dPDs than in non-depressive PDs [115]. Carey et al. [116] reviewed neuroimaging features of anxiety in PD in 2020. They included studies using MRI, PET, and single-photon emission computed tomography. The cortico-striato-thalamo-cortical limbic and fear circuits were found to change depending on the anxiety's severity. Reduced FC between the striatum and ACC, decreased dopaminergic and noradrenergic activity in the striatum, thalamus, and locus coeruleus, and decreased serotoninergic activity in the thalamus have all been observed in the cortico-striato-thalamo-cortical limbic circuit.

5. PATHOANATOMIC STAGING STUDIES ON PARKINSON'S DISEASE-RELATED PATHOLOGY IN THE HABENULA

For many years, PD was considered to have a relatively straightforward neuropathology; however, recent studies have significantly altered this perception. The progression of PD in the human nervous system spans several years, evolving into a fully developed clinical syndrome unless interrupted by death. The disease is marked by a steady increase in intraneuronal lesion severity and predictable distribution changes. These changes occur even in individuals with no recorded motor dysfunction symptoms, indicating presymptomatic and symptomatic phases in PD's progression.

PD primarily affects the limbic, visceromotor, and somatomotor systems and progresses through stages. Each stage is characterized by the ongoing development of distinct inclusion bodies. Braak et al. [117] devised a neuropathological staging scheme for PD based on the progression of Lewy bodies (LBs). They proposed that PD likely originates in the olfactory bulb and the autonomic enteric nervous system, with a retrograde progression of Lewy body pathology (LBP) over time. This pathological spread eventually reaches the SNc, where it is believed to trigger the degeneration of DA neurons, a hallmark of PD. Although there is limited research on the epithalamus's role in PD, it is believed that significant PD-related pathological changes begin to manifest in this region at around Braak stage 4 [117,118]. In PD, it is suggested that the transition from the presymptomatic to the symptomatic phase likely occurs during stages 3-4 [119]. This phase involves severe lesions in the anteromedial temporal mesocortex, crucial for transferring data from sensory areas to the prefrontal cortex via limbic system centers. In a healthy brain, the limbic loop and the neocortex work together, with the neocortex processing sensory information and the limbic loop aiding in memory and importance categorization. Information from sensory areas passes through the temporal mesocortex to limbic structures and then to the prefrontal cortex. Impairments in this pathway can lead to memory and cognitive issues, and reduced limbic input to the prefrontal cortex can affect personal initiative and other cognitive functions [120].

While Braak's hypothesis on PD development receives support from various studies, including in vitro, in vivo, and clinical research, there is also skepticism about its applicability to all PD patients. This doubt stems from variations observed in the disease's progression among different individuals [121]. A subgroup of young-onset, levodopa-responsive PD patients with predominantly motor symptoms and late-stage dementia aligns with Braak's staging. However, this staging does not consistently apply to other levodopa-responsive PD patients, indicating variability in the disease's progression [122]. Given this variability, it is plausible that habenular pathology may also exhibit variations among individuals.

6. THE PATHOBIOLOGICAL BASIS OF THE HABENULA-RELATED MOOD DISORDERS IN PARKINSON'S DISEASE

PD is commonly known for its motor symptoms, but depression is a notable neuropsychiatric aspect of it [123]. During the preclinical phases of PD, the dysfunction of the limbic loop of the basal ganglia and the lateral habenula, along with the interconnected network of dopaminergic, serotonergic, and adrenergic systems, have been implicated in the development of depression. This is discussed in studies by Borgonovo et al. [124] and Wilson et al. [125], which suggest that these neural components and pathways play a critical role in the early onset of depressive symptoms associated with PD.

Studies on rodents have shown that modifications in the dopaminergic pathways are correlated with the exhibition of behaviors that are characteristic of depression. In studies by Lyu et al. [126] and Maillet et al. [127], it was found that the downregulation of astroglial glutamate transporter in the habenula of 6-hydroxydopamine (OHDA)-lesioned rat models could be related to the degeneration of the nigrostriatal pathway, potentially playing a role in PD-related depression. Additionally, Hui et al. [128] observed that unilateral 6-OHDA lesions in the SNc of rats involved presynaptic dopamine D4 receptors in the lateral habenula, which are crucial in regulating PD-related depression. Furthermore, Jiang et al. [129] found that injections of the 5-HT1A receptor-agonist 8-hydroxy-2-(dipropylamino)tetralin hydrobromide into the dorsal hippocampus significantly elevated dopamine and 5-HT levels in the medial prefrontal cortex, habenula, ventral hippocampus, and amygdala, indicating that hippocampal 5-HT1A receptors might influence depression and PD-related depression. Other research has indicated that manipulating 5-HT4 receptor activity in the lateral habenula, either through activation or inhibition, can induce depressive-like behaviors in rats with 6-OHDA lesions [130]. This includes outcomes observed in forced swim tests and sucrose preference tests, suggesting a significant role of 5-HT4 in modulating mood-related responses in the context of PD models. The study by Luo et al. [23] revealed that the lateral habenula plays a pivotal role in connecting the dopaminergic and serotonergic systems, contributing to depressive symptoms in PD rat models. This connection involves the lateral habenula mediating the effects of dopaminergic neurons in the SN on serotonergic neurons in the raphe nuclei, highlighting its significance in the neurobiology of depression in PD.

7. NEUROIMAGING OF THE HABENULA IN PARKINSON'S DISEASE

Only one human study reported on habenular imaging in PD with behavioral impairment. Markovic et al. [131] acquired structural and resting state-fMRI in 22 patients with PD with punding, 30 patients with PD without any impulse control disorders matched for disease stage and duration, motor impairment, and cognitive status, and 30 healthy controls. A seed-based technique was used to examine the resting state-FC of the habenula and amygdala, and volumes of both and cortical thickness were calculated. They found that PD patients with punding had a high FC of the habenula and amygdala with the thalamus and striatum bilaterally and decreased connectivity between the bilateral habenula and the left frontal and precentral cortices. Lower FC between the right amygdala and the hippocampus was also seen in PD patients with punding compared to PD without punding. The volumes of the habenula and amygdala did not differ across groups. The authors concluded that a disruption in connection across critical nodes of the reward circuit (i.e., habenula, amygdala, BG, frontal cortex) might be a contributing cause of punding in PD. A review published by Borgonovo et al. [124] explored the evidence of dysfunction in neural circuitry linked with depression in PD, focusing on the pre-clinical, pre-motor, and early motor stages of the disease. They

argued that the early dysfunction of the neural circuitry related to depression in PD involves the limbic loop of the basal ganglia and LHb. They proposed that changes in the neural circuitry linked with emotional regulation could be indicative of the ongoing neurodegenerative process. New neuroimaging studies with a larger cohort about mood disorders in PD and habenula are needed to clarify the relationship.

5. DISCUSSION AND CONCLUSION

The mood disorders significantly impact the quality of life and prognosis of individuals with PD. According to the most studies, the prevalence of clinically significant depressive symptoms in PD patients ranges from 30 to 35%. The underlying mechanism(s) responsible for the pathophysiology of depression in PD are not well understood, and multiple factors, such as genetic, inflammatory, cellular regulation, and signaling pathways, may be involved. The mood disorders linked with PD are connected to the malfunctioning of dopaminergic neurons in the substantia nigra, as well as alterations in 5-HT neurons in the raphe nuclei. The LHb is among the few areas that plays a role in regulating both DA and 5-HT-containing neurons [132]. There has been significant progress in comprehending the neural circuits and roles of the LHb. The habenula has a key role in the neural mechanisms of depression [133].

The habenula's connections to the brainstem nuclei that modulate key aspects of behavior (e.g., reward, mood, arousal) are well studied. A growing body of research indicates that the habenula, although tiny, is pivotal in enabling adaptive behavior. The capacity to accurately anticipate rewarding or aversive outcomes is a crucial adaptive behavior that promotes survival and facilitates avoidance of hazardous or disagreeable situations. This ability is thought to be attributed to an evolutionarily preserved hedonic neurocircuit, consisting of a network connecting various brain regions involved in the release of monoamines, spanning the forebrain, midbrain, and hindbrain regions. Within this hedonic neurocircuit, the LHb controls monoamine release and monoamine-controlled behaviors, such as reward learning. The complexity of this system has increased with the identification of heterogeneity in the roles of dopaminergic neurons in valence coding. The VTA and SNc contain at least two subpopulations of dopaminergic neurons based on their input-output pathways and cell body locations. These subpopulations include reward-activated and aversion-activated dopaminergic neurons. The LHb may also activate aversion-coding dopaminergic neurons in the medial VTA, which project to the mPFC, as well as inhibit reward-coding dopaminergic neurons. LHb neurons not only encode reward value but also reward probability and magnitude, which can facilitate complex decision-making by highlighting differences in reward magnitude and cost. Hyperactivity of LHb neurons could diminish dopamine-related reward signals, most likely via the intermediary actions of RMTg or by directly activating dopamine neurons that project to the mPFC. Such hyperactivity of these pathways may affect decision-making (e.g.,

overprocessing of aversive events, reduced motivation, or altered learning from positive or negative reinforcement).

Evidence from human and non-human studies shows that LHb dysfunction may play a role in depression. It is possible that LHb hyperactivity contributes to depression and that inhibiting LHb activity may alleviate depressive symptoms. Despite the link between habenula and depression, there are few imaging studies about the role of the habenula in mood disorders, probably due to its small size [12]. In one study, higher habenula volume in first-episode depressive women was shown [13]. Some imaging studies have reported activation of the habenula with aversive stimuli and negative feedback [35,38,40]. Contrarily, in some studies, authors found attenuated habenula activation in depressive patients [39,50,51]. In another study, increased activation in the left habenula during the receipt of punishment was found in healthy individuals, while depressive patients had attenuated left habenula response. They also found greater habenula volumes in depressive patients and an association between greater left habenula activation and depression severity [79]. As seen, the results are inconsistent, which could be related to patient differences, individual factors, disease severity and stages, and medication status. However, it is clear that we need much more studies with larger cohorts to understand the exact mechanism.

The imaging studies about mood disorders in PD mostly showed increased neural activity in the prefrontal regions and impaired FC between the prefrontal-limbic networks in dPD patients. In addition, an inverse relationship between dopaminergic density in the caudate and putamen and the intensity of anxiety symptoms in PD was found in functional imaging studies. However, there is only one imaging study about habenula's role in mood disorders in PD, although the relationship between PD and mood disorders is known. Markovic et al. [131] conducted a study using structural and functional MRI and found that PD patients with punding had increased FC between the habenula and amygdala with the thalamus and striatum bilaterally, as well as decreased connectivity between the bilateral habenula and the left frontal and precentral cortices. However, the volumes of the habenula did not differ across groups. The authors claimed that a disruption in connection across critical nodes of the reward circuit, such as the habenula, amygdala, BG, and frontal cortex, might be a contributing cause of punding in PD. Nevertheless, there is still no human imaging study investigating habenula's role in depressive or anxious PD patients. The LHb, as a link between the dopaminergic and serotonergic systems, is thought to contribute to depressive symptoms in PD. Future studies are important to understand habenula's role in PD patients with mood disorders. Thus, new potential diagnostic and treatment opportunities would be found for mood disorders in PD.

Although extensive research has been conducted on animal models, reports on *in vivo* habenula imaging in humans have been scarce, likely due in part to the habenula's location deep within the brain and its relatively small size in relation to the overall brain volume.

Studies of the human habenular morphology, connectivity, functional activation, and magnetic susceptibility have been added to the growing body of *in vivo* human habenula research thanks to advances in high-resolution MRI. The primary method used in human habenula volumetric studies is manual segmentation. However, the conventional manual-based approaches are considered time-consuming and laborious, especially with large datasets. It is also difficult to produce segmented masks with precision due to the anatomical properties of the habenula. As a result, there is a wide range of variation in the results obtained from manual segmentation of the habenula by different observers, which makes it challenging to establish a definitive standard. Therefore, several semi- or fully automatic segmentation methods have been reported. The suggested automatic segmentation techniques are expected to aid in the automated segmentation and estimation of the volume of the habenula and other critical small brain areas for upcoming psychiatric neuroimaging research. The use of high-resolution MRI-assisted DBS in the habenula region has been suggested and implemented with promising outcomes as a potential treatment for TRD. We believe that neuroimaging studies with high-resolution MRI and a large patient population might make it easy to understand the pathophysiology under the non-motor, especially mood and behavioral findings in PD. Thus, early diagnosis of these findings and predicting the prognosis could be possible.

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The authors confirm that the approval of an institutional review board was not required for this work.

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Author Contribution

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Conflict of Interest

The authors declare no conflict of interest.

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CHAPTER 3

Habenula volume change in Parkinson's disease: A 7T MRI study

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ABSTRACT

Objective: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by motor and early non-motor symptoms. The habenula is implicated in the pathophysiology of depression. This study investigates habenular volume in PD patients without clinical depression to show the changes in PD unrelated to depression.

Methods: The study used high-resolution 7 Tesla MRI data from the TRACK-PD study involving 104 PD patients and 44 healthy controls (HCs). The habenula was manually segmented, and volumes were measured, considering demographic data and depression scores via the Beck Depression Inventory (BDI).

Results: No significant correlation was found between habenular volume and BDI scores in PD patients or HCs. However, the PD group exhibited a significantly larger mean and right habenular volume than HCs. Although PD patients showed higher BDI scores, indicating more subthreshold depression, these did not correlate with the habenular volume.

Conclusion: The results suggest that while the habenula may be involved in the symptoms of PD, its role in depression within this cohort is unclear. The changes might be related to the role of the habenula in motor symptoms. This study provides a new perspective on the role of the habenula in PD, but future research could lead to a greater understanding of the neuroanatomical features of the habenula in PD.

1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease frequently associated with motor and non-motor symptoms such as sleep disorders, hyposmia, pain, constipation, and mood disorders [1 2 3 4]. Depression is a prevalent and incapacitating non-motor symptom of PD, with reported prevalence rates varying widely across studies (2.7%-90%) and can be observed even in the premotor phase of the disease [5]. The estimated 50% prevalence of depression among PD patients reflects a significant proportion of the PD population potentially affected by this symptom.

The habenula has been identified as one of the critical brain regions in the pathophysiology of mood disorders such as depression. Mood disorders often involve dysregulation of the habenula-related reward pathway [6 7], leading to increased habenular activity and metabolism, which correlates with depression symptoms [8 9]. Notably, ablation of the habenula has been found to reduce depression-like behavior in rodent depression models [9 10]. Volumetric changes of the habenula were also reported in human studies of depression. Several studies showed that patients who have major depressive disorder (MDD) and treatment-resistant depression (TRD) had smaller habenula volumes than controls, while the volume was greater in the first episode of depression than that of healthy controls (HCs) and with chronic MDD [11 12 13 14]. Deep brain stimulation (DBS) of the habenula has been demonstrated to alleviate depressive symptoms in a small number of individuals with TRD [15].

Habenula is also known to be directly or indirectly related to some brain regions, such as the subthalamic nucleus and globus pallidus internus, which are essential in PD pathology [16 17]. However, there is minimal data available on habenular function in PD patients with or without depression. One study found alterations in habenular functional connectivity among PD patients with punding [18]. It is unknown whether habenular changes are present among PD patients without clinically relevant depression and potentially predispose PD patients to develop depressive symptoms.

In this study, we investigated habenular volumes in patients with a recent PD diagnosis and without clinical depression using 7 Tesla MRI data from the TRACK-PD study [19]. We hypothesized that habenula volume would be different between PD patients and HCs since the connections of habenula with PD-related areas.

2. MATERIALS AND METHODS

2.1. Participant Selection

One hundred and four PD patients (73 male, 31 female) and 44 HCs (30 male, 14 female) from the TRACK-PD study were recruited. Basic clinical and demographic data, including age, sex, handedness, disease duration, and stage (Hoehn & Yahr Scale) [20] and total levodopa equivalent daily dose (LEDD) [21], were recorded.

The TRACK-PD study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act. It was approved by the Institutional Review Board (IRB) of the Maastricht University Medical Centre (METC AZM/UM 18-027), and written informed consent was obtained from all participants before inclusion.

Participants were included in the TRACK-PD study if they met the following criteria: 1) For the PD group, has been diagnosed with PD according to diagnostic criteria [22] by a neurologist within the last three years before inclusion; 2) Absence of dementia, and a score of ≥24 on the Montreal Cognitive Assessment (MoCA) at baseline; 3) Able to read and understand Dutch; 4) 18 years of age or older; 5) Accepting to be a participant in this study by written informed consent. Participants who had any contraindications for a 7T MRI scan were excluded.

2.2. Beck Depression Inventory

The "Beck Depression Inventory" (BDI), a 21-item questionnaire, was used to assess depression [23]. A total score of 0-8 indicated no depression, 9-15 subthreshold depression (SubD), and ≥ 16 significant depression [24 25].

2.3. MRI Acquisition and Volumetric Analysis

Participants were scanned using a 7T MRI scanner (Magnetom, Siemens, Erlangen, Germany) with a Nova Medical 32-channel head coil. Dielectric pads were applied to improve the signal in the temporal brain regions [26]. Habenula volumes were determined on 7T MRI MP2RAGE (Magnetization Prepared 2 Rapid Acquisition Gradient Echoes) images with Field of View (FoV) 208 x 208 mm, 0.65x0.65x0.65 mm^3 resolution, Repetition Time (TR) 5000 ms, Echo Time (TE) 2.51ms, flip angle (FA) 5 and 3°, and 240 sagittal slices [19]. The habenula was manually segmented using ITK-SNAP version 3.8.0 [27]. Manual segmentation was selected since there is no standardized and common automated segmentation method for habenula yet. The habenula was delineated on coronal slices where this structure was seen bulging into the third ventricle along the ventromedial aspect of the thalamus or lying ventromedial to the stria medullaris of the thalamus [28]. The medial boundary was formed by the cerebrospinal fluid of the third ventricle, and the white matter of the posterior commissure formed the ventral boundary. The dorsal and lateral borders were defined by the

white matter of the stria medullaris of the thalamus in anterior planes or the mediodorsal thalamic nucleus, limitans nucleus, or pretectal area in posterior planes **(Figure 1)**. During the training period of the raters, the habenula segmentation was executed across all three anatomical planes. Initially, segmentation was conducted in the coronal planes, which was accepted as a reference. Subsequently, the accuracy of this segmentation was verified in the axial and sagittal planes. Adjustments were made by adding or removing voxels in these planes to ensure anatomical consistency with the coronal plane reference. Thus, the habenular volumes of each hemisphere were determined, and the mean habenular volume per participant was calculated. After the segmentation training period, the left and the right habenula were segmented for 20 participants by two independent raters (BS, SM) blinded for diagnosis. The Dice Similarity Coefficient for two raters was 0.92 for the left habenula and 0.91 for the right habenula for these 20 subjects. Then, the remaining segmentations were performed by only one rater (BS), blinded for diagnosis. The total brain volume of each participant was calculated using the standard FreeSurfer software v7.1 (http://surfer.nmr.mgh.harvard.edu/). The processing steps included non-uniform signal correction, signal and spatial normalizations, skull stripping, and brain tissue segmentation [29].

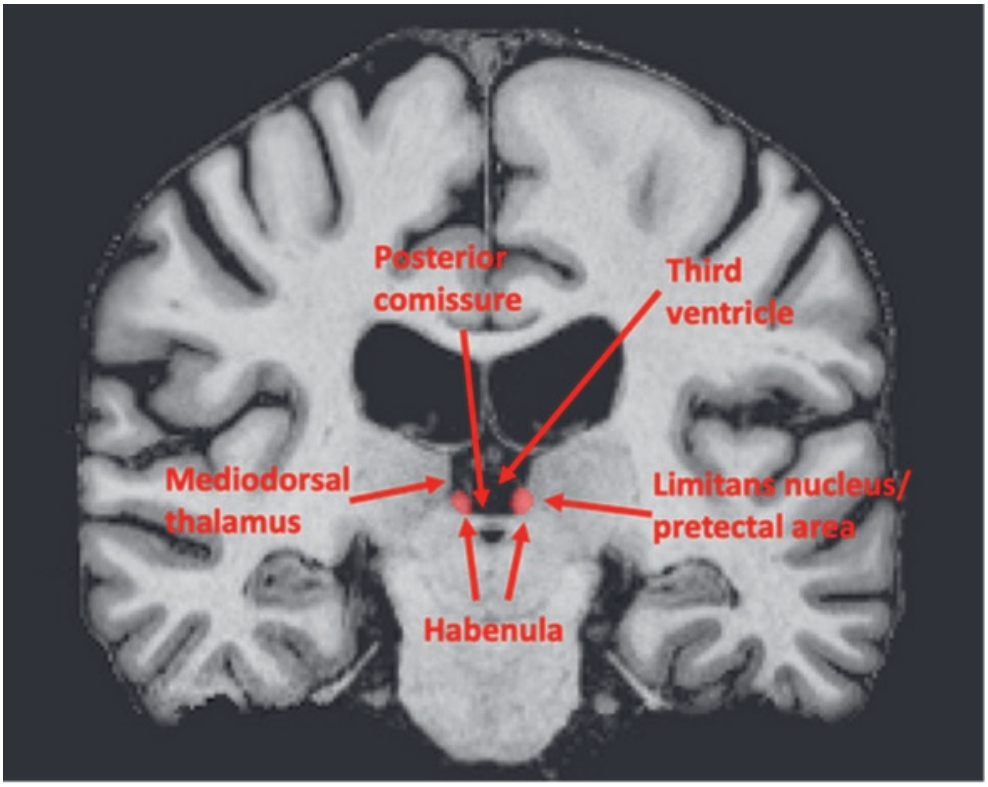

Figure 1. Coronal MRI sections showing the habenula and the local anatomical landmarks for manual segmentation.

2.4. Statistical Analysis

Independent samples t-test was used to define the differences in habenula volume between the groups. MANCOVA design was applied to compare the total volumes. The total brain volume (gray matter and white matter combined) of each participant was calculated using the standard FreeSurfer processing pipeline to correct for differences in head size by including it also as a covariate in the MANCOVA designs. BDI total scores were also accepted as a covariate to find the BDI-unaffected volume differences between PD and HC groups. Normality was confirmed using the Kolmogorov-Smirnov method. Independent samples t-tests or chi-square tests were used to compare the groups on clinical and demographic variables. Pearson or Spearman correlation tests and regression models were used to show if there was an association between habenula volumes and clinical and demographical features, as well as BDI scores.

Before each analysis for imaging data, outliers were defined as data points that lay more than 3 standard deviations away from the mean of their respective group. This criterion is based on a widely accepted statistical practice aimed at minimizing the potential impact of extreme values on the overall analysis. The identification process was conducted using SPSS 28.0, which enabled a meticulous examination of each subject's data across the variables of interest. This rigorous approach ensured that only data points significantly divergent from the group distribution, and thus likely to skew the results, were considered outliers. Five subjects were identified as outliers based on these criteria and subsequently excluded from the analysis.

A p-value <0.05 was considered statistically significant. SPSS 28.0 (IBM, SPSS Inc., New York, United States) package was used for the statistical analysis. GraphPad Prism 9 (GraphPad Software, Massachusetts, United States) was used for graphics.

3. RESULTS

3.1. Clinical and Demographical Results

PD and HC groups were matched by age, sex, handedness, and educational status. Thirty-three PD patients (31.7%) were at Hoehn-Yahr stage 1, 67 (64.4%) were at stage 2, and 4 (3.9%) were at stage 3. The clinical and demographical data of the participants are shown in Table 1.

Table 1. The clinical and demographical data and Beck Depression Inventory scores of the participants.

	PD group (n= 104)	HC group (n=44)	p
Sex (M/F, n)	73/31	30/14	>0.999
Handedness (R/L, n)	89/15	41/3	0.274
Age (years)	62 ± 8.4	60.7 ± 8.16	0.246
Disease duration (month)	19.4 ± 9.34	-	N/A
LEDD	397.3 ± 230.11	-	N/A
Beck Depression Inventory score	7.2 ± 5.05	3.5 ± 3.64	**<0.001**

Noted are the mean ± standard deviation unless otherwise stated. PD: Parkinson's disease, HC: Healthy control, M: Male, F: Female, R: Right, L: Left, LEDD: Levodopa equivalent daily dose.

3.2. Clinical correlations

There was no significant difference between the H&Y stages regarding BDI total score (p=0.320) among PD patients **(Figure 2 for H&Y stages 1 and 2)**. A significant positive correlation was detected between LEDD and BDI total score (r=0.068, p=0.005), while there was no relationship between disease duration and BDI score (p=0.138).

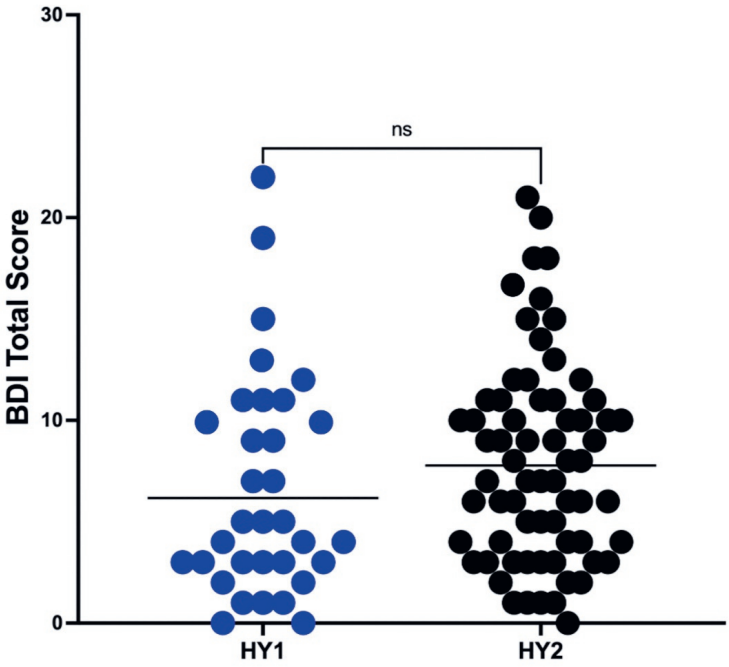

Figure 2. Comparisons of Beck Depression Inventory (BDI) total scores between the Hoehn & Yahr (HY) stages 1 and 2 in PD patients. p=0.320.

3.3. Beck Depression Inventory

The mean BDI total score was significantly higher in the PD group compared to HCs (7.2 ± 5.05 vs. 3.5 ± 3.64; p<0.001) **(Table 1)**. However, the mean BDI total score for both groups remained below 9, indicating the overall absence of relevant depression. In the PD group, there were seven PD patients (6.7%) with depression, 34 patients (32.7%) with subD, and 63 patients (60.6%) had no depression by BDI scores. In the HC group, there were no participants with depression, five participants (11.4%) with subD, and 39 HCs (88.6%) had no depression.

3.4. Imaging Results

3.4.1. Differences of Habenula Volumes Between Hemispheres and Groups

The left habenula was significantly larger compared to the right habenula in both groups (36.6 ± 6.79 for the left and 31.9 ± 5.75 for the right side in the PD group; 34.3 ± 6.05 for the left, and 29.1 ± 5.44 for the right side in HC group; p<0.001 for both groups).

The mean habenula volume was significantly larger in the PD group (34.2 ± 5.91 mm^3 in PD and 31.7 ± 5.29 mm3 in the HC group; Wilks' Lambda = 0.941, F(4, 139)=2.881, p=0.013) **(Figure 3a)**. In addition, the right habenula volume was significantly larger (31.9 ± 5.75 mm^3) in the PD group compared to the HC group (29.1 ± 5.44 mm^3; Wilks' Lambda = 0.941, F(4, 139)=2.881, p=0.004) **(Figure 3b).** Also, the left habenula was slightly insignificantly larger in PD (36.6 ± 6.79 mm^3) compared to HCs (34.3 ± 6.05 mm^3; Wilks' Lambda = 0.941, F(4, 139)=2.881, p=0.059) **(Figure 3c)**. All volumes are shown in **Table 2**. **Figure 4** displays an example of the right and left habenula delineated on all three slices (coronal, sagittal, axial) of the MP2RAGE sequence, depicted for a PD patient **(a, b, c)** and an HC **(d, e, f)**.

Table 2. The habenula volumes of the participants.

	PD group (n= 101)	HC group (n=42)	p
Left habenula volume (mm^3)	36.6 ± 6.79	34.3 ± 6.05	0.059
Right habenula volume (mm^3)	31.9 ± 5.75	29.1 ± 5.44	**0.004**
Mean habenula volume (mm^3)	34.2 ± 5.91	31.7 ± 5.29	**0.013**

Noted are the mean ± standard deviation unless otherwise stated. PD: Parkinson's disease, HC: Healthy control. Beck depression inventory scores were used as covariates in the analysis.

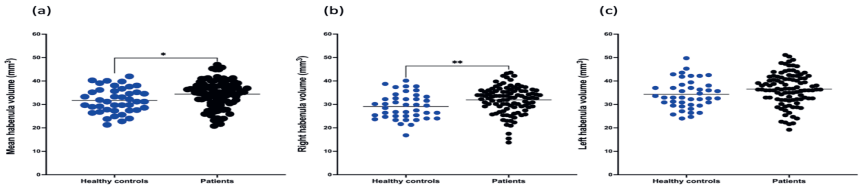

Figure 3. Comparisons of mean (a), right (b), and left (c) habenula volumes between patients with Parkinson's disease and healthy controls. *p=0.013, **p=0.004.

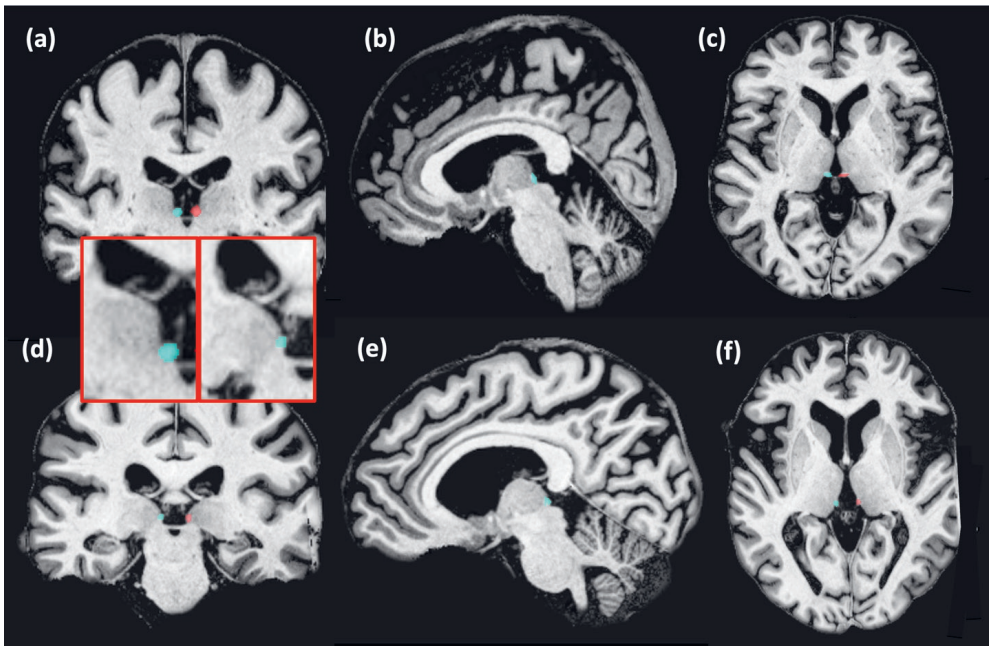

Figure 4. Right and left habenula of a PD patient (a, b, c) and a HC (d, e, f). The right habenula volume in the PD patient is larger than the HC (red-framed area), and the left habenula volume is larger than the right in both this PD and HC patient.

3.4.2. Relationship Between Habenula Volumes, BDI Scores, and Clinical Features

There were no differences in habenula volume in both PD patients and HCs when they were divided into three depression groups (non-depression, subD, and significant depression) by BDI score (p=0.939 for left and p=0.391 for right for PD (**Figure 5a)**; p=0.508 for left and p=0.594 for right for HCs (**Figure 5b**)). After the PD group was divided into two groups by BDI score, a group with ≥ 9 (n=40) and a group with ≤ 8 BDI total score (n=61), there were no significant volumetric differences (p=0.345 for left, and p=0.444 for right) between these groups. There were also 37 non-depressive and five subD participants in the HC group,

and we found no significant volumetric differences between these groups (p=0.508 for the left and p=0.594 for the right). In addition, no significant correlations were found between habenula volumes and BDI total scores (r=0.028 and p=0.737 for left habenula volume, r=0.051 and p=0.544 for right habenula volume).

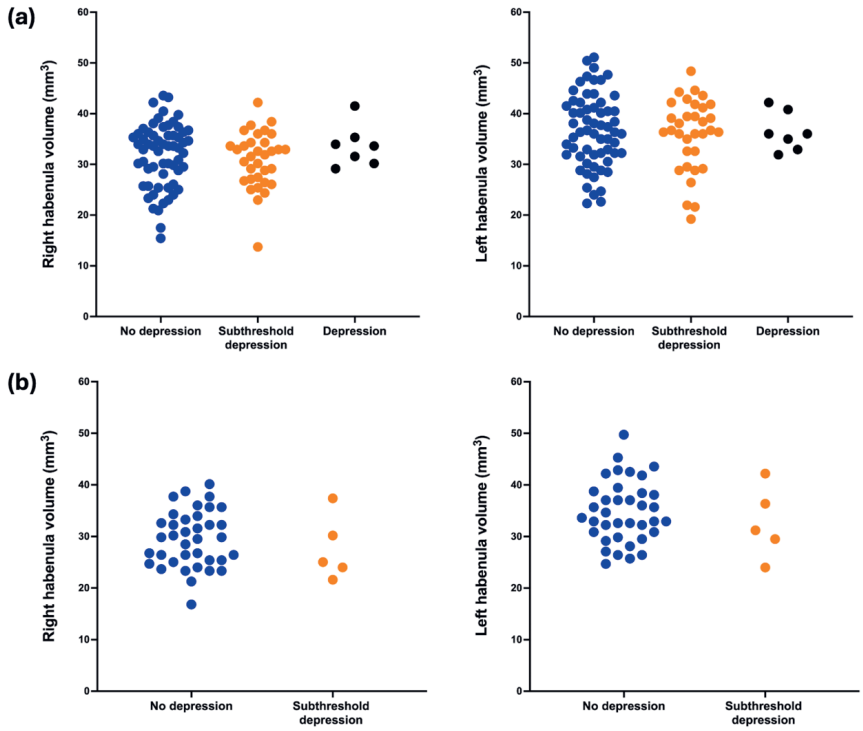

Figure 5. Differences in habenula volumes (mm^3) between PD patients with no depression, subthreshold depression, and significant depression (a), and HCs with no depression and subthreshold depression (b) according to BDI scores. There were no significant differences between the groups.

There were also no volumetric differences between the PD groups by H&Y stage (p=0.243 for left and p=0.291 for right habenula), and no correlation between the volumes with age (r=-0.008 and p=0.928 for left habenula volume, r=0.015 and p=0.855 for right habenula volume), LEDD (r=0.098 and p=0.242 for left habenula volume, r=0.132 and p=0.115 for right habenula volume), and disease duration (r=-0.022 and p=0.825 for left habenula volume, r=0.001 and p=0.990 for right habenula volume).

4. DISCUSSION

This is the first neuroimaging study utilizing a 7T MRI to investigate the habenula volume in PD within a large cohort. In this study, we found that the mean habenula volume was larger in PD patients than in HCs. In particular the right habenula showed larger volumes comparing PD and HC groups. There was no association between habenula volumes and depression scores.

PD patients had considerably higher BDI scores than HCs, and 32.7% of PD patients had a BDI total score consistent with subD (11.4% in the HC group). SubD is characterized by clinically depressive symptoms that do not meet the criteria for MDD, and the prevalence is known to be 8% - 28.8% in PD [25]. However, there is an overlap between mood symptoms and motor symptoms in PD, and diagnosis of subD, a subtle presentation, could be a challenge. Therefore, more PD patients may have undiagnosed subD or depression. We found BDI scores associated with higher LEDD and not H&Y stages. Thus, it is essential to acknowledge depression as a non-motor symptom of PD in all stages of the disease.

The habenula is believed to be involved in the regulation of emotions, particularly negative ones like fear, stress, anxiety, and depression. The lateral subdivision of the habenula, which regulates the activity of serotonin and dopamine neurons, has been linked to the pathophysiology of depression in PD. Within the framework of the monoamine deficiency hypothesis of depression, the lateral habenula couples the dopaminergic and serotonergic systems, and altered basal ganglia activity leads to lateral habenula hyperactivity, which downregulates the serotonergic system, causing depressive symptoms in patients with PD [30].

Neuroimaging research has recently focused on structural alterations of the habenula in mood disorders. Savitz et al. [11] conducted the first MRI volume examination on patients with MDD and bipolar disorder (BD). Currently, depressed women with MDD had smaller habenula volumes than female controls. The study concluded that decreased volume might have functional repercussions that raise the risk of having an affective disorder. Several investigations have demonstrated that MDD or TRD patients had smaller habenula volumes than HCs [12] [13]. Carceller-Sindreu et al. [14] investigated whether habenular volume altered between HCs and patients with varying phases of unipolar MDD. The habenular volume of first-episode depressive women was greater than that of HCs and women with chronic MDD. The authors reported that changes in habenular volume in unipolar MDD could be absent at any stage of the illness, including in patients with severe chronic depression. However, they also claimed that the increased white matter volume observed in women with a first episode indicates that the habenula and its projections play a role in the early phases of recovery and the progression of MDD. Additionally, Liu et al. [31] discovered increased habenula volume in patients with MDD, which was associated with anhedonic symptoms. Schafer et al. [32] examined the differences between BD and HCs and found no longitudinal

volumetric difference between the two groups. The variable results concerning the relationship between habenular volume and mood disorders may be influenced by methodological factors, such as the relatively small number of participants and the segmentation method. In this study, we only had a minimal number of PD patients and HC with clinical manifest depression. This may be the reason why we did not find a relationship between depression scores and volume.

Only one clinical study reported on habenular imaging in PD with behavioral impairment. Markovic et al. [18] obtained structural and resting state-fMRI in 22 PD patients with punding, 30 PD patients without impulse control disorders who were matched for disease stage and duration, motor impairment, and cognitive status, and 30 HCs. A seed-based technique was used to examine the resting state-functional connectivity (FC) of the habenula and amygdala, and the volumes and cortical thickness of both structures were determined. They discovered that PD patients with punding had an increased FC of the habenula and amygdala with the bilateral thalamus and striatum and a decreased FC of the bilateral habenula with the left frontal and precentral cortices. PD patients with punding had a lower FC between the right amygdala and the hippocampus than PD patients without punding. The habenula and amygdala did not differ in size between groups. The authors suggested that a disruption in connectivity between critical nodes of the reward circuit (i.e., habenula, amygdala, basal ganglia (BG), and frontal cortex) may contribute to punding in PD. Borgonovo et al. [33] also published a review of the evidence of early disruption in brain circuitry associated with depression in PD, focusing on the pre-clinical, premotor, and early motor stages of the disease. They asserted that the limbic loop of the BG and lateral habenula play crucial roles in the early development of depression in PD and that alterations in the neural circuitry associated with emotional control may serve as sensitive markers of the ongoing neurodegenerative process. According to the BDI scores, there was no relationship between habenula volumes and BDI scores in both groups in our cohort. With these results, since there are no clinically diagnosed depression patients in our cohort, it can be thought that these changes in the neural circuits involved in the development of depression and changes in habenula volume have not yet occurred in Parkinson's patients during the subclinical depression period. Additionally, we might have yet to find a correlation because we only conducted volumetric analyses. Pathological changes could be detected earlier in connectivity analyses. Further neuroimaging investigations in longitudinal datasets with depressed and non-depressed patients are required to elucidate the link between depression in PD and the habenula.

The human habenula shows asymmetry in size due to cytoarchitectonic organization, neurochemistry, and functional connectivity [34]. Postmortem volumetric analysis [35] and volumetric MRI scans revealed that HCs had increased left habenular volume [11]. In this study, we report, for the first time, a differential volume between the left and right reining nuclei in patients with PD, a finding that echoes previous observations of asymmetry in non-PD subjects but underscores a novel aspect of neuroanatomical variation in PD. This phenomenon dovetails

with the work of Scherfler et al.[36], who highlighted a marked reduction in dopamine transporter binding in the left posterior putamen compared to the right, suggesting a left hemispheric predominance of nigrostriatal dysfunction in PD. Such lateralized neurodegenerative patterns offer a compelling explanation for the asymmetry we observed, proposing that the uneven dopaminergic dysfunction may contribute to differential volumetric changes in the habenula. Theories also suggest an inherently lower level of dopamine in the left versus right nigrostriatal pathway in right-handed patients or a selective vulnerability of left nigrostriatal projections to the pathological mechanisms that characterize PD [37] [38]. These hypotheses align with our findings and suggest that the structural brain changes observed in PD patients—specifically, the volumetric differences in the habenula—may be a direct consequence of the disease's asymmetric impact on dopaminergic innervation. We also found that the right habenula volume was significantly larger in PD patients than in HCs. In the PD group, we demonstrated significantly higher BDI scores than the HCs in our cohort, and more PD patients had subD. It might be hypothesized that larger habenula volumes accompany the first symptoms of depression as a compensatory mechanism and control of neurotransmitter systems. As compensatory mechanisms fail and MDD or TRD occurs, volumes of the habenula may subsequently decrease. However, in our study, we only had a minimal number of patients with clinical depression, and there was no correlation between the volumes and BDI scores. Additionally, it is known that the right habenula has more connections with the substantia nigra and ventral tegmental area (VTA) than the left [39]. Dopamine neurons in the VTA are thought to play essential roles in motivation, reward, various cognitive and emotional processes, and also in negative affect. The nigrostriatal dopaminergic system, in which substantia nigra pars compacta, plays essential roles in sensorimotor functions, including motor execution and habit formation, and also has affective and motivational roles [40]. Hence, this increased volume could be related to motor symptoms in people with PD. The habenula might be overactivated to compensate for dopaminergic disturbances that cause motor and mood symptoms in PD. For future research, it would be prudent to study whether there is an association between the severity of motor and other non-motor symptoms in PD patients and habenula volumes.

A limitation of the current study is the lack of clinically depressed participants and PD patients. A second limitation is that the scores of the patients' detailed motor scales are not included. We also have a smaller HC group than PD. Future studies with depressed PD patients and larger HC groups would be meaningful to understand the relationship between PD and habenula. However, the main strength of the study is that this is the first large-scale examination of the habenula volume in PD using a 7T MRI. Acquiring in vivo habenula imaging data is challenging because of its central location, small size, and close proximity to the third ventricle. The latter causes partial volume effects, especially in clinical MRI scanners. The availability of high-resolution MRI is thought to have aided in detailing habenular morphology, connectivity, functional activation, and reduced magnetic susceptibility.

5. CONCLUSION

This study is the first to demonstrate larger mean and right habenula volumes in PD patients compared to HCs. These results might be significant for understanding the role of habenula in PD pathophysiology. Using high-resolution 7T-MRI in large groups of patients and longitudinal studies could lead to a greater understanding of the neuroanatomical features of the habenula in PD.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this study.

Ethics Approval and Consent to Participate

The TRACK-PD study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act. It was approved by the Institutional Review Board (IRB) of the Maastricht University Medical Centre (METC AZM/UM 18-027). Before inclusion, written informed consent was obtained from all participants.

CRediT authorship contribution statement

Bedia Samanci: Concept and design, Literature review, Investigation, Interpretation, Data processing and analysis, Drafting of the manuscript, Finalizing the manuscript; **Sonny Tan:** Concept and design, Literature review, Investigation, Interpretation, Data processing and analysis, Drafting of the manuscript, Finalizing the manuscript; **Stijn Michielse:** Concept and design, Literature review, Investigation, Interpretation, Data processing and analysis, Drafting of the manuscript, Finalizing the manuscript; **Mark Kuijf:** Concept and design, Interpretation, Critical revision of the manuscript; **Yasin Temel:** Concept and design, Interpretation, Critical revision of the manuscript.

Data Availability Statement

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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4

CHAPTER 4

Exploring habenular structural connectivity in Parkinson's disease: Insights from 7 T MRI study

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ABSTRACT

Background: PD is marked by both motor and non-motor symptoms, with its pathophysiology involving many neural pathways and brain regions beyond the dopaminergic system. While mainly gray matter changes have been noted, white matter changes also exist in PD. Habenula, known for its role in reward processing, mood regulation, motor functions, and cognition, is of interest due to its connection to mood disorders in PD. This study aims to explore diffusion metrics and structural connectivity changes in the habenula of newly diagnosed PD patients using 7 T MRI.

Methods: 84 PDs and 38 HCs were recruited from Maastricht University Medical Centre. Clinical, demographic, and total Beck Depression Inventory (BDI) scores were recorded. A 7 T brain MRI was conducted. Diffusion metrics and structural connectivity were evaluated.

Results: The mean diffusion metrics of Hb were not significantly different between the groups. However, in PD patients, there was an increase in mean structural connectivity from the right Hb to the right hippocampus ($p = 0.006$) and the right fusiform gyrus ($p = 0.007$). On the left side, enhanced connectivity was observed with the left pallidum ($p = 0.040$) and left accumbens ($p = 0.009$). In the PD group, a significant correlation was found between the BDI total score and increased structural connectivity from the right Hb to the left cingulate isthmus ($R2 = 0.090$, $p = 0.003$).

Conclusion: This pioneering study examines diffusion metrics and structural connectivity of Hb in PD patients using highresolution 7 T MRI. Our findings highlight the habenula's potential role in PD pathophysiology, with altered connectivity suggesting early neurodegenerative or compensatory processes. These results underscore the importance of the habenula as a biomarker for PD and its potential as a therapeutic target.

1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor and non-motor symptoms. Although the neuropathology in PD is mainly documented in gray matter (GM), such as the loss of dopaminergic neurons, accumulation of Lewy bodies, and neuroglial cell damage, there are also signs of axonal damage and alterations of white matter (WM). GM primarily consists of neuronal cell bodies and dendrites, whereas WM is predominantly composed of myelinated axons. In PD, the degeneration of dopaminergic neurons and the formation of Lewy bodies are often associated with neuroglial cell damage and axonal demyelination, accompanied by increased concentrations of activated microglia in the extracellular spaces. This suggests that extracellular microstructural abnormalities may be detectable in brain regions rich in dopaminergic neurons, reflecting the underlying neuropathological changes.[1] Diffusion tensor imaging (DTI) enables measurements of WM integrity and can be used to reveal microstructural changes in PD patients.[2] DTI is increasingly viewed as a valuable tool for monitoring disease progression of early-stage PD and possibly in later-stage disease.[3]

The main DTI parameters are fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD).[1] Several studies have reported different results in the context of these diffusion metrics, potentially reflecting disease severity or early compensatory mechanisms.[4-11] A meta-analysis of DTI studies identified consistent neurodegenerative changes in PD patients compared to healthy controls (HC), primarily affecting subcortical regions such as the substantia nigra, as well as the cingulate and temporal cortices, and the corpus callosum. Additionally, atypical alterations in the corticospinal tract were noted, which may reflect compensatory mechanisms or selective neurodegeneration of adjacent white matter fibers.[12] In a recent study, participants with PD were found to have lower structural connectivity in some brain regions, including corpus callosum, fornix, and uncinate fasciculus.[13] The integration of diffusion MRI with tractography allows for in vivo evaluation of brain structural connectivity, mapping the WM tracts that physically link different brain regions. In PD, these studies have identified distinct PD-specific anatomical networks, characterized by disrupted cortical-subcortical loops, reduced modular organization, and compensatory alterations in network structure.[14,15]

The habenula is a small structure with widespread projections to cortical and subcortical regions as well as projections with the brainstem. It is implicated in functions like reward processing, mood regulation, motor actions, cognition, and pain processing. More recently, the habenula has gained attention for its role in mood disorders, which are prevalent in PD.[16,17] Rodent studies have shown that alterations in dopaminergic pathways are linked to depression-like behaviors. Downregulation of astroglial glutamate transporters in the habenula of 6-hydroxydopamine (6-OHDA)-lesioned rats has been associated with nigrostriatal degeneration and PD-related depression.[18,19] Additionally, unilateral 6-OHDA

lesions in the substantia nigra pars compacta were found to involve dopamine D4 receptors in the lateral habenula, which regulate PD-related depression.[20] Furthermore, activation of hippocampal 5-HT1A receptors elevated dopamine and 5-HT levels in several brain regions including habenula, suggesting its role in modulating PD-related depression.[21] However, little is known about the role of the habenula in PD in general and, more specifically, in PD-related mood disorders.[22]

The aim of this study is to investigate the alterations in habenular connectivity in newly diagnosed PD patients compared to healthy controls HCs using DTI from ultra-high field 7T MRI data from the longitudinal observational TRACK-PD study.[23] Our primary variable of interest is the structural connectivity of the habenula, and the main outcome is the identification of potential differences in tractography profiles between PD and HC groups, which may reflect early neurodegenerative changes in PD.

2. MATERIALS AND METHODS

2.1. Participant Selection

Inclusion criteria for the TRACK-PD study were (see Wolters et al.[23] for detailed study protocol): 1) PD diagnosis according to established criteria[24] by a neurologist within the last 3 years; 2) Absence of dementia and a score of ≥24 on the Montreal Cognitive Assessment (MoCA) at baseline; 3) Dutch language proficiency; 4) Age 18 or older; 5) Written informed consent to participate. Those with contraindications for a 7T MRI scan were excluded.

One hundred and six PD patients and 45 HCs were included in the TRACK-PD study. After quality control of MRI sequences used in this study, 84 PD patients (66 male, 18 female) and 38 HCs (27 male, 11 female) were recruited. Basic clinical and demographic data, including age, sex, handedness, disease duration, and Hoehn & Yahr stage (H&Y)[25], Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III (medication-on)[26], total levodopa equivalent daily dose (LEDD)[27], and MoCA scores[28] were recorded.

The study adhered to the Declaration of Helsinki and the Medical Research Involving Human Subjects Act, receiving approval from the Institutional Review Board (IRB) of the Maastricht University Medical Centre (METC AZM/UM 18-027).

2.2. Beck Depression Inventory

The Beck Depression Inventory (BDI), a 21-item questionnaire, was used to assess depression levels.[29] The scoring was categorized as follows: a total score of 0-8 indicated no depression, 9-15 represented subthreshold depression (SubD), and a score of ≥16 was considered indicative of significant depression.[30,31]

2.3. MRI Acquisition and Preprocessing

Participants underwent scanning with a 7T MRI scanner (Magnetom, Siemens, Erlangen, Germany) using a Nova Medical 32-channel head coil, enhanced by dielectric pads to improve signal in temporal brain regions.[32]

Habenula volumes were measured using 7T MRI Magnetization Prepared Two Rapid Acquisition Gradient Echo (MP2RAGE) images, with specifications including a 208 x 208 mm Field of View (FoV), 0.65x0.65x0.65 mm^3 resolution, Repetition Time (TR) of 5000 ms, Echo Time (TE) of 2.51ms, flip angles of 5° and 3°, and 240 sagittal slices.[23]

A localizer sequence was acquired, followed by B0 and B1 mapping, and shimming was used to correct for magnetic field inhomogeneities. A whole-brain diffusion-weighted (DWI) scan was acquired using an echo-planar imaging (EPI) sequence along 66 random directions with an average b-value of the 2000s/mm2 mixed with six B0-volumes. One additional B0 volume and five DWI volumes were recorded with opposite phase-encoding directions. DTI scan parameters included a TE of 60.6 ms, TR of 7000 ms, flip angle of 90°, FoV of 192x192 mm, resolution of 1.5x1.5x1.5 mm^3, 80 slices with axial orientation, GRAPPA acceleration factor of 3, and an acquisition time of 9:48 minutes.[33]

MRI preprocessing involved converting raw DICOM data to NIfTI format, denoising DWI scans, and removing Gibbs ringing artifacts using MRtrix version 3 (https://www.mrtrix.org/). DWI scans were then corrected for susceptibility distortions, eddy currents, and subject motion, and b-matrix reorientation was performed using the FMRIB software library of FSL 6.0.7.3 (https://www.fmrib.ox.ac.uk/fsl). Data quality was ensured by visual inspection and quantification of mean displacement values per volume based on the eddy current correction routine.

2.4. Habenula Tractography

The bilateral habenula was manually segmented on the MP2RAGE sequence as the seed region of interest (ROI), as previously described.[34] With the help of linear transformation (FLIRT), the DWI images were aligned to the MP2RAGE space, enabling the direct use of ROIs during the tractography of the habenula. Probabilistic tractography was used due to its superior handling of inter-individual variations and crossing fibers. This was performed in MRtrix3 using the iFOD2 algorithm[35], with a voxel size of isotropic 0.7 mm and 50,000 streamlines per subject. The FOD map of each subject was seeded at left and right habenula volumes per hemisphere *(Figure 1)*. Tensors were then fitted, and metrics like FA, MD, AD, and RD were calculated in MRtrix3 from the habenula streamlines. In order to generate connectome from the habenula streamlines for structural connectivity, the MP2RAGE image of each subject was parcellated into 86 regions of interest (68 cortical and 18 subcortical regions) of the Desikan atlas[36] using Freesurfer 6.0.0[37] to act as target node labels. Segmentation of these regions in each of the subjects was checked manually. Since the streamlines

have all been seeded from the same location (habenula), only the endpoint of each streamline was assigned based on the Desikan parcellation image. Accordingly, the output was a vector of connectivity values, which represents the number of streamlined endpoints located at the nearest node.

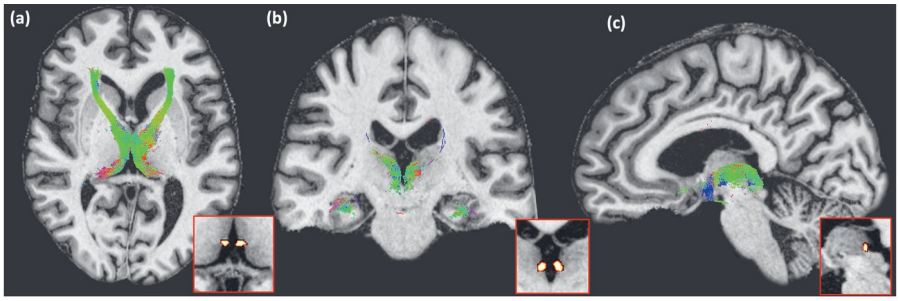

Figure 1. A tractography illustration of a Parkinson's disease patient on axial (a), coronal (b), and sagittal (c) sequences on MP2RAGE image and bilateral habenula as a region of interest (ROI) shown in red squares (b).

2.5. Statistical Analysis

Normality was confirmed using the Kolmogorov-Smirnov method. An independent sample t-test or one-way analysis of variance (ANOVA) was used for normally distributed data, whereas the Kruskal-Wallis H-test or Mann-Whitney U-test was used for non-normally distributed variables for group comparisons. Differences in diffusion measures of the habenula among PD depression subgroups and H&Y stages were assessed using one-way ANOVA with Bonferroni corrections. Paired t-tests were utilized to evaluate differences in diffusion measures between hemispheres. Age, sex, track sizes, BDI scores and antidepressant use were accounted for as covariates in diffusion analysis.

Associations between diffusion and structural connectivity measures with clinical, demographic features, and BDI scores were examined using Spearman correlation tests and regression models. A multiple linear regression with backward elimination was utilized to predict relationships between diffusion measures and variables such as BDI scores, MDS-UPDRS part III scores, LEDD, and MoCA scores. Significance was determined using False Discovery Rate (FDR) corrected p-values, with a threshold of $p<0.05$. Statistical analyses were performed using SPSS 28.0 (IBM, SPSS Inc., New York, United States).

3. RESULTS

3.1. Clinical and Demographical Results

The PD and HC groups were matched in terms of age, sex, handedness, and educational status. Among the PD patients, 26 (31%) were classified as H&Y stage 1, 55 (65.4%) at stage

2, and 3 (3.6%) at stage 3. The clinical and demographical data of the participants are shown in Table 1.

Table 1. Clinical and demographic data of the participants.

	PD group (n=84)	HC group (n=38)	p
Sex (M/F, n)	66/18	27/11	0.496
Handedness (R/L, n)	72/12	36/2	0.222
Age (years)	61.9 ± 8.59	60.4 ± 7.81	0.301
Disease Duration (month)	19.4 ± 8.89	-	N/A
LEDD	395.6 ± 226.42	-	N/A
MDS-UPDRS Part III Score	18.9 ± 7.76	-	N/A
MoCA Total Score	27.92 ± 1.637	27.76 ± 1.567	0.623
BDI Total Score	7.3 ± 5.12	3.6 ± 3.81	***<0.001***

PD: Parkinson's disease, HC: Healthy control, M: Male, F: Female, R: Right, L: Left, LEDD: Levodopa equivalent daily dose, MDS-UPDRS: Movement Disorders Society-Unified Parkinson's Disease Rating Scale, MoCA: Montreal Cognitive Assessment, BDI: Beck Depression Inventory.

We analyzed BDI scores across different H&Y stages to evaluate the relationship between depression severity and PD progression. No significant difference was found in the BDI total scores across different H&Y stages among PD patients (p=0.059). The depression status of each H&Y stage was given in Table 2. However, a significant positive correlation was observed between the BDI total scores and both the LEDD (r=0.316, p=0.003) and disease duration (r=0.217, p=0.047). Additionally, there was a significant negative correlation between the BDI total scores and the MoCA total score (r= -0.261, p=0.016). Conversely, no correlation was found between the BDI total scores and the MDS-UPDRS part III scores.

Table 2. The depression status of each Hoehn&Yahr Stage

Hoehn&Yahr Stage	No Depression, n (%)	Subdepression, n (%)	Depression, n (%)	Total, n (%)
1	19 (73.1 %)	6 (23.1 %)	1 (3.8 %)	26 (31 %)
2	31 (56.4 %)	19 (34.5 %)	5 (9.1 %)	55 (65.4 %)
3	1 (33.3 %)	2 (66.7 %)	0 (0.0 %)	3 (3.6 %)

3.2. Beck Depression Inventory

The mean BDI total score was significantly higher in the PD group compared to HCs (7.3 ± 5.12 vs. 3.6 ± 3.81; p<0.001) *(Table 1)*.

Within the PD group, 6 patients (7.1%) were diagnosed with depression, 27 patients (32.2%) had subD, and 51 patients (60.7%) exhibited no depression according to BDI scores. In the HC group, none were classified with depression, 5 participants (13.2%) fell into the subD

category, and 33 HCs (86.8%) showed no signs of depression. There were 4 PD patients and 2 HCs who used antidepressant treatment (2 selective serotonin reuptake inhibitors (SSRI), 1 amitriptyline, 1 selective serotonin-noradrenaline reuptake inhibitors in PD group, and 2 SSRI in HC group) for various reasons such as pain, posttraumatic stress disorders.

3.3. Imaging Results

3.3.1. FA, MD, AD, and RD Results

The mean FA, MD, AD, and RD of the habenula were not significantly different between PD and HC groups. The diffusion measures are shown in *Table 3*.

No significant differences were observed in diffusion measure values when both PD patients and HCs were categorized into three groups based on their BDI scores, as mentioned in the Method section (p=0.234). Furthermore, no correlations were found between the diffusion metrics and BDI total scores and MoCA total scores in either the PD group or the HCs. Additionally, in the PD group, there was no correlation between the diffusion metrics and LEDD, MDS-UPDRS Part III scores, and disease duration.

Table 3. Diffusion measures in PD and HC groups.

	PD (Mean ± SD)	HC (Mean ± SD)	p
Left FA	0.218±0.0477	0.206±0.0447	0.209
Left MD	0.00083±0.000124	0.00080±0.000089	0.263
Left AD	0.00101±0.000146	0.00097±0.000095	0.120
Left RD	0.00073±0.000119	0.00072±0.000091	0.420
Right FA	0.229±0.0502	0.240±0.0451	0.176
Right MD	0.00085±0.000114	0.00081±0.000071	0.109
Right AD	0.00105±0.000131	0.00102±0.000105	0.326
Right RD	0.00075±0.000112	0.00070±0.000062	0.055

PD: Parkinson's disease, HC: Healthy control, SD: Standard deviation, FA: Fractional anisotropy, MD: Mean diffusivity, AD: Axial diffusivity, RD: Radial diffusivity. Statistical significance threshold: $p<0.05$.

3.3.2. Connectome Results

Structural connectivity of the habenula showed significant differences in the PD group compared to HCs. Specifically, in PD patients, the mean structural connectivity between the habenula and the right hippocampus (p=0.006), as well as the right fusiform gyrus (p=0.007), was increased. On the left side, increased connectivity was noted with the left pallidum (p=0.040) and left accumbens (p=0.009). *Figure 2* illustrates these findings, with *Figure 2a* showing all structural connectivities in the PD group and *Figures 2b, 2c, and 2d* highlighting the significant differences in structural connectivity between the PD and HC groups.

In the PD group, disease duration and left habenula-left pallidum connectivity had significant association ($F(1, 83) = 5.175$, Beta= .251, Adjusted R^2= .051, $p = 0.026$). There were no correlations between these significant areas and LEDD, MDS-UPDRS part III, and MoCA total score.

Within the PD group, a significant association was found between the BDI total score and increased structural connectivity of the right habenula - left isthmus of the cingulate ($F(2, 82) = 9.216$, Beta= .318, Adjusted R^2= .090, $p = 0.003$). However, no significant correlation was found with the structural connectivity of the left habenula. In HCs, BDI total scores showed no significant correlations with structural connectivity of the habenula, either on the right or left side. When both PD patients and HCs were divided into three depression groups based on BDI scores, no differences were observed in structural connectivity analysis.

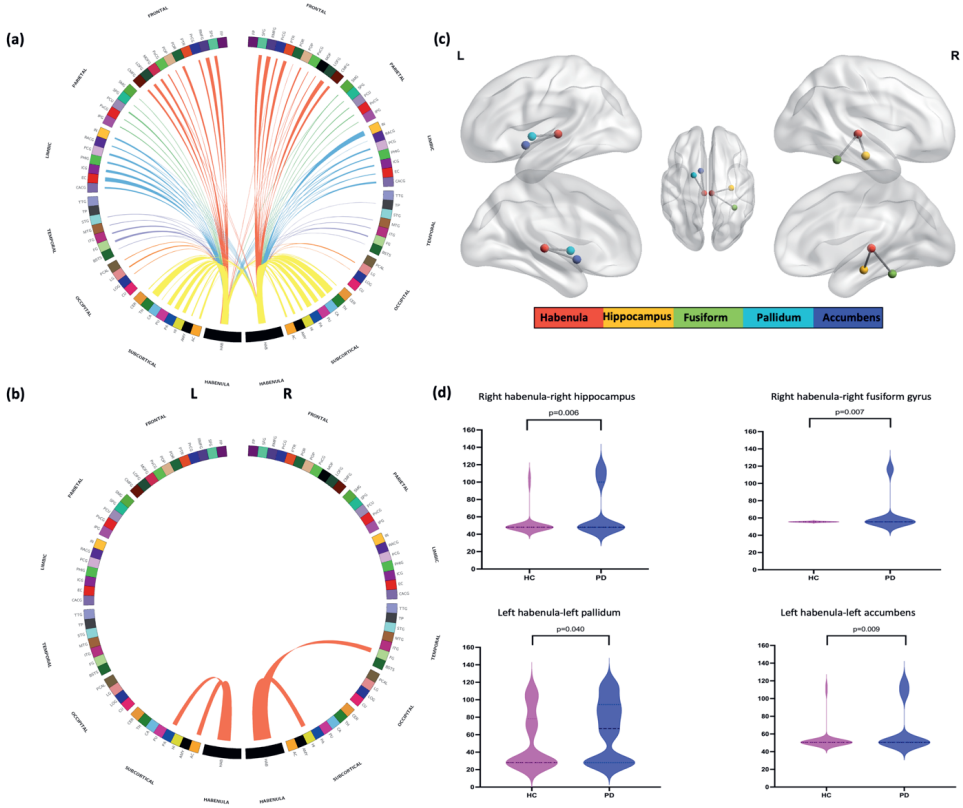

Figure 2. All structural connectivities in PD group **(a),** the regions (left habenula-left accumbens, left habenula-left pallidum, right habenula-right hippocampus, right habenula-right fusiform gyrus) that have increased structural connectivity in PD group compared to HC group **(b, c, d).** The connectogram visualization was created using Circos (http://circos.ca/).

4. DISCUSSION

Our study focused on the diffusion measures and structural connectivity of the habenula in early-stage PD patients, utilizing high-resolution 7T MRI data. We observed increased connectivity in specific habenula-related pathways in the PD group compared to HCs and also some significant association between increased connectivity and clinical features in PD. These findings support the hypothesis of a complex interaction between the structural integrity of the habenula and its connectivity in PD.

Depression, including major depressive disorder (MDD) and subtler subD, is a prevalent non-motor symptom in PD. The prevalence of subD in PD ranges from 8% to 28.8%[31], and its diagnosis can be challenging due to symptom overlap with PD's motor aspects. In this study, the PD group displayed higher BDI scores than HCs, yet not high enough to suggest clinical depression. SubD was observed in 32.2% of PD patients, compared to 13.2% in HCs. BDI scores correlated with higher LEDD, longer disease duration, and lower MoCA scores but not with H&Y stages or MDS-UPDRS part III scores. This underscores the importance of recognizing depression as a pervasive non-motor symptom in PD, linked with disease duration and potentially contributing to cognitive impairment.

DTI techniques are crucial for characterizing microstructural changes in WM fiber tracts in psychiatric and neurological disorders, including PD. Key DTI parameters include FA, MD, AD, and RD. FA reduction indicates impaired WM integrity, while elevated MD is associated with axonal/neuronal loss. Increased RD suggests myelin pathology, and decreased AD points to axonal injury. Studies comparing PD and HCs report varied DTI findings. Most show decreased FA in the PD[4-8], though some suggest early PD stages may exhibit increased FA as a compensatory mechanism.[9] MD is generally higher in the PD[10]; however, there are some lower results that align with the compensatory hypothesis.[11] In general, elevated MD and RD, along with reduced AD, seem to be linked to PD-related myelin and axonal damage.[4-8] Our research revealed no significant differences in habenula diffusion metrics between PD patients and HCs. One possible explanation is that early-stage PD involves subtle WM changes that may not be detectable with current DTI methods or 7T MRI, especially in the habenula. The habenula is a small, complex structure, and the microstructural alterations in PD might be more pronounced in other brain regions known to be affected in PD, such as the substantia nigra, corticospinal tract, or basal ganglia. Additionally, the diffusion metrics we used—FA, MD, AD, and RD—are sensitive to general microstructural integrity but may not reflect specific pathophysiological processes such as axonal degeneration or myelin loss, which are typical in PD. These processes might be better captured by advanced imaging techniques, such as neurite orientation dispersion and density imaging (NODDI) or fixel-based analysis (FBA). Future studies incorporating these techniques could provide more detailed insights into white matter changes in PD. Furthermore, it is important to consider that the study may have been underpowered to detect subtle differences, particularly

given the relatively smaller sample size of the HC group (n=38). Future research with larger cohorts is warranted to fully explore WM alterations in the habenula and other regions in PD. In addition to exploring other regions of interest, future studies might benefit from including additional diffusion metrics or multi-shell diffusion models, which could reveal more specific patterns of microstructural pathology in PD. Such analyses could help to clarify whether the habenula, and its connectivity with other brain regions, plays a role in the early pathophysiology of PD or if these changes are reflective of compensatory mechanisms rather than degeneration. On the other hand, the trend towards higher RD in PD might indicate early myelin pathology without significant axonal or WM tract damage. WM changes in PD are often interpreted as multifactorial due to microvascular structural changes in relation to impaired autoregulation or blood-brain barrier function, whereas α-synuclein actually also accumulates in WM of the motor cortex, reducing myelin proteins.[1] In the early phases of PD, WM alterations may occur before the loss of dopaminergic neurons and could contribute to clinical symptoms of PD.[1] This highlights a potential connection between myelin/oligodendrocyte changes and PD pathology, presenting a potential treatment target. While changes were not statistically significant in our study, they might reflect early neurodegenerative processes in the habenula preceding clinical symptoms, although the habenula also contains gray matter.

In this study, structural connectivity analysis offered more profound insights compared to diffusion metrics. It revealed increased connectivity between the right habenula and both the right hippocampus and right fusiform gyrus. Additionally, increased connectivity was observed between the left habenula and the left pallidum, as well as the left accumbens. While some authors have not found differences between patients and HCs, the majority of studies on facial emotion recognition (FER) in PD have reported poorer performance among patients.[38] FER is a key aspect of social cognition, critical for regulating social and interpersonal behavior. In the study by Slomp et al.[39] including 142 treatment-naïve PD patients and 142 HCs, the authors found that FER deficits are present even at the time of PD diagnosis and that they are associated with self-reported apathy and proxy-reported social behavioral problems, particularly concerning social conventions. On the other hand, emotional processes are often disrupted in depression and given the high prevalence of depression in PD; some researchers have suggested that deficits in FER may not be specific to PD but could be related to mood disorders. Gray and Tickle-Degnen[40] found no evidence that FER deficits in PD are secondary to depression, while only two studies by Baggio et al.[41] and Linn et al.[42] have reported a connection between depression and FER deficits in PD. Regarding our results, the patients in our study were controlled for BDI scores and we believe that the finding of increased connectivity between habenula and right fusiform gyrus could be a compensatory mechanism for FER deficits rather than depression itself, as the fusiform gyrus is crucial for face perception and recognition, along with FER and as there are also studies indicating increased fusiform gyrus activity in PD.[43-47]

The hippocampus, traditionally linked to memory, however, especially the right side, plays a role in scene perception, imagery, and spatial memory, aspects minimally impaired in PD.[48-50] Moreover, its connectivity with other regions, such as the amygdala and anterior cingulate cortex, is crucial for regulating emotions, especially during memory recall and response to positive stimuli.[51] Despite PD patients commonly exhibiting executive and attentional function deficiencies, these might also relate to subclinical visuospatial dysfunction.[52,53] Our findings of increased connectivity, especially in the right hemisphere, may reflect compensatory mechanisms for these subtle deficits in PD, even in the absence of overt cognitive impairments.[54] Lateral habenula interactions with the hippocampus are also important in spatial memory processing, with its inactivation leading to deficits in spatial recognition tasks.[55,56] This suggests a critical role for the habenula-hippocampus connectivity in spatial memory encoding and retrieval.[56] While direct connections between the habenula and the fusiform gyrus are not well documented, their involvement in spatial processes is plausible. Our study's novelty lies in using the habenula as an ROI in DTI analysis, an approach not extensively explored in PD research. The enhanced structural connectivity observed might serve as an early indicator of potential visuospatial and memory deficits in PD patients. In our cohort, there were no PD patients with cognitive/visuospatial complaints and dementia, and all patients had MoCA scores of ≥24. However, correlating structural connectivity data with detailed cognitive evaluations, including visuospatial aspects, could provide further insights into these findings.

The pallidum, which is related to the lateral habenula and is part of the basal ganglia, plays a significant role in the control of movement and also has a role in non-motor symptoms, including sleep disorder, anxiety, and depression.[57] Changes in habenula-pallidum connectivity may indicate neural adaptations to dopaminergic signal loss and motor dysfunctions in PD. Similarly, the accumbens, connected indirectly with the habenula, is involved in reward and motivational behaviors. Its increased connectivity with the habenula could relate to emotional and motivational disturbances in PD, like apathy or depression.[45,58] These findings underscore the habenula's broader involvement in limbic-motor integration, influencing reward-based motor control. The ventral pallidum, a nexus for limbic reward signals, processes these into motor outputs.[59] Mogenson and colleagues[60] highlighted this limbic-motor integration in the accumbens-pallidal system, where ventral pallidal projections to the brainstem convert limbic motivation signals into motor actions. Furthermore, resting state fMRI studies on SubD suggest that pallidum connectivity disruptions relate to dysfunctions like anhedonia.[61] Liu et al.[62] also demonstrated that optogenetic activation of the ventral pallidum-lateral habenula glutamatergic projections triggered aversive behavior and induced depressive-like symptoms following social stress, while inhibition of the circuit produced an antidepressant effect in stress-susceptible mice. The lateral habenula's connections to both the limbic system and the basal ganglia, particularly the globus pallidus internal segment, suggest its convergence role for these circuits.[63] This complex interaction hints at the habenula's role in modulating motor functions through dopamine neuron influence, which is crucial

for reward-based motor control. Additionally, these results could be related to dopaminergic therapy. L-Dopa is known to modulate reversal-related activity in the nucleus accumbens and also have effects on pallidum.[64,65] Correlating structural connectivity data with detailed neuropsychological assessments might offer deeper insights into these associations, potentially unveiling mechanisms for apathy, loss of motivation, or reward-based motor control impairments in PD, as well as the effects of L-Dopa treatment. Besides this, some other addictive drugs and antidepressants are known to impact the structural connectivity and induce neuroplasticity process, especially in fronto-limbic networks.[66,67] However, antidepressant use was accounted for as a covariate in our analysis, which minimizes its potential confounding effect on the observed WM characteristics. As a result, the differences observed between PD and HC groups are unlikely to be influenced by antidepressant use, supporting the robustness of our findings. Our findings also demonstrate a significant positive correlation between disease duration and structural connectivity between the left habenula and left pallidum in patients with PD. This result may reflect neuroplastic changes or compensatory mechanisms in response to progressive dopaminergic neuron loss related to disease duration. In summary, our findings of altered connectivity could reflect early neurodegenerative processes or compensatory mechanisms in PD. The habenula's increased connectivity with both cognitive and motor-related regions could serve as a biomarker for underlying neural mechanisms and therapeutic targets.

The habenula plays a significant role in psychiatric conditions, including depression. Studies using DTI have shown mixed results in PD patients, with some indicating changes in WM tracts in those with depression[68,69], while others show no significant differences.[70,71] Notably, a comparison between patients with MDD and HCs revealed a higher number of WM tracts connecting the right habenula and left mediodorsal thalamus in MDD patients, suggesting a compensatory mechanism in response to functional disintegration in these tracts.[72] Our research did not find a direct link between habenula DTI metrics and BDI scores. This indicates that changes in the habenula's microstructure may not be closely associated with subclinical depressive symptoms in PD. This finding points to a potentially more complex neuropathological process affecting mood disorders in PD beyond the integrity of the habenula. However, we did observe a positive correlation between BDI scores and the structural connectivity of the right habenula-left cingulate isthmus in PD patients. This suggests a possible connection between habenula connectivity and mood. The cingulate cortex, integral to emotional, sensory, motor, and cognitive processes, is linked to various brain regions, and alterations in the isthmus cingulate have been associated with depression.[73,74] Additionally, depressed PD patients often show disruptions in functional brain networks, including the posterior cingulate cortex and temporo-occipital cortex, as well as the prefrontal-limbic network.[75,76] Interestingly, changes in habenula functional connectivity were observed immediately following subcallosal cingulate deep brain stimulation in a study on depression, indicating a potential influence of connections between the habenula and areas like the cingulate cortex and medial prefrontal region.[77] Our findings imply that the role

of the habenula in mood regulation might be more connected to its network connectivity rather than its microstructural integrity. This is important as these regions are involved in reward processing and negative feedback mechanisms. The relationship between depressive symptoms and structural connectivity in the habenula-cingulate regions in PD patients might offer insights into the neural basis of depression in PD. This could help in developing more targeted treatments for depression in PD. Future studies focusing on PD patients with depression are needed to further understand this relationship.

The current study has some limitations, including the absence of comprehensive cognitive assessments covering areas such as executive functions, visuospatial abilities, memory, face perception, and face recognition. Another limitation is the lack of participation from clinically depressed individuals and PD patients. One limitation of our study is the variability within the PD cohort, which may affect the generalizability of the findings. Although we controlled for key variables, some heterogeneity remains due to the inclusion of patients with varying disease severities and symptom profiles. Future studies using more stratified cohorts or additional controls may yield more precise insights into the relationship between PD pathology and structural connectivity. Additionally, the number of HCs in the study is smaller compared to the PD group. Another limitation is the separate analysis of each hemisphere. While this approach was chosen to capture potential asymmetries in brain structure and connectivity, it introduces additional complexity in interpretation and may increase the likelihood of over-adjustment or reduced statistical power. However, given the established lateralization of motor and non-motor symptoms in Parkinson's disease, we believe this approach provides valuable insights into hemisphere-specific alterations that could otherwise be overlooked in a whole-brain analysis. Despite these limitations, a key strength of this research is that it represents the first extensive analysis of DTI metrics and structural connectivity of the habenula in PD patients, utilizing advanced 7T MRI technology.

5. CONCLUSION

The habenula's involvement in both mood and motor functions makes it a potentially significant structure in PD. Our findings add to the emerging evidence that the habenula may have a more intricate role in PD's pathophysiology than previously thought. The impaired structural connectivity of the habenula could serve as an early biomarker for both motor and non-motor symptoms in PD, even in the initial stages of the disease. This insight has potential implications for the development of targeted treatments and the enhancement of PD patient management. Future research focusing on the correlation between the DTI metrics of the habenula and detailed cognitive functions and longitudinal follow-up will further elucidate the habenula's role in PD.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this study.

Ethics Approval and Consent to Participate

The TRACK-PD study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act. The TRACK-PD study was approved by the Institutional Review Board (IRB) of the Maastricht University Medical Centre (METC AZM/UM 18-027). Written informed consent was obtained from all participants before inclusion.

CRediT authorship contribution statement

Bedia Samanci: Concept and design, Literature review, Investigation, Interpretation, Data processing and analysis, Drafting of the manuscript, Finalizing the manuscript; **Ali Bayram:** Literature review, Data processing and analysis, Drafting of the manuscript, Finalizing the manuscript; **Sonny Tan:** Concept and design, Literature review, Investigation, Interpretation, Data processing, Drafting of the manuscript, Finalizing the manuscript; **Meriek Wanders:** Literature review, Investigation, Data processing and analysis, Finalizing the manuscript; **Stijn Michielse:** Literature review, Investigation, Interpretation, Finalizing the manuscript; **Mark Kuijf:** Concept and design, Interpretation, Critical revision of the manuscript, Finalizing the manuscript; **Yasin Temel:** Concept and design, Interpretation, Critical revision of the manuscript, Finalizing the manuscript.

Data Availability Statement

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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CHAPTER 5

Altered habenular and whole brain functional connectivity in early Parkinson's disease using 7 T MRI

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ABSTRACT

Parkinson's disease (PD) is traditionally linked to basal ganglia dysfunction, yet evidence highlights broader network disruptions. Habenula, involved in regulating mood, reward, and motor functions, remains underexplored in PD. This study investigated whole-brain and habenular functional connectivity and their clinical correlates in early-stage PD using 7T MRI. Functional connectivity was analyzed in 104 early-stage PD and 45 healthy controls. Whole-brain analysis revealed increased connectivity in two clusters in PD: the first involved paracentral lobule, middle frontal gyrus, orbital middle frontal gyrus, precentral gyrus, angular gyrus, middle cingulate gyrus, supplementary motor area; the second included middle cingulate gyrus and cerebellum Crus I. Left habenula showed increased connectivity with right middle temporal and angular gyri (p-FDR=0.011). Levodopa equivalent daily dose positively correlated with connectivity between postcentral gyrus and cerebellum (p-FDR=0.020). These findings highlight early motor and cognitive network disruptions and suggest these regions may serve as potential markers of PD-related neurobiological changes.

INTRODUCTION

Parkinson's disease (PD) is traditionally characterized as a movement disorder resulting from significant loss of dopaminergic neurons in the nigrostriatal pathway. However, it is well known that PD also encompasses a wide range of non-motor symptoms, including cognitive impairment and mood disorders, which may involve alterations in multiple brain regions implicated in cognitive and emotional processing[1] . This makes PD clinically and etiologically diverse, and its complex neurobiological foundations are not yet fully understood.

The pathophysiology of PD extends beyond the dopaminergic system, involving multiple neural pathways and brain regions. Studies using restingstate functional magnetic resonance imaging (rs-fMRI) have identified alterations in several large-scale brain networks implicated in both motor and non-motor symptoms of PD, including the default mode network (DMN), sensorimotor network (SMN), salience network (SN), and frontoparietal network (FPN)[2] . Specifically, disruptions within the SMN have been associated with motor symptom severity, while DMN alterations are linked to cognitive decline, and SN abnormalities correlatewithmood disturbances in PD. Among these networks, intrinsic dysfunction within the dorsolateral prefrontal cortex (PFC) has been observed in depressed PD (dPD) patients, suggesting its involvement in mood and executive function impairments[3,4]. Similarly, apathy in PD has been associated with disrupted frontostriatal connectivity, which affects motivation and emotional processing [5,6]. A metaanalysis further revealed that decreased functional connectivity within the DMN consistently differentiates cognitively impaired and unimpaired PD patients [7]. In early-stage, cognitively unimpaired PD patients, decreased medial temporal and inferior parietal connectivity within the DMN correlated with cognitive performance, suggesting that posterior brain disconnection may precede cognitive impairment in PD [8]. Recent studies suggest that these network alterations are not merely a consequence of dopaminergic degeneration but may represent compensatory mechanisms or early pathophysiological changes. For example, structural and functional alterations in the cerebellum have been observed in early-stage PD, indicating that cerebellar changes may contribute to the disease process from its earliest phases [9]. Additionally, alterations in cortical motor areas have been linked to cerebellar hyperconnectivity in early to moderately advanced PD, potentially reflecting compensatory mechanisms for functional recovery [10]. It should also be noted that dopaminergic therapy, particularly levodopa, plays a crucial role in symptom management but has been shown to modulate functional connectivity across both motor and non-motor networks [11,12].

Given the widespread network alterations observed in PD, it is crucial to explore how subcortical structures, such as the habenula (Hb), contribute to both motor and non-motor dysfunctions, particularly in the early stages of the disease. The Hb is a small pair of nuclei located adjacent to the dorsomedial thalamus, with extensive connections to cortical (dorsal anterior cingulate cortex, medial PFC, left inferior frontal sulcus, middle and posterior

anterior cingulate, retrosplenial cortex, primary visual and auditory cortices, and posterior insula) and subcortical (thalamus, putamen, caudate, bed nucleus of the stria terminalis, nucleus basalis of Meynert, posterior hippocampus, septal nuclei, periaqueductal gray, dorsal raphe nuclei, ventral tegmental area and parahippocampal gyrus) [13,14]. It plays a key role in reward processing, learning, motivation, pain perception, aversion, and motor functions [15–18]. Recently, it has gained increasing attention for its role in mood and reward regulation, primarily through its influence on dopaminergic and serotonergic pathways. Given its widespread connectivity, dysfunction of the Hb has been implicated in mood disorders in both PD and non-PD populations; however, functional connectivity changes in the Hb remain underexplored in early-stage PD patients without clinically diagnosed depression [19–23].

In this study, we aimed to investigate (a) whole brain functional connectivity, (b) the functional connectivity between the Hb and the whole brain, and (c) the relationship between connectivity changes and clinical features in early-stage PD patients compared to healthy controls (HCs) using ultra-high field 7 T fMRI data from the longitudinal observational TRACK-PD study designed to investigate functional and structural brain changes in early-stage PD [24]. 7 T MRI offers superior spatial resolution and signal-to-noise ratio compared to conventional field strengths. These advantages enable precise mapping of small structures like the Hb and allow differentiation between its medial and lateral nuclei, enhancing the detection of subtle functional connectivity changes in early PD [25]. We hypothesize that there will be significant differences in whole brain and Hb connectivity between PD patients and HCs, and that these differences will correlate significantly with clinical features, reflecting early neurobiological changes in PD.

RESULTS

Clinical and demographical results

The PD and HC groups were matched for age, sex, handedness, and educational status. Among the PD patients, 32 (30.8%) were categorized as H&Y stage 1, 68 (65.4%) as stage 2, and 4 (3.8%) as stage 3.

A Kruskal-Wallis H-test revealed no significant differences in BDI total scores ($p = 0.092$), MoCA total scores ($p = 0.203$), or LEDD (0.682) across different H&Y stage 1 ($n = 32$), stage 2 ($n = 68$), and stage 3 ($n = 4$) groups. However, within the PD group, BDI total scores showed a significant positive correlation with both LEDD ($r = 0.274$, $p = 0.005$) and disease duration ($r = 0.222$, $p = 0.024$). Additionally, a significant negative correlation was found between BDI total scores and MoCA total scores ($r = -0.242$, $p = 0.013$). Conversely, no correlation was identified between BDI total scores and MDS-UPDRS Part III scores.

The mean BDI total score was significantly higher in the PD group compared to HCs (7.3 ± 5.07 vs. 3.5 ± 3.60; p < 0.001) (Table 1). Among PD patients, 7 (6.7%) were diagnosed with significant depression, 34 (32.7%) had SubD, and 63 (60.6%) exhibited no depression based on BDI scores. In the HC group, none were classified with significant depression, 5 participants (11.1%)fell into the SubD category, and 40 (88.9%) showed no signs of depression. Antidepressant or anxiolytic treatments were used by 5 PD patients (2 selective serotonin reuptake inhibitors (SSRIs), 1 amitriptyline, 1 serotonin-noradrenaline reuptake inhibitor, and 1 oxazepam) and 2 HCs (both on SSRIs).

The clinical and demographic data of the participants are presented in Table 1.

Table 1. Clinical and demographic data of the participants.

	PD group (n=104)	HC group (n=45)	p
Sex (M/F, n)	73/31	31/14	0.874
Handedness (R/L, n)	89/15	42/3	0.182
Age (years)	62.4 ± 8.45	60.7 ± 8.07	0.240
Disease Duration (month)	19.7 ± 9.50	-	N/A
LEDD	402.2 ± 230.5	-	N/A
MDS-UPDRS Part III Score	19.1 ± 7.48	-	N/A
MoCA Total Score	27.9 ± 1.63	27.7 ± 1.56	0.634
BDI Total Score	7.3 ± 5.07	3.5 ± 3.60	<0.001

=PD: Parkinson's disease, HC: Healthy control, M: Male, F: Female, R: Right, L: Left, LEDD: Levodopa equivalent daily dose, MDS-UPDRS: Movement Disorders Society-Unified Parkinson's Disease Rating Scale, MOCA: Montreal Cognitive Assessment, BDI: Beck Depression Inventory.

Comparison of HC and PD groups in whole brain functional connectivity

In the whole-brain ROI-to-ROI comparison between the HC and PD groups, two clusters with significant connectivity changes were identified (Table 2). The first cluster included the right paracentral lobule, bilateral middle frontal gyrus, bilateral orbital middle frontal gyrus, bilateral orbital superior frontal gyrus, left precentral gyrus, right angular gyrus, left middle cingulate gyrus, and left supplementary motor area ($F(4,141) = 6.45$; pFDR = 0.008). The second cluster comprised the left middle cingulate gyrus and bilateral cerebellum Crus I ($F(4,141) = 5.47$; p-FDR = 0.018) (Fig. 1a). All connectivities were significantly higher in the PD group compared to the HC group.

Table 2. Increased connectivity in Parkinson's disease group compared to healthy controls

	T (144)	p-FDR
Cluster 1		
R paracentral lobule – R middle frontal gyrus	3.70	0.011
R paracentral lobule – L middle frontal gyrus, orbital	3.56	0.011
R paracentral lobule – L superior frontal gyrus, orbital	3.52	0.011
R paracentral lobule – R middle frontal gyrus, orbital	3.40	0.013
L precentral gyrus – L middle frontal gyrus, orbital	3.59	0.014
L precentral gyrus – R angular gyrus	3.46	0.014
R paracentral lobule – L middle frontal gyrus	3.30	0.014
L precentral gyrus – R middle frontal gyrus	3.31	0.016
L precentral gyrus – L middle frontal gyrus	3.27	0.016
L middle cingulate gyrus – L superior frontal gyrus, orbital	3.16	0.016
L supplementary motor area – R angular gyrus	3.60	0.021
L supplementary motor area – L middle frontal gyrus	3.46	0.021
L paracentral lobule – R middle frontal gyrus	3.60	0.025
L paracentral lobule – R superior frontal gyrus, orbital	3.32	0.025
L paracentral lobule – L middle frontal gyrus, orbital	3.28	0.025
L precentral gyrus – L superior frontal gyrus, orbital	3.01	0.026
L supplementary motor area – L middle frontal gyrus, orbital	2.95	0.031
L middle cingulate gyrus – R middle frontal gyrus, orbital	2.80	0.034
L precentral gyrus – R middle frontal gyrus, orbital	2.84	0.038
Cluster 2		
L middle cingulate gyrus – L cerebellum crus I	3.85	0.004
L middle cingulate gyrus – R cerebellum crus I	3.84	0.004

R: right, L: left.

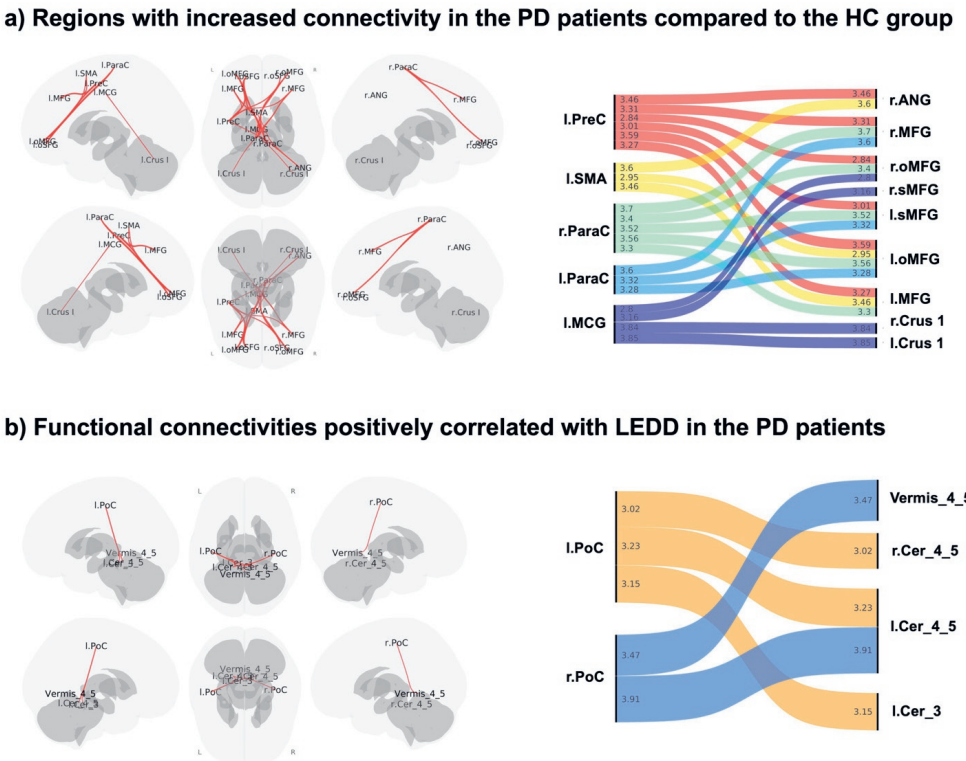

Figure 1. Connectivity alterations in Parkinson's disease (PD). Significant connectivity changes between the PD and healthy control (HC) groups (a). In PD patients, further analyses revealed correlations between functional connectivity and Levodopa Equivalent Daily Dose (LEDD) (b). In the right panels of both figures, the direction of inter-regional connectivity is displayed, along with the t-values corresponding to these connections, indicated by the numbers within the lines. All connectivities were significantly higher in the PD group compared to the HC group. ANG: Angular gyrus, Cer_3: lobule III of cerebellar hemisphere, Cer_4_5: lobule IV and V of cerebellar hemisphere, Crus I: Crus I of cerebellar hemisphere, MCG: Middle cingulate gyrus, MFG: Middle frontal gyrus, mSFG: Superior frontal gyrus, medial part, oMFG: Middle frontal gyrus, orbital part, oSFG: Superior frontal gyrus, orbital part, ParaCG: Paracentral gyrus, PoCG: Postcentral gyrus, PreCG: Precentral gyrus, SFG: Superior frontal gyrus, SMA: Supplementary motor area, Vermis 4_5: Lobule IV and V of vermiş. L: Left, R: Right

The functional connectivity of the habenula

The seed-to-voxel analysis comparing the two groups in terms of Hb connectivity revealed that PD patients exhibited increased functional connectivity of the left Hb compared to the HC group. This increase was observed in a cluster located at the intersection of the right middle temporal gyrus and angular gyrus (MNI coordinates: x = 40, y = -40, z = 22; cluster size = 24 voxels; t(144) = 3.95; p-FDR = 0.011) (Fig. 2). No significant differences were detected between the two groups regarding the functional connectivity of the right Hb.

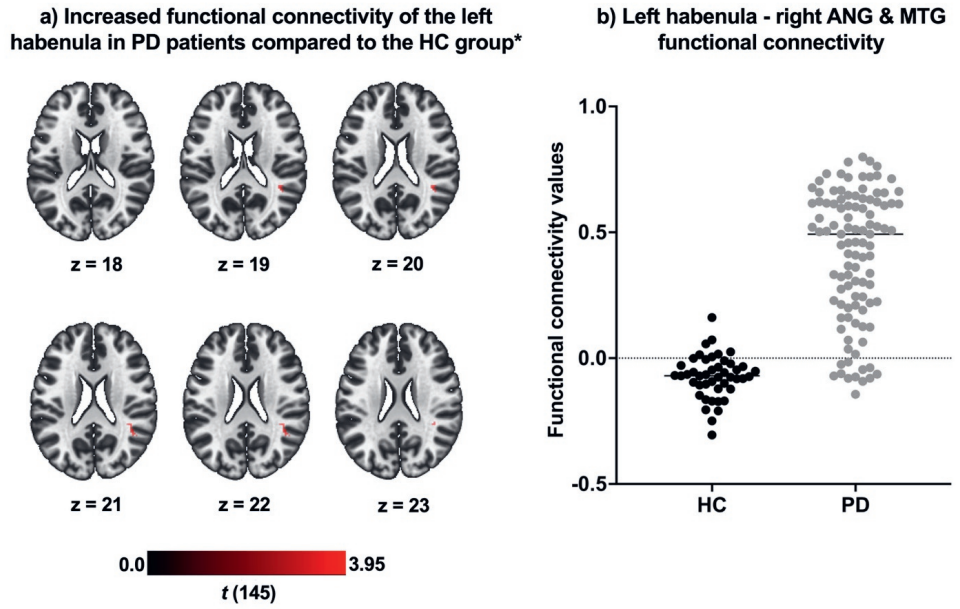

Figure 2. Altered habenula connectivity in Parkinson's disease (PD). a The cluster located at the intersection of the right angular gyrus and middle temporal gyrus, which shows increased connectivity with the left habenula in the PD group compared to the healthy controls (HC) group. b The functional connectivity values of the cluster, indicating enhanced connectivity with the left habenula in PD patients. The mean functional connectivity value in the HC group is -0.07 (indicating almost no connectivity), while the functional connectivity value in the PD group is 0.41. In summary, while there is nearly no connectivity between the right angular gyrus and the middle temporal gyrus in the HC group, this connectivity was found to be significantly increased in the PD group. *Adjusted for age, antidepressant use and Beck Depression Inventory total scores. ANG: Angular gyrus, MTG: Middle temporal gyrus.

Correlations between functional connectivity, BDI scores, and clinical features in PD group

In the whole-brain ROI-to-ROI analysis of the PD group, the analysis of functional connectivity correlating with LEDD revealed a cluster with a positive correlation ($F(3, 96) = 7.88$; p-FDR = 0.020) (Table 3). This cluster included the bilateral postcentral gyrus, bilateral lobules IV and V of the cerebellar hemisphere, lobules IV and V of the vermis, and left lobule III of the cerebellar hemisphere (Fig. 1b). Nofunctional connectivity was found to be negatively correlated with LEDD. There was no correlation with functional connectivity and the other clinical features as BDI total score, MoCa total score, MDS-UPDRS Part III, and disease duration. No correlation wasfound between habenula connectivity and BDI total score, MoCA total score, LEDD, MDS-UPDRS Part III score, or disease duration within the PD group.

Table 3. Functional connectivity positively correlated with levodopa equivalent daily dose in Parkinson's disease group

	T (98)	p-FDR
Cluster 1		
R postcentral – L lobule IV and V of cerebellar hemisphere	3.91	0.010
R postcentral – Lobule IV and V of vermis	3.47	0.022
R postcentral – L lobule III of cerebellar hemisphere	3.15	0.031
L postcentral – L lobule IV and V of cerebellar hemisphere	3.23	0.031
L postcentral – R lobule IV and V of cerebellar hemisphere	3.02	0.038

R: Right, L: Left.

DISCUSSION

This study examined whole-brain and Hb functional connectivity and its links to clinical features in early-stage PD patients compared to HCs. We found altered left Hb connectivity with the right middle temporal and angular gyri, as well as functional changes in brain areas linked to motor and cognitive functions. Connectivity alterations in postcentral gyri and cerebellum correlated with LEDD, reflecting early neurobiological changes in PD. These findings suggest widespread neural network disruptions beyond the nigrostriatal pathway, potentially contributing to early motor and cognitive symptoms in PD.

Depression, including mild forms like SubD, is a common non-motor symptom in PD, affecting 8% to 28.8% of patients and posing diagnostic challenges due to its overlap with PD's motor symptoms [26]. In the present study, PD patients demonstrated higher BDI scores than HCs. There were only 5 PD patients who used antidepressant or anxiolytic treatments. However, SubD was identified in 32.7% of PD patients compared to 11.1% in the HC group. Higher BDI scores were significantly associated with increased LEDD, longer disease duration, and lower MoCA scores, but showed no correlation with H&Y stages or MDS-UPDRS Part III scores. These findings highlight the early prevalence of depression in PD and its association with disease progression and cognitive decline.

The whole-brain analysis identified two clusters with altered connectivity in PD patients. One cluster showed increased connectivity involving motor and cognitive regions such as the paracentral lobule, precentral gyrus, middle cingulate gyrus, supplementary motor area (SMA), superior and middle frontal gyri, and angular gyrus. These areas are integral to motor control, movement planning, postural stability, and cognitive-emotional functions [27]. Dysfunction in these regions is linked to common PD symptoms, including gait and balance difficulties, impaired voluntary movement initiation, apathy, depression, and decision-making challenges. Tuovinen et al.[10] investigated longitudinal functional connectivity changes in PD and found increased connectivity between the cerebellum and the somatomotor network,

along with decreased connectivity between motor regions such as the Rolandic operculum, precentral gyrus, SMA, postcentral gyrus, and middle cingulate cortex in early disease stages. Other studies have also reported both decreased and increased functional connectivity within these areas in PD compared to HCs [27,28]. These regions are part of a broader motor network that includes the basal ganglia, thalamus, and various cortical areas. PD primarily affects the basal ganglia, disrupting dopamine signaling and altering motor circuit outputs to cortical areas like the SMA and precentral gyrus. This disruption not only results in the characteristic motor symptoms but also influences cognitive and emotional aspects of movement. While decreased connectivity often reflects neural dysfunction, increased connectivity may indicate compensatory mechanisms. In our study, increased connectivity between motor and cognitive regions suggests the brain's plasticity in adapting to neurodegeneration by enhancing integration across networks to maintain function.

The second cluster showed increased connectivity between the middle cingulate gyrus and cerebellum Crus I. The middle cingulate gyrus is involved in motor control, pain perception, decision-making, and cognitive processes, while the cerebellum contributes to motor execution, learning, gait, and non-motor functions like cognition [29,30]. Crus I, specifically, is linked to executive functions such as working memory, planning, visuomotor integration, and refining voluntary movements [30–33]. Increased connectivity with Crus I may reflect its role in integrating motor and cognitive processes, adapting to neurodegeneration in PD. Similar findings in prior studies revealed altered cerebellar connectivity with motor and cognitive networks, with increased sensorimotor cerebellar connectivity associated with worsening motor outcomes over time [9,10]. Previous studies have also notedincreased cerebellar connectivity in PD patients compared to controls, as well as disrupted communication between the cerebellum and other areas of the sensorimotor network [34,35]. Our results suggest that the enhanced connectivity between the middle cingulate gyrus and Crus I represents a compensatory mechanism, supporting the cerebellum's expanded role in addressing the motor and cognitive challenges of PD. This adaptation highlights the cerebellum's importance in maintaining complex motor and cognitive functions amidst PD-related disruptions.

Functional neuroimaging studies are crucial for elucidating the contributions of structures beyond the basal ganglia to the pathophysiology of PD. To date, only one study has examined Hb connectivity in PD, finding increased connectivity between the Hb and the amygdala, thalamus, and striatum bilaterally, along with decreased connectivity between the bilateral Hb and the left frontal and precentral cortices in PD patients with punding compared to those without and HCs [36]. Our study revealed increased connectivity between the left Hb and the right middle temporal and angular gyri in PD patients. These regions, integral to language comprehension, socioemotional processing, spatial awareness, and multimodal integration, are key components of the default mode network (DMN), which is often disruptedin PD and other disorders [37–39]. Disrupted DMN activity in PD, linked to dopamine deficiency, may underlie executive dysfunction [40]. Animal studies also suggest Hb-related

DMN disruptions impact connectivity and cognition [7,41]. Our findings of increased Hb connectivity with these regions likely reflect compensatory mechanisms or early maladaptive network reorganization in response to dopaminergic deficits, potentially serving as markers of subtle cognitive changes in early-stage PD.

The relationship between functional connectivity changes and clinical features in PD remains complex. While our study did not find significant correlations between Hb connectivity and features like BDI scores, MoCA scores, MDS-UPDRS Part III scores, or disease duration, whole-brain analysis revealed notable correlations with LEDD. Prior research has linked Hb connectivity changes to SubD in regions such as the amygdala, insula, PFC, mid-cingulate cortex, and entorhinal cortex [42]. We also did not observe a relationship between whole brain connectivity and BDI scores. There are many studies showing reduced connectivity in the medial frontal gyrus, paracentral lobule, precentral gyrus, and SMA in depressed PD patients, which are associated with motor and emotional impairments [43–47]. The precentral gyrus, crucial for initiating voluntary movement, is further burdened in PD by dopaminergic deficits. As depression severity increases, diminished connectivity between motor and frontal cognitive regions may exacerbate motor symptoms like bradykinesia and impair executive functions. These disruptions highlight the intertwined nature of motor and depressive symptoms in PD and underscore the importance of addressing depression as part of PD management [48]. Contrary to our hypothesis, altered habenular connectivity were not significantly correlated with BDI or MoCA scores. This finding suggests that Hb connectivity alterations may reflect mechanisms distinct from depression and cognitive impairment. One possible explanation is that Hb dysfunction in PD may be related to broader motivational, autonomic, or reward-processing abnormalities, which are not fully captured by standard depression or cognitive screening tools. BDI, while widely used in PD research, is a self-report measure that provides only a brief snapshot of depressive symptoms and may not fully capture the complexity of mood disturbances in PD. The BDI does not differentiate between somatic symptoms related to PD (e.g., fatigue, motor slowing) and primary affective symptoms, which could influence the interpretation of our findings. Additionally, we observed that significant connectivity alterations were lateralized to the left Hb, aligning with previous research suggesting potential hemispheric asymmetries in Hb function. While lateralization of habenular activity remains incompletely understood, some studies indicate that the left Hb may be preferentially involved in cognitive and emotional regulation, while the right Hb has stronger links to stress-related responses [23,49]. This asymmetry could suggest that early-stage PD-related connectivity changes preferentially affect cognitive-emotional circuits rather than stress-related pathways. Instead of concluding that Hb connectivity is independent of depression in PD, we acknowledge that multiple factors may have contributed to this negative result, including sample characteristics (mild depressive symptoms in our cohort), potential nonlinear relationships, and interindividual variability in disease progression. This highlights the need for larger, longitudinal studies with detailed neuropsychiatric assessments to better understand the role of Hb connectivity in mood and cognitive symptoms in PD. Addition-

ally, while rs-fMRI provides valuable insights into intrinsic connectivity patterns, it does not capture dynamic task-dependent activity. Given the Hb's role in reward processing, aversion, and decision-making, future studies utilizing taskbased fMRI paradigms—particularly those probing reward anticipation, effort-based decision-making, and emotional regulation—may offer greater specificity in understanding Hb function in PD.

While Hb connectivity showed no significant correlation with LEDD, our whole-brain analysis revealed a positive correlation between LEDD and increased connectivity of the bilateral postcentral gyrus with cerebellar lobules III-IV-V and the vermis (lobules IV-V). The postcentral gyrus, as the primary somatosensory cortex, processes sensory inputs and integrates them with motor commands, while the cerebellum refines motor movements through sensorimotor processing [50]. The functional relationship between the cerebellum and the postcentral gyrus facilitates the integration of sensory data for smooth, coordinated movement. Increased corticocerebellar connectivity in PD has been linked to compensatory responses to basal ganglia dysfunction, particularly in patients with postural instabilitygait difficulty [29,51,52]. Studies, including the earliest study by Wu et al. [53] on the effects of L-DOPA, have shown that L-DOPA partially normalizes cerebellar overactivity, which arises as a compensatory response to basal ganglia abnormalities. Esposito et al. [28] further examined this by conducting a placebo-controlled study with drug-naïve PD patients, and similarly demonstrated that L-DOPA enhances basal ganglia and thalamic involvement in sensorimotor networks. In our study, higher LEDD was associated with increased connectivity between the postcentral gyrus and motor cerebellum, suggesting dopaminergic medication enhances sensory-motor integration. While this connectivity boost may aid motor function, it might also lead to overactivity or maladaptive plasticity, contributing to motor complications like dyskinesias with prolonged treatment. These findings highlight the dual nature of dopaminergic therapy in PD: while improving motor symptoms, it may also reshape brain networks in ways that require careful management. Future research should explore the long-term effects of these connectivity changes and develop strategies to balance motor symptom relief with the minimization of adverse effects. Also, since our PD cohort was scanned while on medication, these findings highlight the importance of considering treatment effects when interpreting connectivity changes. Future studies incorporating OFF-state imaging could help disentangle disease-related and medication-induced alterations in functional connectivity.

The cross-sectional design of this study limits our ability to draw causal conclusions regarding the observed connectivity changes. Without longitudinal data, it remains unclear how these alterations evolve and influence clinical outcomes over time. Future research should focus on longitudinal studies to track these changes and their impact on disease progression. Additionally, investigating the functional significance of Hb connectivity changes in relation to specific cognitive and emotional processes in PD will be crucial for understanding their role in the broader context of disease pathology and symptomatology. While the current

study employed a seedbased connectivity approach via the GLM to test specific hypotheses regarding habenular connectivity in early-stage PD, future studies could benefit from the complementary use of Independent Component Analysis for exploratory network analysis. Our study has also another limitation related to the male-to-female ratio and the lack of detailed ancestry data. Males are approximately 1.5 times more likely to develop PD than females, but emerging evidence suggests that sex differences influence functional connectivity and non-motor symptoms, with women showing higher anxiety and autonomic dysfunction, and men exhibiting greater cognitive impairment [54–58]. Regarding ancestry demographics, our study predominantly included participants of European descent, which may limit generalizability across populations with different genetic and environmental risk factors for PD [59,60]. Future studies with more balanced sex ratios and diverse populations are needed to explore these differences and improve the generalizability of our findings. Despite these limitations, a notable strength of this study is that it marks the first comprehensive exploration of Hb functional connectivity in PD patients, leveraging the capabilities of advanced 7 T MRI technology. The use of 7 T MRI was crucial in detecting the laterality of habenular connectivity changes, which may reflect adaptive network reorganization or compensatory mechanisms in early PD. Its high spatial resolution and enhanced contrast allowed for a more precise mapping of habenular connectivity with both cortical and subcortical regions, revealing subtle network alterations that may not be as easily detected with lower field strengths [25]. These findings underscore the importance of ultrahigh-field imaging not only in advancing our understanding of habenular function in PD but also in developing imaging biomarkers for early diagnosis, disease tracking, and therapeutic response assessment.

Our findings provide novel insights into the functional connectivity alterations of the Hb and cortical regions in early-stage PD. This study is the first to investigate Hb connectivity in a large cohort of early PD patients. We observed increased connectivity between the left Hb and the right middle temporal and angular gyri, alongside widespread cortical connectivity changes, highlighting the complex neural alterations occurring early in PD. This increased connectivity between the Hb and cognition-related regions may serve as early markers for cognitive decline in PD. Furthermore, the altered connectivity between motor regions and the cerebellum underscores the efforts to compensate for dopamine loss, reflecting both the benefits and risks associated with dopaminergic therapy. Recognizing these connectivity changes is crucial for optimizing treatment and understanding PD progression. These findings emphasize that PD extends beyond basal ganglia dysfunction to involve extensive network disruptions. Future longitudinal research is needed to further explore these connectivity changes and refine therapeutic strategies to alleviate symptoms and enhance the quality of life for individuals with PD.

METHODS

Participant selection

The study complied with the Declaration of Helsinki and the Medical Research Involving Human Subjects Act and received ethical approval from the Institutional Review Board (IRB) of Maastricht University Medical Centre (METC AZM/UM 18-027). Written informed consent was obtained from all participants before inclusion.

TRACK-PD is a longitudinal observational study using 7 T MRI in newly diagnosed PD patients [24]. The study aims to provide valuable insights into the diverse clinical presentations of PD and their associated MRI features. Its primary objective is to improve the understanding of PD and identify novel biomarkers for tracking disease progression, ultimately facilitating the development of new therapeutic strategies. The inclusion criteria for the TRACK-PD study were as follows: (a) A Parkinson's disease diagnosis confirmed by a neurologist within the past 3 years following established criteria [61], (b) A baseline Montreal Cognitive Assessment (MoCA) score of ≥24 with no evidence of dementia, (c) Proficiency in Dutch, (d) Age 18 or older and (e) Written informed consent to participate. Participants with contraindications for a 7 T MRI scan were excluded. All PD participants underwent MRI while on their usual dopaminergic medication regimen (on state). This ensures that observed functional connectivity changes reflect disease-related and medication-related effects, rather than acute withdrawal from dopaminergic therapy.

The TRACK-PD study included 106 PD patients and 45 HCs. Following MRI quality control, 104 PD patients (66 males, 18 females) and 45 HCs (31 males, 14 females) were retained. Clinical and demographic data were collected, including age, sex, handedness, disease duration, Hoehn & Yahr stage (H&Y) [62], Movement Disorders SocietyUnified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III (medication-on) [63], total levodopa equivalent daily dose (LEDD) [64], and MoCA scores [65].

Depression levels were evaluated using the Beck Depression Inventory (BDI), a 21-item questionnaire [66]. The BDI assessment and MRI scanning were conducted at the baseline visit to ensure temporal consistency between clinical and neuroimaging data. The cut-off values applied were specifically tailored for PD, consistent with previous studies that also utilized BDI. Scores were categorized as follows: 0-8 indicated no depression, 9-15 represented subthreshold depression (SubD), and ≥16 signified significant depression [26,67].

MRI acquisition and preprocessing

Participants underwent scanning with a 7 T MRI scanner (Magnetom, Siemens, Erlangen, Germany) using a Nova Medical 32-channel head coil, enhanced by dielectric pads to improve signal in temporal brain regions [68].

The whole-brain resting-state fMRI scan consisted of 280 volumes, with an additional five volumes recorded using a reverse phase-encoding direction. During the scan, participants were instructed to focus on a crosshair projected on a screen and to let their minds wander without concentrating on any specific thoughts. The fMRI scan parameters were as follows: echo time (TE) of 18.6 ms, repetition time (TR) of 2000 ms, flip angle of 70°, field of view (FoV) of 200 × 200 mm, resolution of 1.25 × 1.25 × 1.25 mm^3, 92 slices with an axial orientation, and a total acquisition time of 11:12 minutes [24].

The results presented in this manuscript were derived using CONN release 22.a [69] and SPM release 12.7771 [70]. The functional and anatomical data underwent pre-processing through a flexible pipeline that involved several steps: realignment with correction for susceptibility-distortion interactions, outlier detection, indirect segmentation and MNI space normalization, and smoothing. Each scan was co-registered to a referenceimage (the first scan of the first session) using a least-squares approach with a 6-parameter (rigid body) transformation and resampled with B-spline interpolation to correct for motion and magnetization interactions. Potential outlier scans were identified using ART as scans with frame shifts greater than 0.9 mm or global BOLD signal changes greater than 5 standard deviations, and a reference BOLD image was calculated for each subject by averaging all scans without outliers. Finally, the functional data were spatially smoothed using a 2.5 mm full-width half-maximum (FWHM) Gaussian kernel.

Functional data were further denoised using a standard pipeline [71]. This process included regression of potential confounding effects characterized by white matter time series, cerebrospinal fluid (CSF) time series, motion parameters and their first-order derivatives, outlier scans, session effects and their first-order derivatives, and linear trends within each functional run, followed by band-pass frequency filtering of the BOLD time series between 0.008 Hz and 0.09 Hz. Within each subject's eroded segmentation mask, CompCor [72,73] noise components within white matter and CSF were estimated by calculating the mean BOLD signal and the largest principal components orthogonal to the mean BOLD, motion parameters and outlier scans.

Habenula and whole brain functional connectivity

For the whole-brain functional connectivity analysis, ROI-ROI connectivity matrices were estimated to characterize the functional connectivity between each pair of regions among 118 ROIs. This set of ROIs included 116 brain regions from the AAL atlas [74] and the right and left Hb as additional regions. The AAL atlas was selected because it provides a comprehensive and widely used parcellation of the brain into 116 regions.

For the analysis of habenular functional connectivity, seed-based connectivity maps were created to delineate the spatial patterns offunctional connectivity with the seed regions. The seed regions, which were manually segmented for each participant, comprised the right and

left Hb, as previously detailed [75]. The association between the BOLD signal time series of each seed area and the target voxels was modelled by a Fisher-transformed bivariate correlation coefficient from a weighted general linear model estimated separately for each seed area and the target voxels.

Statistical analysis

Statistical analyses were performed using SPSS 28.0 (IBM, SPSS Inc., New York, United States). Normality was confirmed using the KolmogorovSmirnov method. An independent sample t-test or one-way analysis of variance (ANOVA) was used for normally distributed data, whereas the Kruskal-Wallis H-test or Mann-Whitney U-test was used for non-normally distributed variables for group comparisons.

Group-level analyses of habenular functional connectivity were conducted using a general linear model (GLM). For each voxel, a separate GLM was estimated with the first-level connectivity measures at that voxel as the dependent variable and the group as the independent variable. Age, BDI scores and antidepressant use were included as covariates when comparing HC and PD groups. Multivariate parametric statistics with random effects across subjects and sample covariance estimation across multiple measures were employed to evaluate hypotheses at the voxel level. Inferences were drawn at the level of individual clusters of interest. Results were thresholded using a connection significance of $p < 0.001$, combined with a cluster threshold of p-FDR < 0.05 for multiple comparison.

To assess whole brain functional connectivity, the study used a multivariate pattern analysis (MVPA) omnibus test with the CONN toolbox. This approach identifies significant patterns of connectivity across many regions of interest (ROIs), ensuring that both individual connections and clusters of connections match the established significance criteria. In comparing HC and PD groups, age, BDI scores and antidepressant use were included as covariates. We selected age and antidepressant use as covariates based on their potential influence on both functional connectivity and clinical measures. Age was included to accountfor its known effects on brain connectivity and cognitive performance [76]. We included antidepressant use as a covariate due to its potential effects on mood-related brain networks, ensuring that any variability introduced by its use was accounted for [77]. A statistical criterion of $p < 0.05$ was chosen for connection-level inferences, whereas a more stringent cluster-level correction of p-FDR < 0.05 was utilized to account for multiple comparisons [78].

To compare the functional connectivity of the Hb and whole brain between HC and PD groups, and to identify connectivity correlated with BDI, LEDD, UPDRS-III scores specifically in the PD group, a MVPA omnibus test was performed as previously described. Covariates were chosen based on the specific correlation being examined: age, LEDD, antidepressant use, and UPDRS-III scores were used as covariates for BDI scores; age, BDI, antidepressant use, and UPDRS-III scores were used as covariates for LEDD scores; age,

BDI, antidepressant use and LEDD scores were used as covariates for UPDRS-III scores. This approach ensured that relevant confounders were controlled for correlation analyses.

Data Availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Author contributions

B.S., S.T., M.K., and Y.T. contributed to the concept and design of the study. B.S., U.A., and S.T. performed the literature review. B.S. and S.T. conducted the investigation. B.S., U.A., M.K., and S.T. were responsible for data processing and analysis. B.S., M.K., S.T., and Y.T. contributed to data interpretation. B.S., U.A., and S.T. drafted the manuscript. M.K. and Y.T. critically revised the manuscript. B.S., U.A., M.K., S.T., and Y.T. finalized the manuscript. All authors reviewed and approved the final version.

Competing interests

The authors declare no competing interests.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

OVERVIEW

This thesis aimed to investigate the role of the habenula in PD by examining structural, microstructural, and functional changes using advanced neuroimaging approaches, including 7T MRI-based volumetry, diffusion tensor imaging, and functional connectivity analyses. The findings reveal volumetric changes of the habenula, increased structural connectivity with key limbic and motor regions, and altered functional connectivity patterns, suggesting a compensatory role of the habenula in early-stage PD.

The findings of this thesis provide novel insights into the importance of the habenula in PD pathophysiology. These results contribute to an emerging body of research supporting non-dopaminergic mechanisms in PD and highlight the potential role of the habenula as an indicator for non-motor symptoms and biomarker for early diagnosis. Importantly, the observed imaging alterations should not be interpreted exclusively in relation to depressive symptoms. Rather, they should be considered within the broader framework of non-motor dysfunction in PD, including cognitive, motivational, and affective processes that are known to involve habenular circuitry.

In Chapter 3, our 7T MRI volumetric analysis revealed that PD patients exhibited a significantly larger mean and right habenula compared to healthy controls. This finding challenges conventional expectations, as prior studies have often reported habenular atrophy in affective disorders [1] [2]. Notably, we found no correlation between habenular volume and BDI scores, suggesting that habenular alterations in PD may not be exclusively linked to mood disturbances. Instead, it is possible that habenular enlargement reflects other PD-related mechanisms. One potential explanation is that it represents a compensatory neuroplastic response to progressive disruption of dopaminergic input from the SN, given the habenula's reciprocal connectivity with basal ganglia and brainstem monoaminergic nuclei. Alternatively, increased volume may be related to altered activity in motor or reward-processing circuits, which are also affected in PD. While these interpretations remain speculative, they are supported by the habenula's role in integrating motor, cognitive, and emotional information, and by animal studies demonstrating its plasticity in response to dopaminergic dysregulation. Further studies are needed to clarify the functional significance of this finding and its potential role as a disease-stage or phenotype-specific marker.

Several mechanisms could explain this enlargement of the habenula in PD. Given its strong reciprocal connections with the basal ganglia, limbic system, and brainstem monoaminergic centers (Chapter 2, Figure 2) [3], it is possible that habenular volume increase represents a (1) compensatory response to the progressive degeneration of dopaminergic neurons in the SN pars compacta, (2) structural plasticity in response to altered neurotransmitter signaling, or (3) a maladaptive remodeling process associated with disease. Further longitudinal studies

are necessary to determine whether habenular volume increasing represents an adaptive compensatory mechanism or an early biomarker of neurodegeneration.

Diffusion tensor imaging analyses in ***Chapter 4*** provided additional insights into habenular white matter microstructure and connectivity. The results demonstrated that PD patients exhibited increased structural connectivity between the right habenula and the right hippocampus and fusiform gyrus, as well as the left habenula and the left pallidum and nucleus accumbens. These findings suggest that white matter reorganization within the habenular circuit occurs early in PD, likely influencing both cognitive and affective processing. The increased connectivity between the habenula and the hippocampus aligns with previous research demonstrating that habenular dysfunction is associated with memory deficits and executive dysfunction in PD [4]. Additionally, heightened connectivity between the habenula and nucleus accumbens may be associated with reward system dysregulation**,** potentially exacerbating dopamine-related motivational deficits and apathy in PD. Increased connectivity with pallidum might be related to motor impairment. Moreover, a significant correlation was found between higher depression scores and increased habenular connectivity to the cingulate cortex, reinforcing the idea that habenular changes may modulate mood-related circuits in PD. Interestingly, despite diffusion metrics not showing significant differences, the increased structural connectivity patterns in PD suggest that network-level alterations occur before microstructural degeneration. These findings may indicate that white matter changes in PD can precede overt neurodegeneration and may serve as early biomarkers of disease progression.

In Chapter 5, resting-state fMRI analyses revealed significant functional connectivity changes in the habenula in PD patients compared to healthy controls. Specifically, PD patients exhibited increased connectivity between the left habenula and the right middle temporal and angular gyri, alongside widespread cortical connectivity changes, reflecting early neural alterations in PD. The strengthened habenula-cognitive network interactions may serve as early markers of cognitive decline, while altered connectivity between motor regions and the cerebellum likely represents compensatory mechanisms for dopamine depletion, highlighting both the adaptive and maladaptive effects of dopaminergic therapy.

Non-motor symptoms, depression, and the habenula in Parkinson's disease

Non-motor symptoms, including depression, anxiety, apathy, cognitive impairment, and impulse control disorders, are increasingly recognized as core components of the clinical spectrum of PD and may precede the onset of motor manifestations [5]. These symptoms reflect widespread involvement of monoaminergic, limbic, and frontostriatal circuits beyond the nigrostriatal dopaminergic pathway, including serotonergic and noradrenergic systems [5,6].

Within this broader network, the habenula has emerged as an important integrative structure involved in negative reward prediction, aversive learning, motivational regulation, and mood-related processing [7-9].

The role of the habenula is therefore unlikely to be restricted to depression. Through its connections with the prefrontal cortex, basal ganglia, limbic system, and brainstem monoaminergic nuclei, the habenula may also contribute to executive control, reinforcement learning, behavioral flexibility, motivation, and goal-directed behavior [7-9]. These functions are relevant to several non-motor manifestations of PD. Executive deficits such as impaired planning and set-shifting have been associated with disruption of frontostriatal networks[10], whereas apathy and reduced initiative have been linked to impaired reward valuation and motivational drive [11]. Impulse control and compulsive behaviors may similarly involve altered interactions among reward, salience, and inhibitory-control networks in which the habenula may participate [12].

The findings of this thesis should be interpreted within this broader clinical framework. Habenular volumetric and connectivity alterations were observed in patients with early-stage PD who did not have clinical depression, and most imaging findings were not significantly associated with BDI scores. ***In Chapter 3***, increased mean and right habenular volumes were found without a significant relationship with depressive symptom severity. This suggests that structural habenular alterations may occur independently of overt depression and may reflect broader disease-related, compensatory, or network-level processes. ***In Chapter 4***, however, increased structural connectivity between the right habenula and the left cingulate isthmus was associated with higher depressive symptom scores, indicating that specific habenular-limbic pathways may nevertheless be related to affective symptom burden. The connectivity alterations involving the hippocampus, nucleus accumbens, pallidum, cingulate regions, and associative cortical areas may therefore be relevant not only to depressive symptoms but also to motivation, reward processing, cognitive control, and other non-motor domains.

Interpretation of these findings is limited by the clinical characterization available in the present cohort. Non-motor symptom burden was assessed primarily using the BDI, which provides a validated measure of depressive symptom severity but does not comprehensively assess other clinically relevant domains such as anxiety, apathy, anhedonia, compulsive behaviour, reward processing, reinforcement learning, or executive functions including planning and set-shifting. Consequently, the absence of stronger associations between imaging measures and BDI scores should not be interpreted as evidence that habenular alterations are unrelated to non-motor dysfunction. Rather, these imaging abnormalities may be more closely associated with specific motivational, cognitive, and affective processes that were beyond the scope of the present clinical assessment.

Reinforcement learning and reward-based decision-making represent particularly relevant domains for future investigation. Although deficits in these processes in PD are commonly attributed to dopaminergic dysfunction[13], the habenula may also contribute through its regulation of dopaminergic and serotonergic activity and its role in signaling negative outcomes and prediction errors [7-9]. Alterations in habenular networks could therefore contribute to impaired learning from feedback, altered sensitivity to reward and punishment, anhedonia, reduced motivation, or maladaptive decision-making in PD.

Future studies should combine longitudinal multimodal neuroimaging with more comprehensive clinical phenotyping. This should include validated measures of depression, anxiety, apathy, anhedonia, impulse control and compulsive behavior, together with neuropsychological assessment of executive function, planning, set-shifting, and cognitive flexibility. Behavioral paradigms assessing reward consumption, reinforcement learning, punishment sensitivity, and decision-making may be particularly informative. Such an approach would help determine whether specific habenular structural and functional alterations correspond to distinct cognitive, affective, and motivational phenotypes in PD.

Discrepancies Across Structural, Diffusion, and Functional Findings

The advanced neuroimaging approach employed in this thesis provided valuable insights into structural, microstructural, and functional alterations of the habenula in PD. However, while each modality revealed significant findings, the results varied across different imaging techniques. In particular, habenular volumetric alterations were lateralized differently than microstructural findings, and structural connectivity patterns did not entirely overlap with functional connectivity results. These discrepancies raise important questions about the complexity of habenular dysfunction in PD and suggest that different imaging modalities may capture distinct aspects of neural reorganization and disease adaptation. They also may arise from methodological differences, compensatory mechanisms, disease heterogeneity, and the complex role of the habenula in different functional domains of PD. This section discusses these differences and their potential explanations.

Lateralization Differences Between Volumetric and Connectivity Findings

One of the most notable differences between the volumetric analysis, structural and functional connectivity results was the lateralization of habenular changes. The volumetric analysis revealed increased volume of the right habenula in PD patients compared to controls, while we found increased structural connectivity in the right and left habenula and functional connectivity in the left habenula. Several potential explanations can account for these findings.

This difference may be related to functional specialization of the left and right habenula [14]. The left and right habenula may serve distinct functional roles in motor and non-motor

symptomatology. Previous studies in rodents and primates suggest that the right habenula is more involved in motor control, while the left habenula plays a stronger role in reward-based learning, cognitive, and affective regulation [4,15]. The observed right habenular volume increasing in the volumetric study may reflect a compensatory response to dopaminergic degeneration in motor circuits, while the right and left habenular structural connectivity changes observed in DTI and left in fMRI may be related to motor and also non-motor symptoms such as cognitive impairment.

Volumetric MRI, DTI and fMRI have different sensitivities. MRI volumetric analysis primarily detects macrostructural changes, which often result from long-term neuroplasticity, neurodegeneration, or compensatory hypertrophy. It is possible that structural hypertrophy of the right habenula is a compensatory response to early dopamine depletion, attempting to regulate dopaminergic tone in the basal ganglia. In contrast, DTI assesses microstructural integrity and connectivity, which may not always correlate with gross volume alterations but instead reflect subtle reorganizations in habenular connectivity before significant structural atrophy occurs, and fMRI reflects real-time network interactions. These changes can reflect early-stage axonal changes and network reorganization that may not be detectable via volumetric measurements. At the same time, the bilateral DTI changes may reflect early compensatory alterations in habenula-related white matter tracts, possibly indicating adaptive restructuring in response to progressive neurodegeneration in the mesolimbic system. The fact that DTI and fMRI revealed increased connectivity in the left and right habenula suggests that microstructural and network-based reorganization may precede significant atrophy or hypertrophy in PD. This distinction may explain why volumetric increases were observed on the right while connectivity changes were more pronounced on the left. Each imaging technique may be capturing different aspects of habenular adaptation in PD.

Additionally, PD itself is known for asymmetric neurodegeneration, often beginning unilaterally in the SN and later progressing to both hemispheres [16,17]. Some studies have suggested that asymmetries in habenular dysfunction may arise due to asymmetric dopaminergic depletion [18]. This could mean that different PD subtypes (e.g., left vs. right onset PD) might experience differential habenular involvement, which could explain why structural and diffusion-based alterations do not perfectly align. Longitudinal studies that will include different PD subtypes will help to understand these differences.

Differences Between Structural and Functional Connectivity Patterns

Another major discrepancy observed in this thesis was the difference in habenular connectivity patterns between DTI-based structural connectivity and fMRI-based functional connectivity. DTI showed increased structural connectivity between the habenula and the hippocampus, fusiform gyrus, nucleus accumbens, and pallidum. Notably, these connections were primarily subcortical and limbic, suggesting that habenular involvement in PD may extend beyond the traditional basal ganglia circuits into emotion, motor, and memory net-

works. fMRI revealed increased functional connectivity between the habenula and cortical regions, including the middle temporal gyrus, angular gyrus, and motor-related areas such as the cingulate cortex and precentral gyrus. The observed changes were more widespread than those seen in DTI-based connectivity, involving higher-order associative cortices and motor regions, indicating a broader functional reorganization of habenular circuits in PD. These findings raise the question why diffusion-based connectivity and functional connectivity yield different results.

One of the possible explanations may be that DTI connectivity reflects anatomical pathways [19], while fMRI connectivity captures dynamic network activity [20]. DTI measures white matter tract integrity and anatomical connections, reflecting long-term adaptations in white matter tracts and how strongly the habenula is structurally connected to other regions. Increased connectivity on DTI does not necessarily indicate increased functional interactions between these areas. In contrast, fMRI reflects real-time network interactions, meaning it may capture more dynamic and transient changes in brain function that are not yet reflected in white matter microstructure. It measures correlated activity patterns, which can be influenced by dynamic interactions between regions, neurotransmitter changes, and compensatory plasticity. This could explain why functional connectivity alterations were more widespread and involved higher-order cortical areas, whereas structural connectivity changes were more confined to limbic and subcortical structures. The habenula may undergo early-stage structural reorganization (observed in DTI), but its functional interactions may shift dynamically over time depending on disease severity, medication effects, and compensatory mechanisms.

Functional connectivity may be more sensitive to early disease-related changes. Since functional changes often precede structural degeneration, the observed functional connectivity increases in cortical regions may represent early compensatory mechanisms, whereas structural connectivity increases in limbic regions could indicate a more chronic adaptation process. This may suggest that functional network reorganization is an early response to neurodegeneration, whereas structural disconnection follows at later disease stages.

One key factor influencing fMRI-based connectivity results is dopaminergic treatment. The observed correlation between LEDD and habenular functional connectivity with the cerebellum and postcentral gyrus suggests that dopaminergic medication modulates habenular activity in ways that are not captured by structural imaging techniques like DTI. Since DTI measures structural rather than dynamic functional alterations, these medication-related changes are less apparent in structural connectivity analyses.

Different imaging modalities capture different aspects of habenular dysfunction, with volumetric hypertrophy likely reflecting compensatory mechanisms, structural connectivity changes indicating compensation or long-term reorganization, and functional connectivity increases representing real-time compensatory processes. The increased structural and func-

tional connectivity with motor and non-motor regions in the early stages, along with the observation of this phenomenon beyond the habenula in functional connectivity, suggests that reorganization and compensatory mechanisms may be at play in the early stages of Parkinson's disease. The broader cortical involvement observed in fMRI but not DTI suggests that functional changes may precede anatomical reorganization, reinforcing the importance of longitudinal studies to track these changes over time. The lateralization of these findings may reflect distinct roles of the habenula in motor vs. non-motor symptoms in PD.

CONCLUSION

This thesis presents the first advanced neuroimaging investigation of the habenula in PD, demonstrating structural, microstructural, and functional changes that emerge early in the disease. By integrating 7 T MRI-based volumetry, DTI, and resting-state fMRI, this work reveals that the habenula undergoes complex neuroplastic adaptations, influencing both motor and non-motor symptoms.

While some discrepancies emerged between volumetric, DTI, and fMRI findings, these variations likely reflect the habenula's diverse functional roles, as well as the differing sensitivities of imaging modalities to distinct neurobiological mechanisms. The results suggest that compensatory plasticity, neurotransmitter depletion, and methodological factors contribute to these differences, underscoring the importance of advanced neuroimaging techniques in capturing the complexity of PD-related brain changes.

Beyond its immediate findings, this thesis advances the field by positioning the habenula as a key structure in PD pathophysiology, expanding the traditional dopaminergic-centric model of the disease. It highlights the potential of habenular alterations as early biomarkers and emphasizes the need for longitudinal studies to track its progression over time. Additionally, it lays the groundwork for future investigations into neuromodulation strategies, such as deep brain stimulation (DBS), targeting the habenula to alleviate both motor and non-motor symptoms.

Ultimately, this work bridges the gap between fundamental neurobiology and clinical applications, offering new perspectives for personalized diagnostic and therapeutic approaches in PD. By deepening our understanding of habenular involvement in neurodegeneration, this research contributes to a paradigm shift in PD studies, paving the way for innovative treatment strategies that may enhance the quality of life for patients.

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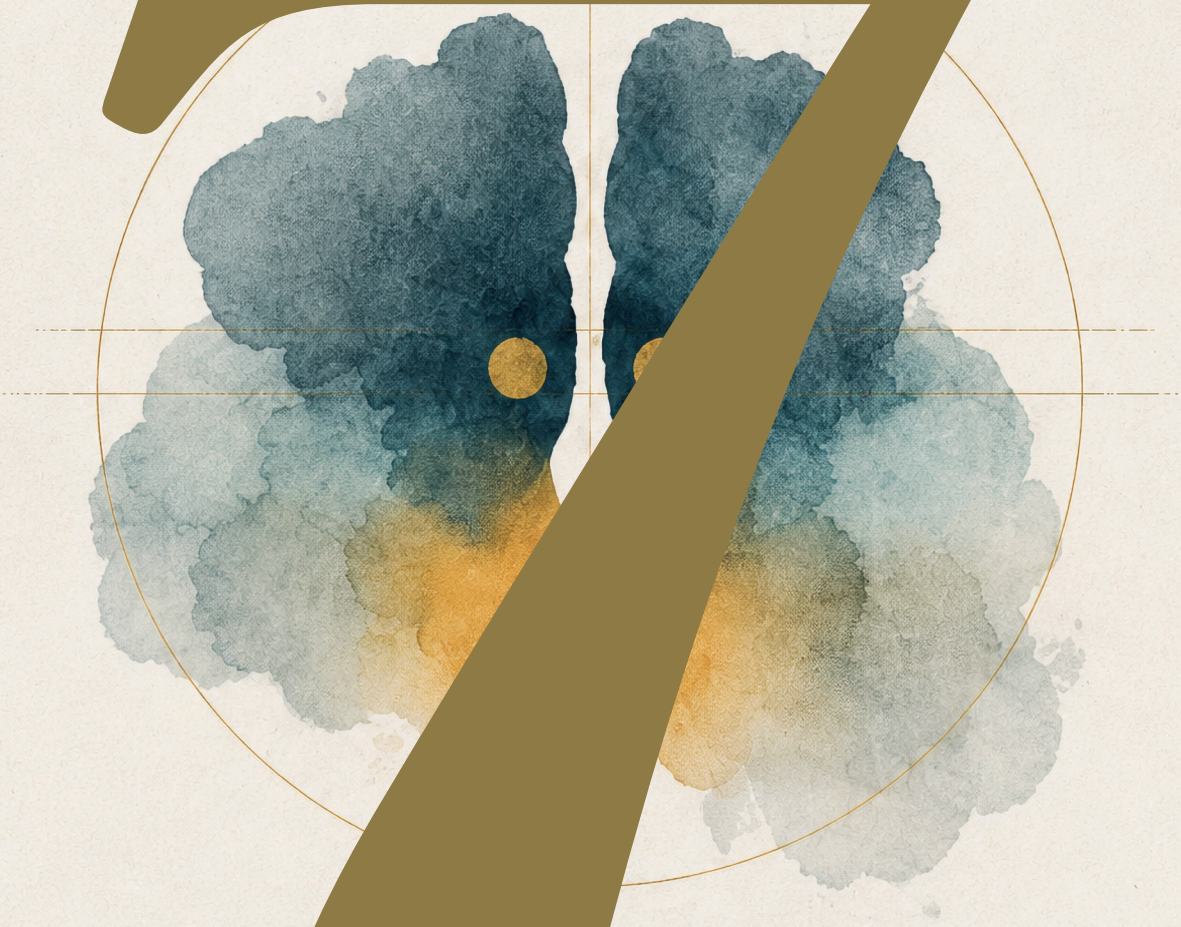

CHAPTER 7

VALORIZATION ADDENDUM

The findings presented in this thesis contribute to the understanding of PD, particularly in the context of the habenula's structural and functional roles. While the primary focus has been scientific exploration, it is imperative to discuss the broader societal and clinical implications of this research. The valorization addendum highlights how these findings can translate into tangible benefits for healthcare systems, patients, and future research directions.

SCIENTIFIC IMPACT

This thesis provides a comprehensive investigation of the habenula in PD, marking one of the first in-depth neuroimaging studies focusing on this structure in PD. By integrating 7T MRI-based volumetric analysis, DTI, and resting state fMRI, this work highlights significant alterations in the habenula's volume, structural and functional connectivities.

The findings of habenular hypertrophy, increased structural and functional connectivity patterns challenge the traditional dopaminergic-centric view of PD and suggest that non-dopaminergic mechanisms play a critical role in disease pathophysiology. This research provides evidence that early compensatory adaptations in the habenula may contribute to both motor and non-motor symptoms in PD, thereby expanding our understanding of PD beyond the basal ganglia-thalamocortical circuit.

From a scientific standpoint, the results of this thesis offer novel insights into the role of the habenula as a regulator of dopamine and serotonin systems in PD, reinforcing the need for further research into habenular function in neurodegenerative diseases. These results also highlight the importance of limbic-motor interactions in PD, as evidenced by increased connectivity between the habenula, hippocampus, pallidum, and nucleus accumbens, suggesting a potential role in motor, motivation deficits, apathy, and cognitive dysfunction. Furthermore, these findings are important for establish the foundation for future studies investigating habenular-targeted therapies, including neuromodulation strategies such as deep brain stimulation (DBS) and pharmacological interventions aimed at modulating habenular activity to alleviate motor and non-motor symptoms in PD.

CLINICAL IMPACT

One of the most significant contributions of this thesis is its potential clinical relevance in early disease detection, possible progression monitoring, and therapeutic advancements in PD.

Detecting habenular changes through advanced imaging techniques could facilitate earlier diagnosis and intervention, potentially altering the disease trajectory. One of the most criti-

cal outcomes of this thesis is the identification of the habenula as a potential biomarker for early-stage PD. Structural and functional alterations in the habenula, detected via advanced neuroimaging techniques, could facilitate earlier and more accurate diagnoses. Given the challenges in diagnosing PD before motor symptoms become prominent, structural and functional changes in the habenula could provide a novel target for early-stage screening and disease stratification. The correlation between habenular connectivity and LEDD suggests that individual differences in habenular function may influence treatment response, further supporting its potential as a biomarker for personalized medicine approaches in PD. Given the habenula's role in dopamine regulation and maladaptive plasticity in response to levodopa treatment, pharmacological interventions targeting habenular circuits may help mitigate levodopa induced dyskinesias and improve motor symptoms in PD patients. Earlier diagnosis could enable timely interventions, potentially slowing disease progression and improving patient outcomes. The development of standardized imaging protocols for assessing habenular changes in clinical settings would be a logical next step to operationalize these findings.

Depression and apathy are among the most debilitating non-motor symptoms of PD, affecting quality of life and disease burden. This thesis provides evidence that habenular dysfunction may contribute to these symptoms via disrupted interactions with the limbic system, reinforcing the importance of assessing habenular function in PD patients with cognitive and mood impairment. The observed increased habenula-hippocampus connectivity in PD patients may also have implications for cognitive impairment, as the habenula plays a role in learning, memory, and decision-making. Understanding these mechanisms may aid in the development of early diagnostic and therapeutic strategies targeting cognitive decline in PD.

The findings suggest that neuromodulation of the habenula such as DBS could be explored as a therapeutic strategy in PD. Previous studies have demonstrated that habenular DBS may alleviate depressive symptoms in treatment-resistant [1], suggesting a similar approach could be investigated in PD patients suffering from severe apathy and mood disturbances. This approach would be beneficial for also motor symptoms due to the habenula's connections with motor areas.

Collaboration between neuroscientists, clinicians, and pharmaceutical companies will be essential to translate these therapeutic ideas into clinical trials and, eventually, clinical practice.

SOCIETAL AND ECONOMIC IMPACT

PD is a growing global health burden, with the number of affected individuals expected to double by 2040 due to aging populations worldwide. Motor and non-motor symptoms contribute together significantly to the disease burden, caregiver stress, and healthcare costs. The

findings of this thesis have implications for reducing this burden through earlier diagnosis and more effective treatments.

Economic Benefits of Early Diagnosis and Intervention

Earlier detection of PD through habenular imaging biomarkers could facilitate earlier interventions, potentially slowing disease progression and reducing long-term healthcare costs associated with advanced PD and dementia. The potential for habenular-based biomarkers to refine PD subtypes may lead to more personalized treatment approaches, improving patient outcomes and reducing unnecessary medication side effects. Early diagnosis and targeted treatments could reduce hospitalizations, complications, and the need for long-term care, resulting in significant cost savings for healthcare systems. Investments in advanced imaging technologies and therapeutic research, as suggested by this thesis, could also stimulate economic growth by fostering innovation in the medical and biotechnology sectors. Governments and private entities should consider funding these avenues to capitalize on the dual benefits of improved patient care and economic development.

IMPACT ON CAREGIVERS AND QUALITY OF LIFE

PD is a debilitating condition that significantly impacts patients' quality of life. Non-motor symptoms are often underdiagnosed and undertreated, exacerbating the disease burden. By identifying habenular dysfunction as a contributing factor, this thesis provides a foundation for targeted interventions that could enhance the quality of life for both patients and caregivers.

Neuromodulation strategies targeting the habenula may offer non-pharmacological treatment options for mood disorders in PD, reducing reliance on antidepressants and their associated side effects. Improved management of non-motor symptoms would not only enhance patient well-being but also reduce caregiver stress and healthcare costs. Moreover, addressing these symptoms could enable patients to maintain their independence and participate more fully in societal activities for longer periods.

The findings reinforce the need for interdisciplinary collaboration between neurologists, psychiatrists, and neuroscientists in treating PD, highlighting the importance of non-dopaminergic targets in disease management.

FUTURE DIRECTIONS AND TRANSLATIONAL POTENTIAL

The findings of this thesis open several avenues for future research, particularly in translational applications aimed at improving diagnostic accuracy, disease monitoring, and treatment strategies in PD.

The cross-sectional nature of these studies limits the ability to establish causality. Future longitudinal studies should assess whether habenular hypertrophy and increased connectivity are transient compensatory changes or whether they progress to atrophy and decreased connectivity over time. These studies aim to determine whether habenular alterations precede symptom onset, making it a potential early biomarker for PD.

The findings from this thesis have significant implications for developing targeted therapies. The habenula's involvement in both motor and non-motor symptoms makes it a promising candidate for therapeutic interventions. DBS could be explored for PD-related mood or motor impairments. Additionally, pharmacological modulation of habenular activity, targeting glutamatergic or GABAergic pathways, may offer novel treatment avenues, particularly in patients with depression or impulse control disorder.

Since no significant association was found between habenular volume and depression scores or global cognitive scores, future research should examine whether other symptom domains, such as apathy, impulsivity, visuospatial and executive dysfunction, are more closely related to habenular alterations. In particular, validated instruments targeting apathy, anxiety, anhedonia, impulse control, and compulsive behaviours, together with detailed assessment of executive functions such as planning, set-shifting, and cognitive flexibility, may help determine which aspects of the non-motor syndrome are most closely associated with habenular structure and connectivity. Assessing habenular function across different PD subtypes is also important to determine whether habenular alterations are associated with disease progression or specific symptom profiles. Furthermore, investigating habenular connectivity during reward-based learning, motor inhibition, or emotional processing tasks may provide additional insights into how habenular dysfunction contributes to specific PD symptoms.

Exploring the habenula's role beyond PD could provide broader insights into its involvement in other neuropsychiatric and neurodegenerative disorders. Comparative studies across conditions such as depression, anxiety, and Alzheimer's disease could reveal shared mechanisms and therapeutic targets.

Integrating structural, functional, and molecular imaging modalities could provide a more comprehensive understanding of the habenula's role in PD. Molecular imaging of neu-

rotransmitter activity, combined with connectivity analyses, could uncover novel insights into the biochemical underpinnings of PD symptoms.

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During the writing process of this thesis, I used Grammarly to support grammar and spelling correction. This tool was employed solely to improve language clarity and did not contribute to the scientific content, analysis, or interpretation presented in this thesis. I did not use any artificial intelligence (AI) tools. All intellectual contributions, analyses, and interpretations presented are my own. No AI-generated content was included without critical review and revision.

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CURRICULUM VITAE

Bedia Samanci graduated from medical school in 2009 and became a certified neurology specialist in 2016. She worked as a research fellow at the Athinoula A. Martinos Biomedical Imaging Center, Boston, in 2015-2016. Since 2019, she has been working at the Behavioral Neurology and Movement Disorders Unit, Department of Neurology, Istanbul Faculty of Medicine, Istanbul University. She started her PhD training at Maastricht University, School for Mental Health and Neuroscience in September 2021 under the supervision of Prof. Dr. Yasin Temel.

She has been a board member of the Turkish Alzheimer Association and the Turkish Neuropsychiatric Society since 2021, National Deep Brain Stimulation Society since 2024, and is also a member of the European Academy of Neurology, Movement Disorders Society, Deep Brain Stimulation Society, Turkish Parkinson's Disease Society and the Turkish Neurological Society. She is also an active panel member of Movement Disorders and Dementia Panels of European Academy of Neurology. She also has been elected to the EAN Dementia and Cognitive Disorders and Neuroimaging Management Groups for the 2026–2028 term.

Her research centers on neurodegenerative disorders, particularly Parkinson's disease, Alzheimer's disease, and other movement disorders and dementias. Additionally, she has a strong interest in neuroimaging and biomarkers in these fields.

She has authored and co-authored over 50 peer-reviewed articles in both international and national journals, as well as contributed to various books.

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