Diffusion Tensor Imaging Investigation of Uncinate Fasciculus Anatomy in Healthy Controls: Description of a Subgenual Stem

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Keywords

Diffusion tensor imaging · Uncinate fasciculus · Brodmann area 25 · Subgenual stem · Neuroanatomy

Abstract

The uncinate fasciculus is the largest white matter association tract connecting the prefrontal cortex and the antero-medial temporal lobe. The traditional anatomical description outlines a temporal stem that hooks around the posterior insula, a subinsular body, and 2 prefrontal stems extending to the lateral orbital gyri and the frontopolar cortex. Recent imaging studies of the white matter tracts deep to the subgenual cingulate gyrus (Brodmann area 25: BA25) suggest the presence of white matter fibers extending from BA25 to the amygdala, via a route that would run in close proximity to the uncinate fasciculus, that are of functional importance in mood disorders. We hypothesized that these fibers represent a third, medial prefrontal stem of the uncinate fasciculus. Using diffusion tensor imaging in 74 healthy volunteer humans, we seeded the uncinate fasciculus using 2 regions of interest centered over the temporal stem and the caudal body of the uncinate fasciculus in the coronal plane at the level of the anterior commissure. A medial pre-

frontal stem extending to the subgenual cingulate gyrus was demonstrated in 65/74 left and 70/74 right cerebral hemispheres, and had a mean fractional anisotropy value of 0.43 (95% CI 0.40–0.47). The medial subgenual stem fibers were inseparable from the caudal body and temporal stem of the main uncinate fasciculus and followed the same hook-shaped morphology. A probable medial subgenual prefrontal stem of the uncinate fasciculus was demonstrated in a cohort of healthy volunteers and is of potential significance in our understanding of neuropsychiatry and mood disorders.

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Introduction

Background

The uncinate fasciculus (UF) is the largest white matter association tract connecting the prefrontal cortex with the anteromedial temporal lobe [1]. Functionally, the UF is considered key in the prefrontal inhibition of the medial temporal lobe structures [2–5]. Numerous studies have demonstrated reduced connectivity in the UF across a range of neuropsychiatric conditions including mood disorders [6, 7], anxiety disorders [8], psychotic disorders

Fig. 1. a Anatomical structures: coronal T1-weighted anatomical image demonstrating the important anatomical structures surrounding the uncinate fasciculus including the medial temporal lobe, insula, and subgenual cingulate gyrus. **b** Regions of interest (ROIs): 2 customized ovoid coronal plane ROIs used to seed the

uncinate fasciculus overlaid onto an individual subject's coronal T1-weighted anatomical image. One temporal ROI (blue shading) was placed over the left temporal lobe white matter stem, and a second subcaudate ROI (red shading) was placed over the subcaudate white matter.

[9], antisocial personality disorder/psychopathy [10], and in the frontotemporal dementias [11]. In addition, the UF is one of the last association tracts to completely mature, with final myelination extending from the second and late into the third decade of life [4], corresponding to the age of onset of many of the implicated psychiatric conditions.

Anatomy of the UF

Structurally, the UF has a hook-shaped morphology that curves around the posterior insula within the temporal stem [1, 5, 12]. The temporal component connects with the uncus, anterior temporal pole, and basolateral amygdala [13], and extends posteriorly/caudally prior to the U-shaped hook. From here the tract runs medially to the insula and ventrally/inferiorly to the head of the caudate nucleus (a location that would have likely been targeted in the once performed subcaudate tractotomy) – see Figure 1a [1, 14]. Upon arrival in the prefrontal region, the traditional description has been a division into 2 prefrontal stems, extending to the lateral orbital gyri and the frontopolar cortex, respectively [15].

White Matter Tracts Deep to the Subgenual Cingulate Gyrus

Recent interest in the subgenual cingulate gyrus (Brodmann area 25: BA25) as a deep brain stimulation (DBS) target for depression has triggered a wealth of neuroimaging research based on the hypothesis that BA25 and the white matter underlying it may represent a key central node of corticolimbic communication that underlies the pathophysiology of depression [16–23]. This hypothesis has been supported by studies demonstrating increased cortical blood flow in BA25 in depressed patients (which was reduced with antidepressant treatment) [24, 25], reduced connectivity in the cingulum bundle connecting to BA25 in depressed patients [20, 26], and a moderate response rate to DBS for previously treatment-resistant patients [16–18]. Johansen-Berg et al. [21] demonstrated that the white matter connections that were likely activated in DBS responders differed in location and distribution to those in nonresponders, with the responder group activating (amongst others) white matter fibers extending from BA25 towards the amygdala and anterior temporal pole. The presence of these fibers was also confirmed by our prior published work on target selection for

DBS using diffusion tensor imaging (DTI) [27]. By anatomical necessity, these fibers would run in close proximity to the subinsular/subcaudate body of the UF and would also curve in a hook-shaped manner around the posterior insula to reach the mesial temporal structures.

Hypothesis

Based on the anatomical proximity of these fibers to the UF as well as the demonstrated reduced connectivity of the UF in depressed patients [6, 7, 27], we hypothesize that these fibers are in fact a medial/subgenual prefrontal stem of the UF, and that there are 3 rather than 2 prefrontal stems of the UF. We have tested this hypothesis using DTI, a noninvasive indirect method of imaging white matter fibers with magnetic resonance imaging (MRI) founded on the tendency of water molecules to diffuse parallel rather than perpendicularly to the long axis of white matter tracts.

Materials and Methods

Ethics

Ethics approval was received for this research from the Human Research Ethics Committees of St. Vincent's Hospital, Sydney, and the University of Sydney, and was performed in accordance with the principles of the Declaration of Helsinki. All imaging acquisition was undertaken with the understanding and written consent of the volunteer subjects.

Recruitment

Seventy-four nondepressed adult volunteers (ages 18–78) were recruited by the Department of Anatomy, University of Sydney, to form a bank of control subjects for a variety of future MRI studies. Volunteers were screened for symptoms of depression using the Beck Depression Inventory 2nd edition (BDI-II), a 21-question self-reporting inventory first described in 1961 and updated in 1996 that has been shown to be a valid and reliable tool for the assessment of depression severity [28, 29]. Using this scoring system, a score of >13 is consistent with depression; all of our recruited volunteers had a BDI-II score of ≤13. In addition, volunteers were excluded from the study if they self-reported with a screening questionnaire any history of a major psychiatric disorder, neurodegenerative disorder, or major prior neurological injury.

MRI Acquisition

All volunteers underwent MRI of the brain using a 3-tesla MRI scanner (Philips, Acheiva). Volunteers lay supine within the scanner with their head immobilized in a head coil. High-resolution 3-dimensional T1-weighted anatomical image sets were acquired (TE = 2.5 ms, TR = 5,600 ms, flip angle = 8°, voxel size = 0.8 × 0.8 × 0.8 mm). T2-weighted image sets were then acquired (TE = 100 ms, TR = 3,000 ms, voxel size = 2 × 2 × 2.5 mm). DTI data were acquired with a whole-brain single-shot echo-planar pulse sequence (TR = 8,788 ms, flip angle = 90°, 112 × 112 matrix size, 224 × 224 mm FOV, 2.5 mm slice thickness, 55 axial slices) with 4 im-

age sets collected for each subject. For each slice, diffusion gradients were applied along 32 independent orientations with $b = 1,000$ s/mm² after the acquisition of the $b = 0$ s/mm² images.

Image Processing

Images were skull-stripped and coregistered using FSL Version 4.1 Diffusion Toolbox (Functional Magnetic Resonance Imaging of the Brain Centre, John Radcliffe Hospital, University of Oxford, Oxford, UK, 2008), and tractography was performed using Diffusion Toolkit Version 0.6 (Wang R, Wedeen VJ, Massachusetts General Hospital, 2010). Image analysis was undertaken using TrackVis Version 0.6.0.1 (Wang R, Wedeen VJ, Massachusetts General Hospital, 2015).

Region of Interest Selection

Using fused T1-weighted and DTI tractography sequences, 2 customized ovoid regions of interest (ROIs) were created in the coronal plane (on a single coronal slice) for each cerebral hemisphere at the level of the anterior commissure as seen on a midline sagittal slice (Fig. 1b). The temporal ROI (ovoid 16 mm x-axis, 12.5 mm z-axis) was placed over the white matter temporal stem as it extended caudally (posteriorly) towards the caudal margin of the insula, whilst the subcaudate ROI (ovoid 24 mm x-axis, 10 mm z-axis) was placed over the white matter ventrally (inferiorly) to the caudate head with wide medial and lateral extensions to the medial cortex and the claustrum, respectively. These large ROIs were created to ensure complete capture of all fibers related to the UF, which is the only major association tract that would cross both of these locations, with an “and” algorithm used to include only fibers that crossed both ROIs. The placement of a subcaudate ROI in such a caudal/posterior location differs with many prior studies (which selected subcaudate fibers more rostrally) because we wished to ensure inclusion of any potential medial fibers to the subgenual cingulate gyrus that would likely exit the main body of the tract more caudally than the frontopolar or lateral orbital gyrus fibers. A length filter requiring a minimum tract length of 20 mm was applied, to aid exclusion of artificial tracts from the analysis.

ROI Seeding and Tract Analysis

These ROIs were applied in a standardized fashion to the imaging of all volunteers in both hemispheres, and the corresponding white matter tracts were subsequently seeded and analyzed in 3 dimensions. Each hemisphere was assessed to ensure that the UF was clearly demonstrated, that the inferior occipitofrontal fasciculus had been reliably excluded, and that there was no significant susceptibility artifact in these regions that would obscure analysis. From here the UFs were assessed for the presence or absence of a temporal stem, subinsular body, medial subgenual stem, frontopolar stem, and lateral orbital gyrus stem. After initial analysis of the first 10 sets of imaging, a variant of the medial subgenual stem was demonstrated and then assessed for in all image sets, which extended ventrally from the main UF body towards the medial orbital gyri, caudal to the other frontal stems. For the purposes of data collection and analysis, the various stems were labeled temporal, subgenual, medial orbital (variant), frontopolar, and lateral orbital, respectively.

Subgenual and Medial Orbital Stem Definition

The definition of the subgenual stem for this study was a set of fibers running medially from (and intimately associated with) the body of the UF and extending to the subgenual cingulate cortex

(caudal and ventral to the genu of the corpus callosum rostral margin, rostral to the lamina terminalis). In order to qualify as a medial subgenual stem, the fibers involved had to be selected with a third ovoid ROI placed in the sagittal plane 5 mm lateral to the subgenual cingulate cortex (and centered on the proposed fiber stem) using an “and” algorithm, with clear demonstration of extension along the body and hook of the UF, along the temporal stem, and running adjacent to the basolateral amygdala. For a variant medial orbital stem there were similar criteria applied, except that the third ROI was placed in the axial plane 5 mm dorsal to the cortex of the medial orbital gyri.

Topographic Assessment

In addition to assessing for the presence or absence of the various stems, we also analyzed the topographical location of those fibers associated with each stem within the body and temporal components of the UF by undertaking selective fiber tractography using a third ROI with an “and” algorithm over the distal prefrontal aspect of each stem, respectively (in order to visually exclude fibers from the other stems).

Fractional Anisotropy Assessment

The mean fractional anisotropy values for each analyzed tract were measured using TrackViz Version 0.6.0.1 (Wang R, Wedeen VJ, Massachusetts General Hospital, 2015), in order to serve as a bank of control data for any future studies of these tracts in pathological processes. To explore the effects of age we also assessed the fractional anisotropy of each tract in those between 18 and 33 years old ($n = 37$) and those between 35 and 78 years old ($n = 37$). We determined whether there were any significant differences between these age groups using 2-sample t tests ($p < 0.05$).

Results

Demographics and Image Quality

Detailed demographic details of the study volunteers are described in Table 1. Seventy-four adult volunteers participated in this study (38 male, 36 female) with an age range of 18–78 years (mean 39.2 years; median 34 years). A comparison of mean fractional anisotropy values of the UF stems between the younger half of the age range (18–33 years) and the older half (35–78 years) showed no significant difference using a Student t test with a preset α -value of 0.05. The healthy volunteers included students and staff of the University, as well as members of the general public predominantly living within inner Sydney. The BDI-II scores range from 0 to 13 with a mean of 4.1 (i.e., no volunteers had a score of >13 to indicate the presence of depression). All volunteers included in this study had satisfactory image quality with no significant susceptibility artifacts obscuring the temporal and frontal lobes (volunteers with such artifacts did not undergo completion of their DTI sequences and were not included in the imaging control bank).

Table 1. Demographic details of the healthy volunteer cohort

Variable		Descriptors
Age, years	Range	18–78
	Interquartile range	24–57
	Mean	39.2
	Median	34
	Mode	21
	Standard deviation	16.9
Gender	Male	38
	Female	36
BDI-II scores	Range	0–13
	Interquartile range	2–9
	Mean	4.1
	Median	4
	Mode	3
	Standard deviation	2.3
BDI-II, Beck Depression Inventory 2nd edition.		

Temporal Stem and Body

The results of this study are summarized in Table 2. The temporal stems and subinsular bodies of the UFs were present in both cerebral hemispheres in all 74 volunteers (Fig. 2). Fibers of the temporal stem were consistently seen extending towards the basolateral surface of the amygdala as well as more laterally and rostrally towards the temporal pole.

Lateral Orbital Gyrus Stem

The lateral orbital gyrus stem was identified in 63 of 74 left cerebral hemispheres and 60 of 74 right cerebral hemispheres. There was nonvisualization/absence of the lateral stem bilaterally in 2 volunteers.

The topographic location of the lateral stem fibers was found consistently in the lateral and ventral aspect of the temporal stem (extending towards the temporal pole), lateral and caudal aspect of the hook, and lateral and dorsal aspect of the body of the UF. There was a consistent ventral and lateral angulation of the more distal aspect of the lateral stem towards the lateral orbital gyrus cortex.

Frontopolar Stem

The frontopolar stem was identified in all 73 of 74 left cerebral hemispheres and in all 74 right cerebral hemispheres. The volunteer with an absent left frontopolar stem had both a medial subgenual stem and a medial orbital gyrus stem, as well as a visualized lateral orbital gyrus stem.

Fig. 2. Uncinate fasciculus: temporal stem, hook, and body. The uncinatus fasciculus overlaid onto an individual subject's sagittal T1-weighted anatomical image. Fibers of the temporal stem were consistently seen extending towards the basolateral surface of the amygdala as well as more laterally and rostrally towards the temporal pole. Furthermore, note the prefrontal stems extending rostrally from the body. The locations of the temporal and subcaudate ROIs are indicated by the blue and red shadings, respectively.

Fig. 3. Axial view of the prefrontal stems of the uncinatus fasciculus. The uncinatus fasciculus overlaid onto an individual subject's axial T1-weighted anatomical image. Note the presence of 3 clearly separate prefrontal stems extending to the lateral orbital gyri, the frontopolar cortex, and subgenual cingulate cortex.

Table 2. DTI analysis and fractional anisotropy (FA) values of the uncinatus fasciculus and its stems in 74 healthy volunteers

	Temporal stem	Body	Lateral orbital stem	Frontopolar stem	Subgenual stem	Medial orbital stem
Left cerebrum	74/74	74/74	63/74	73/74	65/74	7/74
Mean FA	0.44	0.46	0.43	0.44	0.43	0.41
95% CI	0.41–0.49	0.43–0.49	0.40–0.48	0.40–0.49	0.40–0.47	
Age 18–33 years	0.43	0.46	0.44	0.44	0.44	
Age 35–78 years	0.44	0.45	0.42	0.44	0.43	
<i>p</i> value	0.105	0.267	0.093	0.673	0.231	
Right cerebrum	74/74	74/74	60/74	74/74	70/74	6/74
Mean FA	0.45	0.46	0.43	0.43	0.42	0.41
95% CI	0.41–0.45	0.42–0.49	0.40–0.49	0.40–0.48	0.39–0.48	
Age 18–33 years	0.46	0.46	0.44	0.44	0.43	
Age 35–78 years	0.44	0.45	0.43	0.43	0.41	
<i>p</i> value	0.087	0.255	0.159	0.316	0.077	

The topographic location of the frontopolar stem fibers was consistently more medial and dorsal than the lateral stem fibers with the temporal stem (extending more dorsomedially with the temporal pole), more rostral within the hook, and more medial within the rostral body of the UF. In this way, the frontopolar fibers were

consistently flanked externally by the lateral stem fibers up to the level of bifurcation between these 2 stems.

Subgenual Cingulate Gyrus Stem

A subgenual cingulate gyrus stem, which satisfied the requirements set out in the methodology above, was dem-

Fig. 4. Coronal view of the prefrontal stems of the uncinate fasciculus. The uncinate fasciculus overlaid onto an individual subject's coronal T1-weighted anatomical image. Note the medial and subcaudate course of the subgenual stem towards the subgenual cingulate cortex, as well as its caudal location in relation to the frontopolar and lateral orbital stems.

Fig. 5. Coronal view of the subgenual prefrontal stem fibers of the uncinate fasciculus. The subgenual prefrontal stem of the uncinate fasciculus overlaid onto an individual subject's coronal T1-weighted anatomical image – the T1-weighted image has been partially subtracted to highlight the 3-dimensional course of the pathway. Note the course of the fibers from the subgenual cingulate cortex in a subcaudate fashion towards the hook of the uncinate fasciculus, and the final destination adjacent to the basolateral amygdala.

onstrated in 65 of 74 left cerebral hemispheres and 70 of 74 right cerebral hemispheres. In all demonstrated cases, the stem extended laterally from the subgenual cingulate cortex to merge with the caudal body of the UF, and extended via the temporal stem to the region just lateral to the basolateral amygdala (Fig. 3, 4).

The topographic location of the subgenual cingulate gyrus stems was always the most medial within the temporal stem, extending towards the basolateral surface of the amygdala rather than the temporal pole (Fig. 5). These fibers remained the most medial and rostral with the hook, and the most medial within the caudal body. These were the first fibers to separate from the body of the UF, usually separating approximately 6–10 mm rostral to the coronal plane of the anterior commissure (caudal surface). From here they angled medially and with mild rostral extension towards the subgenual component of the cingulate gyrus, running along the ventral (inferior) surface of the putamen and caudate head.

Medial Orbital Gyrus Stem

A medial orbital gyrus stem, which satisfied the requirements set out in the methodology above, was demonstrated in 7 of 74 left cerebral hemispheres and 6 of 74 right cerebral hemispheres. Of the 7 positive left cerebral hemispheres, 4 hemispheres did not demonstrate a subgenual cingulate gyrus stem. Of the 6 positive right cerebral hemispheres, 2 did not demonstrate a subgenual cingulate gyrus stem.

The topographic location of the medial orbital gyrus stem was again in the medial aspect of the temporal stem, medial and rostral aspect of the hook, and also demonstrated early separation from the body of the UF. After separation from the body, these fibers curved sharply ventrally towards the caudal aspect of the medial orbital gyrus cortex. In those cases where both a medial orbital gyrus stem and a subgenual cingulate gyrus stem were present, the fibers of the 2 stems were very difficult to separate/differentiate within the temporal stem, but the medial orbital gyrus stem was more rostral within the hook, and more lateral and ventral within the caudal UF body.

Discussion

Background

Prior studies on the white matter tracts underlying the subgenual cingulate gyrus (BA25) have demonstrated the presence of fibers extending towards the amygdala [21, 22, 27, 30] and that these fibers are amongst those likely activated in responders rather than nonresponders to DBS of BA25 [19, 21, 22]. In fact, Riva-Posse et al. [19] demonstrated that the volume of activated tissue in BA25 DBS responders consistently involved fibers of the UF using seed tractography of an activated tissue model ROI [30].

Consistent with our tractography results, fibers extending between BA25 and the amygdala have also been demonstrated in postmortem human, nonhuman primate, and rodent anatomical studies. Ebeling and von Cramon [31], in a microdissection study of 10 adult non-diseased postmortem human brains, described the frontal fibers of the UF spreading in a fan-like fashion towards 3 regions of the prefrontal cortex: the gyrus rectus (area 11), medial retro-orbital cortex (area 12), and the subcallosal gyrus (area 25). Our DTI results are consistent with this finding, demonstrating these latter fibers as a distinct medial/subgenual prefrontal stem of the UF.

Tract tracing studies in macaque monkeys [32] and in rodents [33] also demonstrate the presence of fibers extending between the infralimbic cortex (homologous to BA25 in humans) and the amygdaloid nuclei, with topography comparable to the course of the fibers we demonstrate. These direct connections have also been reported in human DTI studies that seeded BA25 and demonstrated connections to the amygdaloid nuclei via a subcaudate and subinsular course (as described above) [19, 21, 34, 35]. Houenou et al. [34] in fact stated that they believed these fibers were probably originating from the UF based on correlation with white matter atlases.

A major unique feature of our study was to demonstrate these same fibers using the UF as a seed rather than seeding BA25, demonstrating that they are in fact a component of the UF. By using a larger and more caudally located subcaudate ROI than those used in many prior DTI studies of the UF, we aimed to demonstrate medial/subgenual fibers that branch off more caudally than the other well-described prefrontal stems.

Salient Findings

In this study we have demonstrated the probable presence of a medial prefrontal stem of the UF extending towards the cortex of the subgenual cingulate gyrus (BA25) in humans using DTI. Our findings indicate that this

probable tract is present in at minimum a high proportion of human brains (65/74 left hemispheres, 70/74 right hemispheres) and is intimately associated with (and to our assessment inseparable from) the caudal body and the temporal stem of the UF. The anatomical location of this subgenual cingulate gyrus stem likely corresponds to the fibers identified by Johansen-Berg et al. [19], Riva-Posse et al. [21], and Vergani et al. [30] that extend towards the amygdala and are amongst those activated in DBS responders.

Hypothesis of Functional Significance

Given the location of these probable fibers between BA25, a cortical region heavily implicated in mood regulation and the pathophysiology of depression, and the basolateral amygdala, where the amygdala receives the majority of its input fibers, it would be reasonable to hypothesize that the subgenual stem of the UF plays a role in the circuitry underlying the regulation of mood and prefrontal control over the emotional expression of mood (our affect). This hypothesis could be potentially tested using functional BOLD-MRI or PET imaging in combination with tractography. In addition, lesioning studies in primates may also prove useful to further test this hypothesis.

Limitations

The major limitation of this study is the indirect nature of white matter tract anatomical assessment inherent in diffusion tensor tractography (which is based on a probabilistic determination of tract location resulting from the calculated diffusion vectors of protons). This limitation can manifest in the appearance of nonexistent/artificial tracts that reflect the calculation of vectors from skull base, CSF pulsation, or patient motion artifacts rather than true white matter tracts. We have attempted to reduce the impact of such tracts on this study through the application of a length filter (requiring a minimum tract length of 20 mm), as the majority of such artificial tracts is short (usually <15 mm). In addition, the reproducibility of the tracts analyzed in this study between volunteers (in a sample size of 74 volunteers, 148 hemispheres) would markedly reduce the likelihood that these represented artificial tracts. Future tracer studies on nonhuman primates could be used to interrogate these tracts further in a more direct fashion.

Another limitation of DTI is the associated difficulties of separating crossing fibers in multiple directions, and as such tracer studies or use of diffusion spectrum imaging may be of use.

The sample size of this study ($n = 74$, 148 hemispheres) renders it an initial assessment only of the presence or absence of the tracts of interest, and it would be useful to confirm our findings in a larger cohort of healthy volunteers.

Relevance

The results of this study are of relevance not only to furthering our potential understanding of mood regulation circuitry and neurosurgical targeting, but also in improving and refining our knowledge of the functional neuroanatomy of the prefrontal lobe and its interactions with the limbic system. A detailed analysis of the white matter anatomy of the frontal lobe will be essential for future progress in understanding the pathophysiology and treatment of psychiatric diseases. In this study we have demonstrated the probable presence of a medial/subgenual prefrontal stem of the UF that appears to communicate between a major mood regulation hub (the subgenual cingulate gyrus: BA25) and the input region of the amygdala (involved in emotional expression of mood).

Further validation of our findings is required in 2 formats. Firstly, since the acquisition of imaging for our study there have been advances in spatial resolution/voxel size for DTI sequences, and such sequences (potentially in combination with 64-directional acquisition) should

be used in follow-up studies to ensure that the medial subgenual stem fibers are truly inseparable from the remainder of the UF. Secondly, given the indirect nature of DTI and the potential for artificial tract visualization, confirmation of the presence of these tracts with further anatomical tracer studies would be useful.

Conclusion

A previously undescribed medial prefrontal stem of the UF extending between the subgenual cingulate gyrus (BA25) and the basolateral amygdala was demonstrated using DTI. The fibers of this stem were inseparable from the body and temporal stem of the UF. We hypothesize that these fibers would play an important role in mood regulation and depression pathophysiology, and could be further researched using functional neuroimaging and anatomical tracer studies.

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