Collothalamic projections to the human amygdala: hemispheric asymmetry modulates trait anxiety

Authors: Robert D. Rafal[a,c]*, Kristin Koller [b,c,]

Affiliations:

[a]Department of Psychological and Brain Sciences, University of Delaware, USA.

[b]Cardiff University Brain Imaging Research Centre, Wales, UK.

[c]School of Psychology, Bangor University, Wales, UK.

*Correspondence to: Robert Rafal
Department of Psychological and Brain Sciences
University of Delaware
Wolf Hall, the Green, University of Delaware, Newark, DE 19716
610 241-2582
rrafal@psych.udel.edu

Key Word: anxiety, medial geniculate nucleus, superior colliculus, stria terminalis, suprageniculate nucleus, threat, ventral amygdalofugal

Running Title: Collothalamic projections to the human amygdala

Abstract:

In 19 people probabilistic DTI tractography was used to visualize the topographic relationships between three white matter components of a fascicle, the supraventricular temporal bundle, that traverses above the temporal horn of the lateral ventricle: collothalamic auditory and visual projections to the amygdala via the posterior thalamus, and the amygdalofugal stria terminalis. This bundle constitutes a subcortical, 'low road' pathway that transmits threat signals to the amygdala, and that projects signals that bias orienting toward visual threat to the bed nucleus of the stria terminalis. The course of the visual streamline passes below the brachium of the superior colliculus through the position two thalamic nuclei that have been shown to both receive afferents from the superficial layers of the superior colliculus and to also project to the amygdala: the suprageniculate nucleus and the inferior pulvinar. The visual streamline passes laterally dorsal to the auditory streamline and both collothalamic streamlines then traverse together above the temporal horn of the lateral ventricle, dorsal to the stria terminalis, with the auditory streamline dorsal to visual streamline, and entering the lateral amygdala dorsal and medial to it. Individual differences in the degree of hemispheric asymmetry of the fractional anisotropy of the visual streamline, but not the auditory streamline, predicted trait anxiety: weaker left hemisphere connectivity relative to those in the right hemisphere, was associated with higher trait anxiety. There was no correlation between individual differences in the microstructure of either the stria terminalis or the ventral amygdalofugal pathway and trait anxiety.

Word Count: 248

New & Noteworthy

- Three components of a white matter bundle, auditory and visual collothalamic projections to the amygdala and the stria terminalis, traverse above the temporal horn of the lateral ventricle.

- This bundle constitutes a ‘low road’ pathway that transmits threat signals to the amygdala, via the posterior thalamus, and that biases spatial orienting toward visual threat.
- Hemispheric asymmetry of the microstructure of the visual pathway predicts individual differences in trait anxiety.

Introduction

Motivated by the need to “escape from the shackles of subjectivity”, LeDoux (4) (p.156) advanced the phenomenon of fear conditioning as the key to a renaissance of interest of emotion within neuroscience. He and his colleagues demonstrated collothalamic projections, in rats, from auditory thalamus (medial geniculate nucleus, posterior intralaminar nucleus and suprageniculate nucleus) to the lateral amygdala (5) that were shown to be critical for conditioning fear responses to auditory threat (6).

More recently it has been hypothesized that there is also a subcortical, visual pathway that transmits threat signals from the superior colliculus to the amygdala (7). This hypothesis was advanced to account for observations that some cortically blind patients can process the emotional valence of visual stimuli (8,9) (10) (11) (12), and that fearful faces presented in the hemianopic field can facilitate visual discrimination in the seeing visual field (13).

Specifically, this hypothesis posits a subcortical (“low road”) pathway that transmits visual threat signals – outside of explicit awareness, and with shorter latencies than cortical relays – from the retina to the superficial layers of the superior colliculus; and that these signals are transmitted to the amygdala via a monosynaptic connection in the pulvinar (7). Pessoa and Adolphs (14) raised several challenges to this hypothesis: 1) Is the processing mediated by this putative pathway unconscious? 2) Is it a faster route than circuits transmitting affective signals via the cerebral cortex to the amygdala? 3) Is there anatomical evidence that such a pathway exists in the primate brain? With regard to the last query, they noted that tracer studies had not, at that time, shown that any pulvinar nucleus that receives retino-recipient signals from the superficial layers of the superior colliculus also project to the amygdala.

Since Pessoa and Adolphs' (14) review of this hypothesis, evidence has accumulated, from neuroimaging studies, supporting the veridicality of a subcortical threat pathway in humans; and more recent anatomical advances from diffusion tractography, and from animal tracer studies, have supported the anatomical plausibility of such a pathway.

Using deterministic diffusion imaging tractography, Tamietto *et al.* (15) virtually dissected a streamline, passing through the pulvinar, that connected the superior colliculus to the amygdala. Moreover, they showed that this pathway had stronger connections (indexed as higher fractional anisotropy (FA)) of the streamline in the damaged hemisphere of a hemianopic patients with blindsight who had suffered from destruction of primary visual cortex in childhood. This neuroplastic compensation presumably contributes to a heightening of surveillance attention to the hemianopic field.

Using probabalistic tractography in humans and macaque monkeys, Rafal *et al.* (1) demonstrated the same streamline, and showed that it traversed above the temporal horn of the lateral ventricle enroute to the amygdala positioned dorsal to the stria terminalis. Moreover, they showed that this pathway had been destroyed in a patient, with unilateral pulvinar damage, who was unable to recognize angry facial expressions in pictures shown to him in his contralesional visual field (16). In three other patients with pulvinar damage, in whom the pathway was spared, emotional faces were recognized in both visual fields.

Using a dot-probe task in another patient who had damage to the ventro-medial pulvinar, Ward, Danziger and Bamford, (17) showed that the patient was delayed in orienting to visually threatening stimuli in the contralesional field; and Rafal *et al*. (1) confirmed that the pathway connecting the superior colliculus to the amygdala had been destroyed in that patient, but not in three other patients in whom the recognition of threatening faces was intact in the contralesional visual field. This observation, that the orienting effect toward threat was delayed in the contralesional field of that patient compared to the ipsilesional field or to controls, supported the claim that the subcortical pathway does mediate faster orienting toward threat than does the cortical route.

Koller *et al*. (2) provided further evidence for the anatomical veracity of this pathway and its role in orienting toward visual threat. Unlike conventional approaches to studying fear conditioning in rodents, which assay fear by measuring autonomically mediated freezing or startle brain stem responses, Koller *et al*. (2) employed a temporal order judgment, saccade decision task that measured spatially selective orienting responses. Participants were tested monocularly. They were presented two pictures, one in each visual field, on each trial that could onset either simultaneously, or with equal probability, one picture (left or right field picture) could onset 57ms before the other. Participants were instructed to make an eye movement toward the picture that appeared first. Although not task relevant, one picture was a threatening image (e.g. a charging, wide-mouthed predator) and the other was a pleasant image (e.g. a bunny). Results showed that participants had a small but reliable bias to saccade toward the threatening picture, regardless of their temporal onset asymmetry; but this orienting bias toward threat was only observed on trials in which the threatening image was presented in the temporal hemifield. The temporal hemifield advantage for the threat orienting bias is consistent with the hypothesis that the visual afferents mediating the bias are transmitted via the retinotectal tract to the superior colliculus. Unlike the geniculostriate pathway that transmits visual signals from each eye equally to each visual cortex, the retinotectal tract to the superior colliculus receives dominantly crossed projections from the temporal hemifield (nasal hemiretina) of each eye (18) (see van Koningsbruggen, Koller and Rafal (19) for a review). The temporal hemifield advantage for the bias to orient toward threat is suggestive of mediation via the retinotectal tract to the superior colliculus. Finally, Koller *et al.* (1,2) showed that the white matter microstructure, as indexed by fractional anisotropy (FA) of the streamline, predicted individual differences in orienting bias toward threat.

Notably, though, this effect was only evident for the streamline in the right hemisphere.

The current research had two major goals:

1) To demonstrate the anatomical relationship, in the human brain, between the visual and auditory collothalamic pathways to the amygdala, and the topographic trajectories of these two collothalamic pathways in relation to the stria terminalis as these three pathways traverse above the temporal horn.

Probabalistic tractography has previously been used to virtually dissect the auditory streamline in mice (20) and humans (21), and to show that it plays a role in the pathophysiology of tinnitus (21); but neither communication demonstrated the topographic anatomy of the streamline in relation to the temporal horn of the lateral ventricle, or to neighboring white matter pathways. Here we have virtually dissected the auditory streamline and confirmed that the visual and auditory pathways form adjacent parallel fiber tracts enroute to the amygdala as they traverse above the temporal horn. We demonstrate that these two collothalamic pathways to the amygdala, and the stria terminalis amygdalo-fugal efferents to the bed nucleus of the stria terminalis, together form a supraventricular white matter bundle traversing above the temporal horn of the lateral ventricle.

2) To determine which posterior thalamic nuclei that the streamline depicting the putative visual pathway traverses which has been shown to both: 1) receive projections from the retinorecipient superficial layers of the superior colliculus and; 2) to project to the lateral amygdala.

Since Pessoa and Adolphs (14) raised this second question, and since streamlines visualizing a putative visual pathway from the colliculus to the amygdala were first identified a decade ago, evidence for such connectivity between the retino-recipient superficial layers of the superior colliculus and the amygdala, via the dorsal pulvinar, has been demonstrated in tree shrews (22). Moreover, advances have been made in the anatomical parcellation of the pulvinar in primates and other mammals (23)(24), and the connectivity of pulvinar nuclei with the superficial and deep layers of the superior colliculus and with the amygdala. In experimental animals, posterior thalamic nuclei that receive projections from the retino-recipient layers of the superior colliculus can be by labeling with vesicular glutamate transporter 2 (24); and two other tracer studies in macaque monkeys have specifically sought to address this question. These two tracer studies have identified posterior thalamic nuclei that both receive input from the superficial layers of the superior colliculus, and that also send projections to the lateral amygdala (25,26).

The current research exploits the unique potential of diffusion tractography to not only demonstrate anatomical connectivity between brain structures, but to also visualize the topography of these connections, and their anatomical relation to neighboring pathways, in humans and other non-human primates. Conventional tracer studies in primates provide a gold standard for confirming anatomical connectivity between brain structures. However, it is not practical (or ethical) to employ tracer studies in primates to trace the entire course of the white matter fascicles connecting brain regions, or their anatomical relationship to other white matter tracts. To do so would require the sacrifice of many animals over many days to track the course of the tracer down the axon. By contrast, while diffusion tractography cannot identify synaptic connections in a pathway, cannot provide information about the direction of information transmission in axons, and may reveal spurious connectivity, it does afford the potential to visualize the full anatomic topography of white matter connections, and their anatomical relationships to one another.

Our goal was to map the anatomical course of the visual streamline, as it traverses through the posterior thalamus, onto contemporary maps of the pulvinar (24) and onto findings from the more recent tracer studies in non-human primates (25,26) (26). This was achieved by virtually dissecting the brachium of the superior colliculus as a landmark

In addition to these two major goals, we examined whether individual differences in the white matter microstructure (indexed by fractional anisotropy -FA) of each of the collothalamic pathways, or of the two amygdalofugal projections to the bed nucleus of the stria terminalis (the stria terminalis the ventral amygdalofugal pathway) predicted individual differences in trait anxiety.and

Materials and Methods

Participants

The virtual dissections reported here were performed on the same imaging data set from 19 individuals who had previously participated in studies examining the role of subcortical projections from the superior colliculus to the amygdala (1,2) and of the stria terminalis (3) in mediating orienting toward threatening visual stimuli. Those participants (9 male, age range 18–47) were recruited from the student population at Bangor University. Participants had no known neurological, psychological, psychiatric or cognitive impairments. Participants had completed the State-Trait Anxiety Inventory to exclude individuals with clinical anxiety disorder. The study was advertised on student online forums and via word of mouth. Participants were screened against exclusion criteria related to MR safety. Payment of £ 10/h was provided for participation in MRI scanning and £6/h for the saccade decision task with eye tracking. Participants had normal or corrected to normal vision. Prior to commencement of this study, this research was approved by the Bangor University Ethics Committee, and the research was conducted in compliance with its regulations. All participants were competent to give informed consent. Oral and written consent was obtained prior to participation from all participants. Participants were informed that their data, and their personal demographic information, would be kept anonymous.

Previous reports using this data set for tractography visualized a collothalamic streamline connecting the superior colliculus with the amygdala (1,2) and the stria terminalis (3) and provided evidence that white matter microstructure both the collothalamic visual pathway and the stria terminalis predicted a bias to orient toward visual threats presented in the temporal hemifield.

In the current communication PROBABILISTIC tractography was used to virtually dissect three other white matter tracts to further test the validity of the low road hypothesis of threat detection:

1) **A collothalamic auditory pathway projecting from the inferior colliculus to the amygdala via the medial geniculate nucleus.** This collothalamic pathway has been demonstrated in rodents by LeDoux and colleagues (4), and has been shown to be critical in fear conditioning (5). While such a pathway presumably also exists in primates, we do not provide direct evidence here that the auditory pathway we visualize in the current research transmits threat signals. However, if the auditory streamline can be shown to be part of the same supraventricular temporal white matter bundle as the visual streamline, the anatomical proximity of the auditory and visual pathways would be consistent with the hypothesis that the two pathways evolved as part of a circuit with a common function.

2) The brachium of the superior colliculus was virtually dissected as a landmark to identify the posterior thalamic nuclei that the visual pathway passes through.

3) **The ventral amygdalofugal pathway.** Having previously demonstrated that the other major amygdalofugal projection to the bed nucleus of the stria terminalis mediates orienting toward threat (3), we virtually dissected a streamline depicting the ventral amygdalofugal pathway here to examine whether or not the ventral amygdalofugal pathway also might transmit threat signals from the amygdala to the bed nucleus.

We also submitted both visual and auditory collothalamic pathways, and both amygdalofugal pathways, to statistical analyses to test whether their microstructure predicted individual difference in trait anxiety in our sample. We also examined whether hemispheric asymmetries in the microstructure of each of the four pathways correlated with trait anxiety.

Neuroimaging Methods

Data acquisition

A Phillips 3 Tesla Achieva magnetic resonance (MR) scanner at the Bangor Imaging Unit at Bangor University was used to acquire T1-weighted anatomical and diffusion weighted images.

High resolution multi-echo T1 weighted images (0.7x0.7x0.7 mm isotropic voxel resolution) were acquired using an MPRAGE (magnetization prepared gradient echo) sequence.

DWI-EP (diffusion weighted imaging – echo planar) images were collected at 2x2x2mm with the following parameters: b-values = 0 (averaged four volumes) and 2000, b-directions = 61, slices = 76, section thickness = 2mm, TR = 2 s, TE = 35ms.

Subsequent data pre-processing was carried out using the FSL-FDT toolbox (28). After correction of diffusion images for head movement and eddy current correction, diffusion tensor models were fitted at each voxel using the DTI-FIT tool in FSL. Diffusion parameters were calculated using the Markov Chain Monte Carlo sampling method. The DTI data was then prepared for probabilistic tractography via the BEDPOSTX tool in FSL's FDT toolbox. Probabilistic tracking was carried out using the PROBTRACKX tool in FSL's FDT toolbox. Prior to running the BEDPOSTX process, the non-diffusion brain image was extracted from the skull (using the FSL brain extraction tool (BET)), and a brain mask was formed. Anatomical T1- weighted scans were brain extracted using the BET-tool, and were registered with the B0 diffusion brain image, using FSL's FLIRT utility.

Probabilistic tractography

Probabilistic tractography was implemented using ProbtrackX from the FSL FDT toolbox (28) (curvature threshold = 0.2, number of samples = 5,000). To generate streamlines between the medial geniculate nucleus and the amygdala, a seed mask in each medial geniculate nucleus was manually drawn in native diffusion space of each individual and a target (waypoint and terminal) mask on the ipsilateral amygdala (Figure 1B). Connections between the inferior colliculus and the medial geniculate nucleus were also visualized by generating a streamline using a seed mask manually drawn on inferior colliculus in native space in each hemisphere of each participant and a target (waypoint and termination) mask on the medial geniculate nucleus. As previously described, masks on the superior colliculus and the amygdala had been used to demonstrate connections between the SC and the amygdala (2). The stria terminalis streamline had been generated using a seed mask on the amygdala, a target and termination mask on the bed nucleus of the stria terminalis, and a waypoint mask on the stria terminalis (3).

In the current research the ventral amygdalofugal streamline was generated using the same amygdala and bed nucleus seed and target masks that were used for virtual dissection of the stria terminalis as described by Koller *et al*. (3), but with a different exclusion mask (Figure 1B). For tractography of the stria terminalis a sagittal exclusion mask had been drawn in each hemisphere to exclude the ventral amygdalofugal tract and the fornix; whereas, for the ventral amygdalofugal tract, the exclusion mask was drawn through an axial section to exclude the stria terminalis.

FIGURE 1 NEAR HERE

The brachium of the superior colliculus was virtually dissected in two stages. (Figure 2). Masks were drawn manually in native space of each hemisphere of each subject on the superior colliculus (as described in (2), on the calcarine cortex in the coronal plane (Figure 2, top right), and on the optic tract in the coronal plane (Figure 2, top left). In the first stage, a streamline was generated between a mask drawn on calcarine cortex and the superior colliculus; and a second streamline was generated between a mask on the optic tract and the calcarine cortex to visualize the optic tract and the geniculostriate tract. The bottom panel shows the voxels that were present only in the second streamline (between the optic tract and the calcarine cortex) are shown in red; and the voxels common to both streamlines in yellow. Second, all voxels in the second streamline (shown in red and yellow) were subtracted from the streamline connecting the superior colliculus and the calcarine cortex. The residual voxels (green) visualized the brachium of the superior colliculus.

FIGURE 2 NEAR HERE

After thresholding each streamline at 10%, each individual's streamline, in native diffusion space, was used for computation of mean FA (by using the streamline to mask the FA image generated with FSL's DTIFIT utility). For visualization purposes, composite streamlines were generated by adding each of the streamlines for all subjects after normalization in MNI space.

Results

Topography of the collothalamic projections to the amygdala

Figure 3 shows the composite streamlines generated between the superior colliculus and the amygdala (red) and between the medial geniculate nucleus and the amygdala (blue). Both collothalamic projections traverse above the temporal horn of the lateral ventricle dorsal to the stria terminalis (yellow). Emerging from the midbrain, the visual pathway from the superior colliculus passes over the auditory pathway and is positioned ventral to the auditory pathway as they traverse above the temporal horn dorsal to the stria terminalis (which is positioned immediately dorsal to the temporal horn.) The auditory pathway enters the superior border of the lateral amygdala positioned ventromedial to the visual pathway.

FIGURE 3 and 4 NEAR HERE

Comparison of white matter microstructure of the visual and auditory pathways connecting the colliculi and the amygdala

Mean FA for each streamline, auditory and visual, in each hemisphere for each participant was submitted to a 2 x 2 ANOVA with streamline (auditory/visual) x hemisphere (left/right) as within subject factors. Figure 5 shows the mean fractional for the auditory and visual collothalamic projections to the amygdala in each hemisphere. There was a main effect of modality: Mean FA for the visual pathway was lower for the auditory streamline than for the visual streamline (F[1,18] =11.8, *p=.003)*.This difference may partly reflect the fact that the visual streamline passed through the gray matter of the pulvinar whereas the auditory streamline did not. There was also an interaction between modality and hemisphere: as reported by Koller *et al*. (2), FA of the SC-amygdala pathway in the left hemisphere was higher compared to that in the right (*t[18]= 3.3, p* = 004). There was no hemispheric

FA asymmetry for the auditory pathway (*t*[18] = - 1.6, ns).

FIGURE 5 NEAR HERE

Unsurprisingly individual differences in connectivity strength (indexed by FA) of the auditory projections from the thalamus to the amygdala did not reveal any correlation with the bias to orient toward threatening visual stimuli in these participants for the streamlines in either the left hemisphere (r = 0.35) or the right hemisphere (r=.11).

Role of visual collothalamic projections to the amygdala in modulating trait anxiety

Each participant in these experiments had also completed the State Trait Anxiety Inventory. Individual differences in bias to orient toward threat, reported by Koller et al. (2), did not correlate with trait anxiety (r= 0.1689). Mean FA values for individual participants in either the left or right hemisphere also did not correlate with trait anxiety for either the visual or auditory collothalamic pathways (Table 1).

However, for the visual streamline, individual differences in hemispheric asymmetry between FA values of right compared to left hemisphere visual streamlines (calculated by subtracting right hemisphere mean FA from left hemisphere mean FA) correlated with trait anxiety for the connections between the SC and the amygdala (Figure 6A). Thus, although mean FA of SC-amygdala streamline in the left hemisphere streamline was higher, in this sample, than the mean FA of the streamline in the right hemisphere, those individuals in whom this asymmetry was smallest (i.e., with stronger connectivity in the right hemisphere relative to the left) had higher trait anxiety scores. By contrast, hemispheric FA asymmetry for the medial geniculate-amygdala auditory streamline did not correlate with trait anxiety (Figure 6B).

TABLE 1 NEAR HERE

Role of amygdalo-fugal pathways in modulating trait anxiety and in orienting toward visual threat

Figure 7 shows the streamlines connecting the amygdala to the bed nucleus of the stria terminalis. The stria terminalis is shown in blue and the ventral amygdalofugal pathway in red. We have previously reported (3) that connectivity strength of the stria terminalis, in both the left and right hemispheres, predicted individual differences in orienting toward threatening visual stimuli. Paired samples t-tests revealed a hemispheric asymmetry in mean FA of the ventral amygdalofugal pathway, with mean FA of the left hemisphere streamline being higher (FA = 0.33, SD = 0.04) than on the right (FA = 0.31, SD = 0.04) (t[1,17] = 3.94, $p < .0003$) (Table 1). There was no hemispheric asymmetry in mean FA for the stria terminalis (left hemisphere mean FA = 0.328, SD 0.04; right hemisphere mean FA 0.294, SD = 0.04 (t[1,18] = 1.47).

FIGURE 7 NEAR HERE

In contrast with the mean fractional anisotropy of the stria terminalis, which predicted individual difference in orienting toward threat in the saccade decision task (3), there was no correlation between the mean FA of the ventral amygdalofugal pathway, in either hemisphere, with orienting bias toward threat (left hemisphere $r = -0.10$; right hemisphere $r = -0.09$). Moreover, although in this sample mean FA was higher in the left hemisphere for both the SC-posterior thalamus-amygdala streamline and the ventral amygdalofugal streamline, across individuals the FA asymmetries of these two streamlines were not correlated with one another ($r=0.23$).

Hemispheric FA asymmetry did not correlate with trait anxiety for either the stria terminalis, nor for the ventral amygdalofugal pathway (Figure 8).

FIGURE 8 NEAR HERE

Discussion

The supraventricular temporal bundle: Collothalamic projections to the amygdala in the human brain mediate orienting toward threat

In the current communication, probabilistic DTI tractography was used to virtually dissect, in the human brain, a streamline corresponding to a collothalamic pathway, from the medial geniculate nucleus to the lateral amygdala in rodents (5), that LeDoux, Sakaguch & Reis (6) showed to be critical for mediating fear conditioning. We demonstrate that this pathway can be visualized in the human brain as a component of the supraventricular temporal bundle, (Figure 9). This bundle is comprised of collothalamic projections that transmit auditory (5) and visual (1,2,29) threat signals from the thalamus to the amygdala, and of the stria terminalis, that transmits visual threat signals from the amygdala to the bed nucleus of the stria terminalis (3).

FIGURE 9 NEAR HERE

In addition to transmitting signals of looming visual threat from wide-field vertical neurons in the superficial layers of the superior colliculus (see below), it has now been shown in humans that the visual collothalamic pathway transmits other threat signals including facial expressions of fear or anger. It has been shown that newborn human infants, in whom synaptogenesis has not yet occurred in the primary visual cortex, are ready at birth to discriminate simple features that ensure that the baby can begin to bond with its parent. Newborns will track a basic face-like stimulus consisting of an oval containing dark circles forming an inverted triangle (30). It has now also been shown that the visual collothalamic pathway is capable of transmitting facial features signaling social threat such as angry or fearful faces (1,29), and that these signals are activated by low spatial frequency information (32, 34, 50).

To date, then, several of the purported features of the hypothesized “fast, subcortical ‘low road’ pathway to the amygdala for orienting toward threat” have received provisional experimental validation: 1]. The preservation of emotional blindsight in hemianopic patients (8,9) (10) (11) (12) confirms that a putative subcortical pathway could mediate responses to threats that are not consciously perceived. Moreover, Bertini et al. have shown that hemianopic patients who also have damage to the pulvinar do not manifest affective blindsight (13). 2]. The observation, that selective transection of the streamline, described by Tamietto *et al*. (15) and Rafal *et al.* (1) selectively impairs processing potential visual threats, implicates a pathway connecting the superior colliculus to the amygdala that passes through the pulvinar. 3]. The observation that destruction of this pathway delays orienting toward contralesional threat in a dot probe task (17) provides support for the proposal that this pathway provides a faster route than does the cortical route to the amygdala. Moreover, it has been recently demonstrated in monkeys that ensemble responses of amygdala neurons in monkeys carry robust information about open-mouthed, presumably threatening, faces within 50 ms after stimulus onset. This short-latency signal was not found in the visual cortex, suggesting a subcortical origin (25). Using a novel pathway identification pattern recognition fMRI method Kragle et al. (33) have shown evidence for a superior colliculus-amygdala pathway, and have shown that activity in that pathway encodes the intensity of emotional response to negative images and sounds but not pleasant images or pain. However, this study could not distinguish collicular signals transmitted from the deeper layers of the colliculus, which connect to the medial pulvinar, from signals transmitted from the superficial layers, which project to the inferior pulvinar nuclei. Intracranial recordings in humans have recorded short latency (74ms) responses in the amygdala to pictures of fearful facial expressions but not to neutral of happy facial expressions or to other arousing scenes, and these short latency amygdala responses were elicited specifically by low spatial frequency components of fearful faces (34). 4]. The observation of a temporal hemifield advantage for orienting toward threatening pictures in the temporal order saccade decision task (2) supports the proposal that the orienting bias toward threat is mediated by collothalamic projection of visual signals transmitted to the superficial layers of the superior colliculus via the retinotectal tract and; 5]. The observation that individual differences in bias to orient toward threat was predicted by individual difference in white matter microstructure of the streamline (27) support the hypothesis that orienting bias toward threat is mediated by a collothalamic visual pathway to the amygdala corresponding to the streamline.

More recently, McFadyen *et al*. (29) conducted a multi-modal neuroimaging study in which the streamline connecting the superior colliculus to the amygdala via the pulvinar was reconstructed with probabalistic tractography using data from the Human Connectome Project; and they also computationally modeled the flow of hemodynamic activity during a face-viewing task. While tractographic data does not reveal the direction of information transmission in a streamline, hemodynamic modeling provided evidence for a functionally afferent pathway from the pulvinar to the amygdala; and critically, individuals with greater fibre density in this pathway also had stronger dynamic coupling and enhanced fearful face recognition.

We have also previously shown that the third component of the supraventricular bundle, the stria terminalis is also involved in selective spatial orienting toward visual threat (3). Research on fear conditioning in rodents had not previously ascribed any role for stria terminalis projections to the bed nucleus in mediating responses to threat signals. For example stria terminalis lesions have no effect on fear-potentiated startle (35). However, fear-potentiated startle is mediated by projections from the central nucleus of the amygdala to brain stem autonomic centers. And, as described above, disruption of the visual pathway in humans also delays freezing-like responses in a dot-probe task. In the dot-probe task, a task-irrelevant threatening stimulus delays responding to the probe target, and this effect was delayed in responding to probe targets in the visual field contralateral to a lesion in the posterior thalamus that interrupted the visual collothalamic pathway (1).

By contrast to brain-stem, autonomically mediated alerting, freezing and startle reactions, that have been used to probe for fear responses in experimental animals (and presumably in the dot-probe task employed in humans), the saccade decision task employed in the study reported by Koller et al

(1,27,29) examined spatially selective orienting. In that study, white matter microstructure (indexed by FA) of the stria terminalis in both hemispheres was shown to predict a spatial orienting bias toward threat.

Notably, for the visual collothalamic pathway to the amygdala, only the pathway in the right hemisphere was observed to predict individual difference in threat orienting bias. This asymmetry may reflect a right hemisphere dominance in controlling spatial orienting. Although the visual collothalamic pathway in the left hemisphere was not shown to be involved in mediating spatial orienting, that does not mean that the pathway in the left hemisphere does not transmit threat signals. In fact, both patients with interruption of the visual collothalamic pathway studied by Ward and his colleagues – the patient who manifested a delay in the alerting/distracting effect elicited by threat in the contralesional hemifield in the dot probe task (17), and the patient who could not recognize fearful faces in his contralesional field (16) – had lesions in the left hemisphere. So, there is evidence that the left hemisphere pathway is involved in processing threatening stimuli, and in being aroused by them; but since spatial orienting responses are more dependent on processing in the right hemisphere, the collothalamic projections to the right hemisphere may be more involved in spatial orienting.

In the current research we also virtually dissected a streamline corresponding to the ventral amygdalofugal pathway. Unlike the stria terminalis, whose microstructure (as indexed by fractional anisotropy) predicted individual differences in the bias to orient toward visual threat (3) that of the ventral amygdalofugal pathway did not.

Table 2 summarizes our key findings reported here and elsewhere (2,3) for visual and auditory collothalamic projections to the amygdala, as well as for the two amygdalofugal projections to the bed nucleus of the stria terminalis (the stria terminalis and the ventral amygdalofugal pathway).

TABLE 2 NEAR HERE

Anatomical connectivity in the pulvinar between the retino-recipient layers of the superior colliculus and the amygdala: Course of the visual streamline through the posterior thalamus

As noted in the introduction, Pessoa and Adolphs (14) had challenged the hypothesis that a ‘fast, subcortical pathway’ transmitting visual threat signals from the superior colliculus could be relayed to the amygdala via the pulvinar because, at the time, tracer studies in animals had not demonstrated any pulvinar nucleus that both received retinorecipient signals from the superficial layers of the superior colliculus and that also sent projections to the lateral amygdala. Since the time of publication of Pessoa and Adolphs’ critique, more recent anatomical studies have revised our understanding of the anatomy of pulvinar nuclei (23,24) in primates and tracer studies in animals (25,26) have identified candidate posterior thalamic nuclei that both receive projections from the superficial layers of the superior colliculus and that also project to the amygdala.

One of the key objectives of the current research was to analyze the anatomical, topographic trajectory of the visual streamline connecting the superior colliculus with the amygdala in relation to these more recent anatomical studies in primates. Our goal was to identify the most likely candidates that could serve as a thalamic relay nucleus transmitting visual threat signals from the superficial layers of the superior colliculus to the amygdala in the human brain. While tractographic streamlines provide no information about possible synaptic connections, tractography was used here to identify which posterior thalamic nuclei that the streamline passes through have been shown, by recent tracer studies, to both receive retino-recipient afferents from the superficial layers of the colliculus and to also project to the amygdala. For this purpose, we virtually dissected the brachium of the superior colliculus as an anatomical landmark, and we demonstrated that the streamline passes entirely ventral to the brachium.

Our anatomical analysis of the course and potential thalamic connectivity of the visual pathway connecting the superior colliculus to the amygdala considers contemporary views of the comparative anatomy of the posterior thalamus, and the nature of the visual threat signals that it transmits. The name of the pulvinar, cushion in English, derives from its appearance as a rounded bulge of the caudal thalamus. Noting that this gross anatomical feature is only evident in primates, Les Gros Clark (36) identified it as a distinguishing anatomical feature of the primate order. It is now understood that the pulvinar constitutes a collection of posterior thalamic nuclei whose homologue is present in many mammalian species (23,24). Most of these nuclei are visual and are now understood to serve as the thalamic hub for the extrastriate cortex in the same way that the lateral geniculate nucleus serves as a thalamic hub for primary visual cortex (23,24).

In rodents it is now understood that Les Gros Clark was correct that the homologue of the primate pulvinar is lateral posterior nucleus (37). Collicular neurons projecting to the thalamus have large visual receptive fields and constitute a visual channel that samples visual signals at a much larger and broader spatial scale than do signals transmitted through magno and parvo-cellular projections to visual cortex. Consider that visual receptive field sizes in the retina range from about 1/3° - 1° in the fovea and in the peripheral retina are about 2°, and that receptive field sizes in the primary visual cortex are about 1°. By contrast, receptive field sizes in the superior colliculus range from about 1° for neurons with foveal receptive fields at the rostral pole to 20° in the visual periphery in the caudal colliculus.

Collicular neurons that project to the mouse pulvinar originate from a unique class of neurons called wide-field vertical cells (38). Their cell bodies are located in the deepest part of the superficial layers, and their dendrites spread vertically throughout the superficial layers to form tufts that cover large areas of the colliculus. These neurons respond to small moving stimuli but also to looming visual stimulation– the primordial visual threat. Via a disynaptic pathway transmit signals of overhead, looming threat to the amygdala via the lateral posterior nucleus of the thalamus (49).

As noted earlier, LeDoux and colleagues speculated that collothalamic visual threat signals could, like acoustic signals, be transmitted via posterior thalamic nuclei to the amygdala. The suprageniculate nucleus is a polymodal structure that responds better to visual than auditory stimulation in cats, and that receives input from the visual superficial layers of the superior colliculus in the rat (39,40). LeDoux *et al.* (3) demonstrated retrograde labeling of the suprageniculate nucleus after injections of the lateral amygdala in rats (p 1217, Fig.5). Linke *et al.* (41) also demonstrated, in double-labeling experiments in rats, projections from the superficial layers of the superior colliculus to the suprageniculate that also projected to the lateral amygdala. They suggested that these connections may represent a subcortical pathway for visual information transfer to the amygdala, but they did not provide evidence that this pathway transmitted threat signals.

In support of this premise, a recent study (25) reported involvement of the suprageniculate nucleus in the non-human primates in transmitting rapid, real time visual threat signals to the amygdala. Single unit recordings in the amygdala′s lateral nucleus and dorsal tip of the basal nucleus traced connectivity with subcortical regions as monkeys viewed displays of three different facial expressions, including open mouth expression of threat. Connectivity was, in addition, tracked with rabies virus injections emanating from the basal and lateral amygdala nuclei. Variable distributions of label were found in inferior and lateral pulvinar subdivisions, and dense labeling was also observed in the suprageniculate nucleus.

In the current virtual dissections, the course of the visual streamline, demonstrated by DTI tractography, is consistent with a pathway from the superficial visual layers of the superior colliculus to the amygdala relayed through the either the inferior pulvinar nuclei or, possibly, through the suprageniculate nucleus. Exiting the superior colliculus, the streamline courses medial and then superior to the medial geniculate nucleus (Figure 4B) through the location of the suprageniculate nucleus. It then arcs dorsally through the pulvinar, below the brachium of the superior colliculus, before turning rostro-laterally and exiting the lateral pulvinar to take position above the temporal horn *enroute* to the lateral amygdala.

The current research also sought to determine whether collicular projections in the visual streamline also pass through any posterior thalamic nuclei that Elorette *et al.* (26) have shown to also project to the amygdala. They injected retrograde tracers into the amygdala and anterograde tracers into the colliculus and identified zones of overlap of these projections within the pulvinar of the macaque monkeys. Zones of overlap were seen in the suprageniculate nucleus and in areas of the medial and inferior pulvinar nuclei that were concentrated in the vicinity of the brachium of the superior colliculus. In one monkey in whom tracer was injected in the superficial layers of the superior colliculus, labeling was observed in the inferior pulvinar nucleus (Fig. 4, p. 7). Although, as noted earlier, the superficial layers of the superior colliculus have been shown to project to the amygdala in rats (41), Elorette *et al.* (26) did not observe labeling in the suprageniculate nucleus in this monkey. In that monkey, injection of tracer into the intermediate and deep layers of the superior colliculus revealed overlap with efferents to the amygdala in the suprageniculate nucleus (Fig. 3, p. 6). In a second monkey, the collicular injection sites were in the intermediate and deeper layers of the colliculus. In that monkey overlap of thalamic afferents from the superior colliculus and efferents to the amygdala were observed only in pulvinar nuclei above the brachium of the superior colliculus.

Berman and Wurtz (42) demonstrated, with electro-anatomy, that the colliculus transmits signals to the inferior pulvinar nucleus that are relayed to motion-selective neurons in cortical area MT/V5. It is possible that the proximal portion of the visual tractography streamline also contains fibers that transmit these motion signals to MT as well as fibers projecting to the amygdala.

Both Inagaki *et al.* (25) and Elorette *et al.* (26) have reported dense labeling of the medial pulvinar after tracer injections in the amygdala. However, the medial pulvinar nucleus is dorsal to the brachium of the inferior colliculus, and the tractographic streamline courses ventral to the brachium. The medial pulvinar nucleus receives projections from the deep (motor) layers of the superior colliculus that are consistent with the tracer data from both Inagaki et al. (25) and Elorette *et al.* (26) and well as Burton and Jones (43). However, Burton and Jones (43) showed that projections from the medial pulvinar to the amygdala pass through the external capsule and not above the temporal horn. Although the superior colliculus seed masks used in our tractography studies encompassed the full depth of the colliculus, we did not visualize any projections passing through the medial pulvinar. Thus, our tractographic data did not visualize collothalamic pathways that transmit oculomotor signals from the deep layers of the colliculus to the amygdala via the medial pulvinar. Since the deep layers of the superior colliculus send axon collaterals signaling that an eye movement has been made to a salient target, the relay of these signals to the amygdala via the medial pulvinar also may convey information about potential threat, but in our data, we were not able to visualize this pathway. The collothalamic pathway visualized with tractography therefore likely only transmits visual signals from the superficial layers of the superior colliculus.

While the streamline passes through the ventral lateral pulvinar, no direct connections have been demonstrated in monkeys between lateral pulvinar and the amygdala (44). Efferents from the amygdala to the thalamus have only been demonstrated to the mediodorsal thalamus and other midline thalamic nuclei (45), not to the pulvinar. Elorette *et al.* (26) demonstrated that the lateral pulvinar receives projections from the intermediate and deep layers of the colliculus but does not project to the amygdala.

Given these known anatomical constraints, the topography of the visual streamline being considered here is most consistent with the proposal that the projections from the superficial layers of the superior colliculus synapse in the suprageniculate nucleus and/or inferior pulvinar which then projects to the lateral amygdala. It should be noted that the inferior pulvinar is now known to have three subdivisions two of which, the posterior inferior and inferior central medial divisions, stain with vesicular glutamate transporter 2 indicating that these subdivisions receive afferents from the superficial layers of the superior colliculus (24).

Role of collothalamic projections to the amygdala in modulating anxiety

Another new finding of the current research was that individual differences in the hemispheric balance of the visual colliculo-thalamo-amygdala projections was a predictor of trait anxiety. Specifically, stronger connectivity of this subcortical visual pathway to the amygdala in the left hemisphere, relative to those on the right, predicted lower levels of trait anxiety. Individual differences in connectivity of the auditory subcortical pathway to the amygdala did not correlate with trait anxiety. While our research has implicated this subcortical visual pathway in both orienting toward threat and in modulating anxiety, we have not found that individual differences in orienting bias toward threat correlate with individual differences in trait anxiety.

Fractional anisotropy of the neither of the two amygdala efferent pathways to the bed nucleus of the stria terminalis, the stria terminalis or the ventral amygdalofugal pathway, predicted individual differences in trait anxiety. It must be acknowledged that this null result cannot rule out that either of these pathways are uninvolved in transmitting signals that modulate anxiety. The bed nucleus has been shown to be involved in defensive preparedness that can be manifest as anxiety in both experimental animals (46,47) and humans (48). It is also possible that transmission of threat signals from the amygdala that modulate anxiety in the bed nucleus may be routed indirectly through projections from the amygdala, via the uncinate fasciculus, to the medial prefrontal cortex which then projects to the bed nucleus of the stria terminalis.

In summary we provide an *in vivo* demonstration in humans of two collo-thalamo-amygdalar pathways: an auditory pathway corresponding to that implicated in fear conditioning by in rodents (4); and a visual pathway from the superior colliculus to the amygdala, that is most likely transmitted via the suprageniculate nucleus or the inferior pulvinar nuclei, that has been implicated in transmitting visual threat signals to the amygdala (1,2,27) These two collothalamic pathways are component of a white matter bundle, traversing above the temporal horn of the lateral ventricle that, along with the stria terminalis, are part of a circuit for mediating spatial orienting responses toward threat. We further show that individual differences in hemispheric asymmetry of the microstructure of this visual pathway is a predictor of trait anxiety.

Acknowledgments Thanks to Gail Berger for helpful comments and suggestions on an earlier draft of this manuscript.

Author Contributions: Kristin Koller collected the MRI sequences, performed tractography, computed mean FA values, and acquired STAI questionnaires. Robert Rafal and Kristin Koller both contributed to conceptualization of the research. Robert Rafal manually drew the seed, waypoint and target masks, conducted the statistical analyses and wrote the first draft of the manuscript.

Disclosures : The authors declare no competing interests. All data is available in the main text or the supplementary materials.

Open Practices Statement: 1) Anonymized imaging data (the four tractography streamlines in MNI coordinates) are available as Supplementary Material. 2) This study was not pre-registered.

References

1. Rafal RD, Koller K, Bultitude JH, Mullins P, Ward R, Mitchell AS, et al. Connectivity between the superior colliculus and the amygdala in humans and macaque monkeys: virtual dissection with probabilistic DTI tractography. Journal of Neurophysiology [Internet]. 2015 Sep 22;114(3):1947–62. Available from: http://jn.physiology.org/lookup/doi/10.1152/jn.01016.2014

2. Koller K, Rafal RD, Platt A, Mitchell ND. Orienting toward threat: Contributions of a subcortical pathway transmitting retinal afferents to the amygdala via the superior colliculus and pulvinar. Neuropsychologia. 2019;128:78–86.

3. Koller K, Hatton CM, Rogers RD, Rafal RD. Stria terminalis microstructure in humans predicts variability in orienting towards threat. Eur J Neurosci. 2019;50(11):3804–13.

4. LeDoux JE. Emotion Circuits in the Brain. http://dx.doi.org/101146/annurev.neuro231155 [Internet]. 2003 Nov 28;23(1):155–84. Available from: http://www.annualreviews.org/doi/10.1146/annurev.neuro.23.1.155

5. LeDoux JE, Farb C, Ruggiero DA. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J Neurosci [Internet]. 1990 Apr 1;10(4):1043–54. Available from: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.10-04-01043.1990

6. LeDoux JE, Sakaguchi A, Reis DJ. Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. J Neurosci [Internet]. 1984 Mar 1;4(3):683–98. Available from: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.04-03-00683.1984

7. Tamietto M, Gelder BD. Neural bases of the non-conscious perception of emotional signals. Nat Rev Neurosci [Internet]. 2010 Oct 2;11(10):697–709. Available from: http://dx.doi.org/10.1038/nrn2889

8. Hamm AO. Affective blindsight: intact fear conditioning to a visual cue in a cortically blind patient. Brain [Internet]. 2003 Feb 1;126(2):267–75. Available from: http://www.brain.oupjournals.org/cgi/doi/10.1093/brain/awg037

9. Morris JS, Öhman A, Dolan RJ. A subcortical pathway to the right amygdala mediating “unseen” fear. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 1999 Feb 16;96(4):1680–5. Available from: http://www.pnas.org/content/96/4/1680.full

10. Morris JS, DeGelder B, Weiskrantz L, Dolan RJ. Differential extrageniculostriate and amygdala responses to presentation of emotional faces in a cortically blind field. 2017.

11. Pegna AJ, Khateb A, Lazeyras F, Seghier ML. Discriminating emotional faces without primary visual cortices involves the right amygdala. Nat Neurosci [Internet]. 2004 Dec 12;8(1):24–5. Available from: http://www.nature.com/doifinder/10.1038/nn1364

12. Striemer CL, Whitwell RL, Goodale MA. Affective blindsight in the absence of input from face processing regions in occipital-temporal cortex. Neuropsychologia. 2017;128:50–7.

13. Bertini C, Pietrelli M, Braghittoni D, Làdavas E. Pulvinar Lesions Disrupt Fear-Related Implicit Visual Processing in Hemianopic Patients. Front Psychol. 2018;9:2329.

14. Pessoa L, Adolphs R. Emotion processing and the amygdala: from a “low road” to “many roads” of evaluating biological significance. Nat Rev Neurosci [Internet]. 2010 Nov 1;11(11):773–82. Available from: https://www.nature.com/articles/nrn2920

15. Tamietto M, Pullens P, Gelder BD, Weiskrantz L, Goebel R. Subcortical Connections to Human Amygdala and Changes following Destruction of the Visual Cortex. Curr Biol [Internet]. 2012 Aug 7;22(15):1449–55. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960982212006513

16. Ward R, Calder A, Parker M, Arend I. Emotion recognition following human pulvinar damage. Neuropsychologia [Internet]. 2007 Apr 8;45(8):1973–8. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17250857

17. Ward R, Danziger S, Bamford S. Response to Visual Threat Following Damage to the Pulvinar. Curr Biol. 2005;15(6):571–3.

18. Sylvester R, Josephs O, Driver J, Rees G. Visual FMRI responses in human superior colliculus show a temporal-nasal asymmetry that is absent in lateral geniculate and visual cortex. Journal of Neurophysiology [Internet]. 2007;97(2):1495–502. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=17135475&retmode=ref&cmd=prlinks

19. Koningsbruggen M van, Koller K, Rafal RD. Deafferentation of the Superior Colliculus Abolishes Spatial Summation of Redundant Visual Signals. Front Syst Neurosci [Internet]. 2017 Feb 24;11:1077–8. Available from: http://journal.frontiersin.org/article/10.3389/fnsys.2017.00009/full

20. Keifer OP, Gutman DA, Hecht EE, Keilholz SD, Ressler KJ. A comparative analysis of mouse and human medial geniculate nucleus connectivity: A DTI and anterograde tracing study. NeuroImage. 2015;105:53–66.

21. Crippa A, Lanting CP, Dijk P van, Roerdink JBTM. A Diffusion Tensor Imaging Study on the Auditory System and Tinnitus. Open Neuroimaging J. 2010;4:16–25.

22. Day-Brown JD, Wei H, Chomsung RD, Petry HM, Bickford ME. Pulvinar Projections to the Striatum and Amygdala in the Tree Shrew. Front Neuroanat [Internet]. 2010;4:143–143. Available from: http://www.frontiersin.org/Neuroanatomy/10.3389/fnana.2010.00143/abstract

23. Grieve KL, Acuña C, Cudeiro J. The primate pulvinar nuclei: vision and action. Trends Neurosci. 2000;23(1):35–9.

24. Baldwin MKL, Balaram P, Kaas JH. The evolution and functions of nuclei of the visual pulvinar in primates. J Comp Neurol. 2017;525(15):3207–26.

25. Inagaki M, Inoue K ichi, Tanabe S, Kimura K, Takada M, Fujita I. Rapid processing of threatening faces in the amygdala of nonhuman primates: subcortical inputs and dual roles. Cereb Cortex. 2022;33(3):895–915.

26. Elorette C, Forcelli PA, Saunders RC, Malkova L. Colocalization of Tectal Inputs With Amygdala-Projecting Neurons in the Macaque Pulvinar. Front Neural Circuits. 2018;12:91.

27. Koller K, Rafal RD, Platt A, Mitchell ND. Orienting toward threat: Contributions of a subcortical pathway transmitting retinal afferents to the amygdala via the superior colliculus and pulvinar. Neuropsychologia. 2019;128:78–86.

28. Behrens TEJ, Berg HJ, Jbabdi S, Rushworth MFS, Woolrich MW. Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? NeuroImage [Internet]. 2007 Jan 1;34(1):144–55. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=17070705&retmode=ref&cmd=prlinks

29. McFadyen J, Mattingley JB, Garrido MI. An afferent white matter pathway from the pulvinar to the amygdala facilitates fear recognition. eLife. 2019;8:e40766.

30. Johnson M, Morton J. Biology and Cognitive Development: The Case of Face Recognition. 1991.

31. Vuilleumier P, Armony JL, Driver J, Dolan RJ. Distinct spatial frequency sensitivities for processing faces and emotional expressions. Nat Neurosci [Internet]. 2003 May 12;6(6):624–31. Available from: http://www.nature.com/doifinder/10.1038/nn1057

32. Vuilleumier P, Armony JL, Driver J, Dolan RJ. Distinct spatial frequency sensitivities for processing faces and emotional expressions. Nat Neurosci. 2003;6(6):624–31.

33. Kragel PA, Čeko M, Theriault J, Chen D, Satpute AB, Wald LW, et al. A human colliculus-pulvinar-amygdala pathway encodes negative emotion. Neuron. 2021;109(15):2404-2412.e5.

34. Méndez-Bértolo C, Moratti S, Toledano R, Lopez-Sosa F, Martínez-Alvarez R, Mah YH, et al. A fast pathway for fear in human amygdala. Nat Neurosci [Internet]. 2016 Aug 1;19(8):1041–9. Available from: http://www.nature.com/doifinder/10.1038/nn.4324

35. Hitchcock JM, Davis M. Efferent Pathway of the Amygdala Involved in Conditioned Fear as Measured With the Fear-Potentiated Startle Paradigm. 2018.

36. Clark WE. The Thalamus of Tarsius. J Anat. 1930;64(Pt 4):371–414.

37. Harting JK, Hall WC, Diamond IT. Evolution of the Pulvinar (Part 2 of 2). Brain, Behav Evol. 1972;6(1–6):439–52.

38. Savier E, McHaney K, Chen H, Cang J. Characterization of visual response properties and connectivity of Wide-field vertical neurons in the mouse superior colliculus. J Vis. 2022;22(14):3044.

39. Linke R. Differential projection patterns of superior and inferior collicular neurons onto posterior paralaminar nuclei of the thalamus surrounding the medial geniculate body in the rat. Eur J Neurosci [Internet]. 2001 Dec 24;11(1):187–203. Available from: http://doi.wiley.com/10.1046/j.1460-9568.1999.00422.x

40. Hicks TP, Watanabe S, Miyake A, Shoumura K. Organization and properties of visually responsive neurones in the suprageniculate nucleus of the cat. Exp Brain Res. 1984;55(2):359–67.

41. Linke R, Lima ADD, Schwegler H, Pape H -C. Direct synaptic connections of axons from superior colliculus with identified thalamo-amygdaloid projection neurons in the rat: Possible substrates of a subcortical visual pathway to the amygdala. J Comp Neurol. 1999;403(2):158–70.

42. Berman RA, Wurtz RH. Functional Identification of a Pulvinar Path from Superior Colliculus to Cortical Area MT. Journal of Neuroscience [Internet]. 2010 May 5;30(18):6342–54. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.6176-09.2010

43. Burton H, Jones EG. The posterior thalamic region and its cortical projection in new world and old world monkeys. J Comp Neurol [Internet]. 1976 Jul 15;168(2):249–301. Available from: http://doi.wiley.com/10.1002/cne.901680204

44. Benevento LA, Standage GP. The organization of projections of the retinorecipient and nonretinorecipient nuclei of the pretectal complex and layers of the superior colliculus to the lateral pulvinar and medial pulvinar in the macaque monkey. J Comp Neurol [Internet]. 1983 Jul 1;217(3):307–36. Available from: http://doi.wiley.com/10.1002/cne.902170307

45. Aggleton JP, Mishkin M. Projections of the amygdala to the thalamus in the cynomolgus monkey. J Comp Neurol [Internet]. 1984 Jan 1;222(1):56–68. Available from: http://doi.wiley.com/10.1002/cne.902220106

46. Fox AS, Oler JA, Tromp DPM, Fudge JL, Kalin NH. Extending the amygdala in theories of threat processing. Trends in Neurosciences [Internet]. 2015 May 1;38(5):319–29. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0166223615000612

47. Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. Eur J Pharmacol. 2003;463(1–3):199–216.

48. Somerville LH, Whalen PJ, Kelley WM. Human Bed Nucleus of the Stria Terminalis Indexes Hypervigilant Threat Monitoring. Biological Psychiatry [Internet]. 2010;68(5):416–24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006322310003458

49. Wei P, Liu N, Zhang Z, Liu X, Tang Y, He X, et al. Processing of visually evoked innate fear by a non-canonical thalamic pathway. Nat Commun. 2015;6(1):6756.

50. Burra N, Hervais-Adelman A, Celeghin A, Gelder B de, Pegna AJ. Affective blindsight relies on low spatial frequencies. Neuropsychologia. 2019;128:44–9.

Supplemental Material

All the streamlines published in this paper are available at https://doi.org/10.17605/OSF.IO/W9UFZ
.

Supplemental Material

The streamlines visualized in Figure 3 and 7 are available (in MNI space) in Supplementary Material.

Figure Legends

Figure 1. A. Masks of the medial geniculate nuclei (light blue) and superior colliculi (yellow). B. Masks used for tractography of the stria terminalis and the ventral amygdalofugal pathway shown, from top to bottom, in sagittal, coronal and axial sections. Left column: Amygdala mask (blue); Middle column: Mask of the bed nucleus of the stria terminalis (red); Right column shows the exclusion masks used for tractography of the stria terminalis (green) and the ventral amygdalofugal pathway (red).

Figure 2. Virtual dissection of the brachium of the superior colliculus was achieved in two stages. First, a streamline was generated between a mask drawn over the calcarine cortex (top right) and the superior colliculus; and a second streamline was generated between a mask on the optic tract (top left) and the calcarine cortex (top right) to visualize the optic tract and the geniculostriate tract. The bottom figure shows the voxels that were present only in the second streamline (between the optic tract and the calcarine cortex) in red and the voxels common to both streamlines in yellow. Second, all voxels in the second streamline (shown in red and yellow) were subtracted from the streamline connecting the superior colliculus and the optic tract. The residual (green) voxels visualized the brachium of the superior colliculus.

Figure 3: A. Composite streamlines from all participants in MNI space showing voxels through which the streamlines passed in 75% of participants. From top left to bottom right (y = -34, -32, -27, -23, -20, -18, -16, -11, -6) are coronal slices from posterior to anterior showing the anatomical relations of the stria terminalis (yellow) and the brachium of the inferior colliculus (light blue, the auditory (dark blue) and visual (red) collothalamic projections from the midbrain to the amygdala. B. The streamlines shown in sagittal (left, x= -27), and axial (right, y= -2) planes. C. The same streamlines are shown in a

3D reconstruction. The superior colliculus is shown in pink, the inferior colliculus in copper and the amygdala in green.

Fig. 4: A. Coronal section from posterior to anterior showing the visual streamline (red) emerging from the superior colliculus and traversing above the medial geniculate nucleus (blue) through the location of the suprageniculate nucleus. B. Sagittal slices through the thalamus, from medial to lateral, showing the anatomical relationship between the visual pathway (red) connecting the superior colliculus and the amygdala, and the brachium of the superior colliculus (green). Voxels common to both streamlines are shown in yellow.

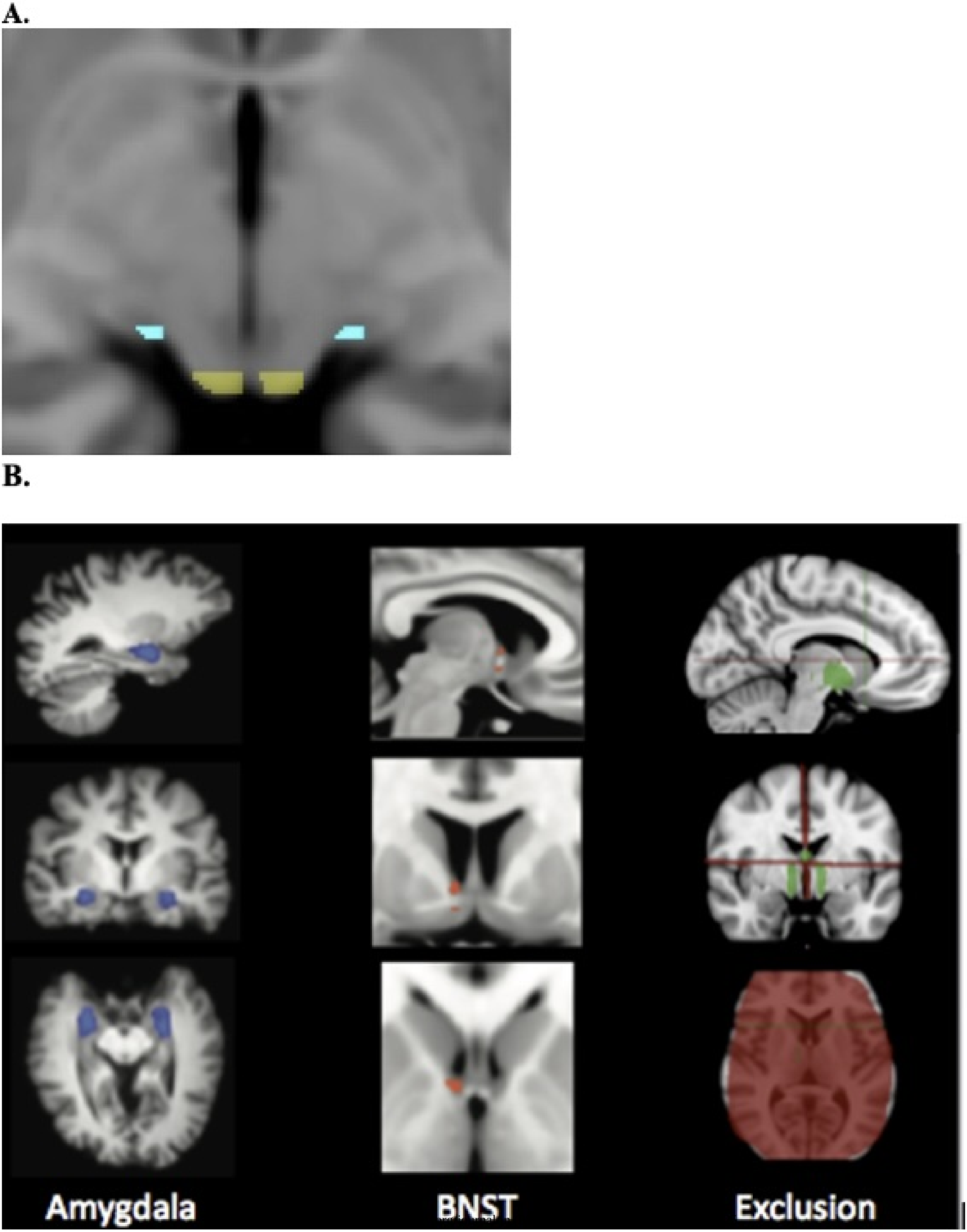
Figure 5: Fractional anisotropy of visual streamline (between SC and amygdala) and the auditory streamline (between the MGn and amygdala) in each hemisphere. Error bars = SEM.

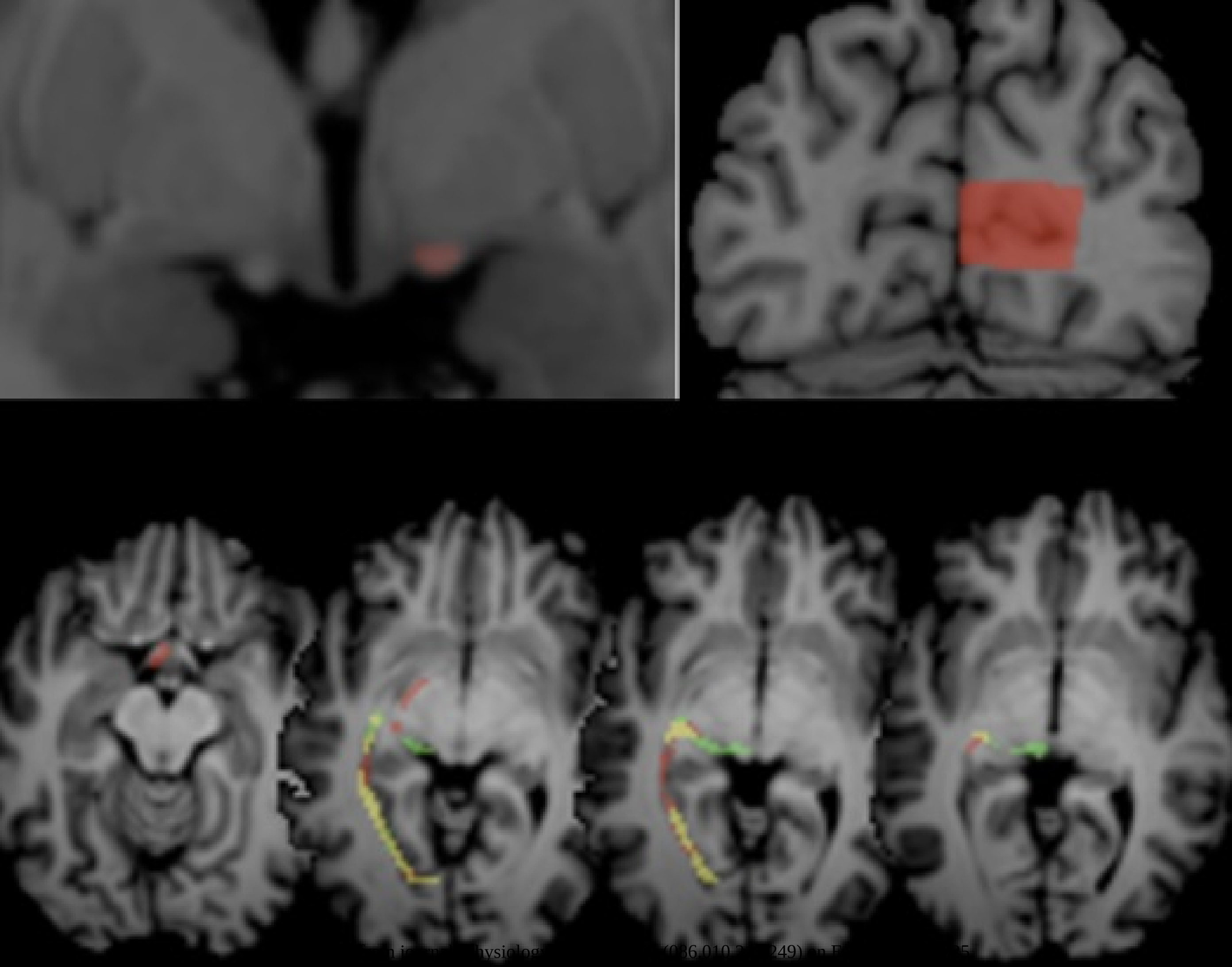
Figure 6: Hemispheric asymmetry in streamline connectivity (FA) correlations with trait anxiety for collothalamic projections to the amygdala. A. Visual streamline (SC-posterior thalamus-amygdala). B Auditory streamline (MGn-amygdala).

Figure 7. Composite streamlines (showing overlap of 100% of the participants in MNI space) depicting the stria terminalis (blue) and the ventral amygdalofugal pathway (red).

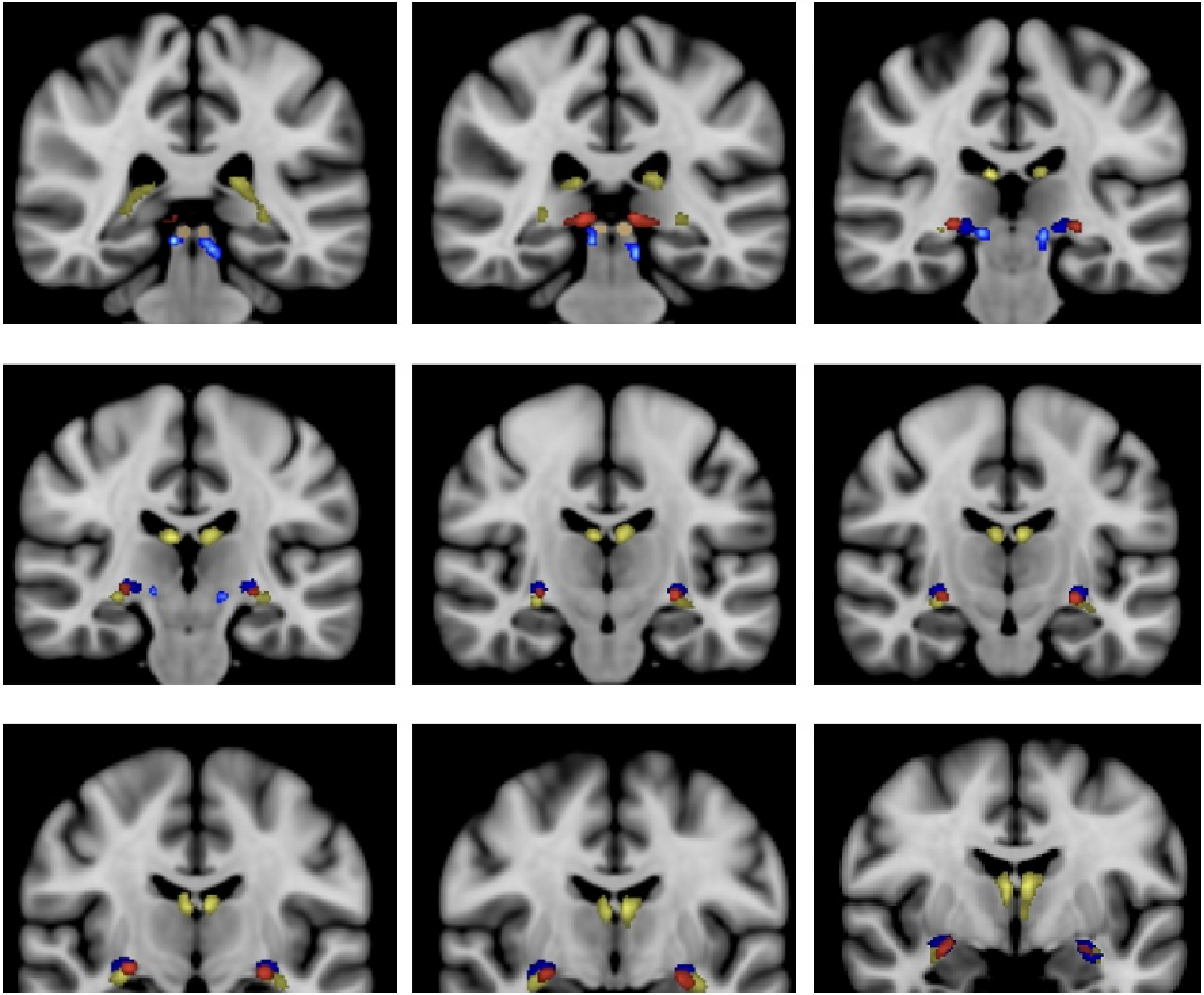
Fig. 8: Hemispheric asymmetry in streamline connectivity (FA) correlations with trait anxiety for A. the stria terminalis, and B. the ventral amygdalofugal pathway.

Figure 9: The supraventricular temporal bundle (arrows) in sagittal (left) and coronal (right) views. A fractional anisotropy image is superimposed on a semi-opaque T1-weigthed image.

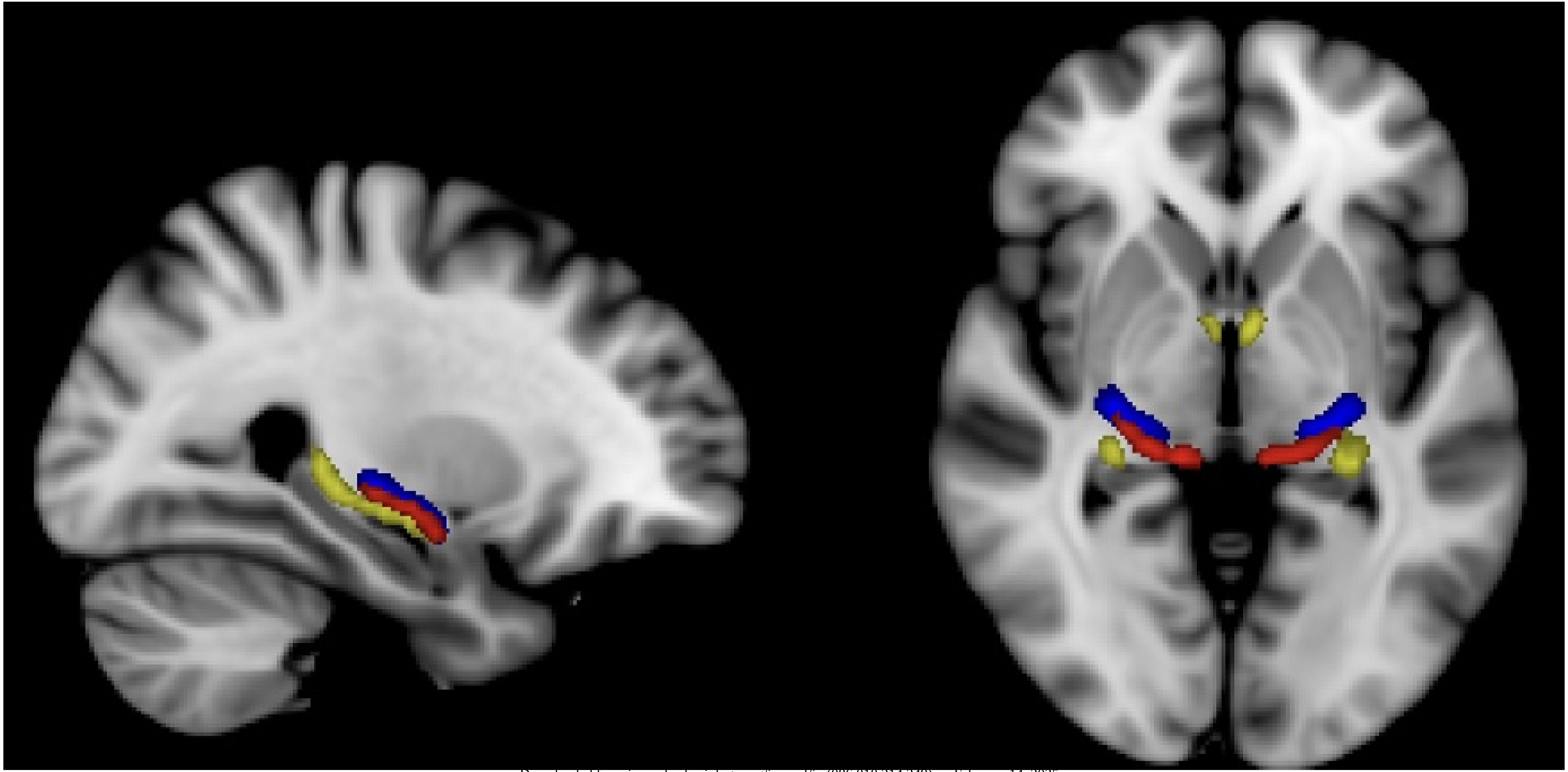

A.
B.
Amygdala
BNST
Exclusion



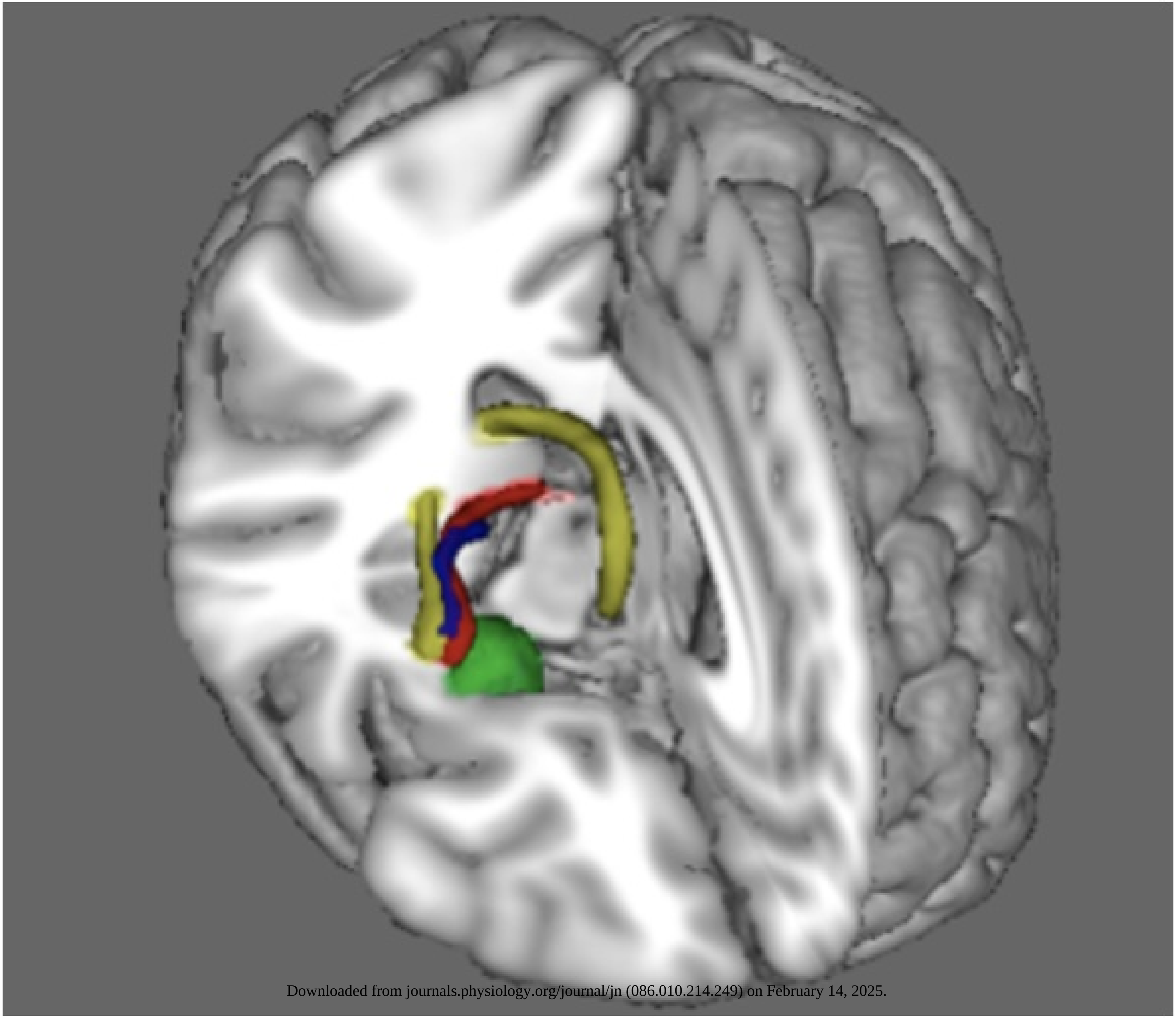
A.



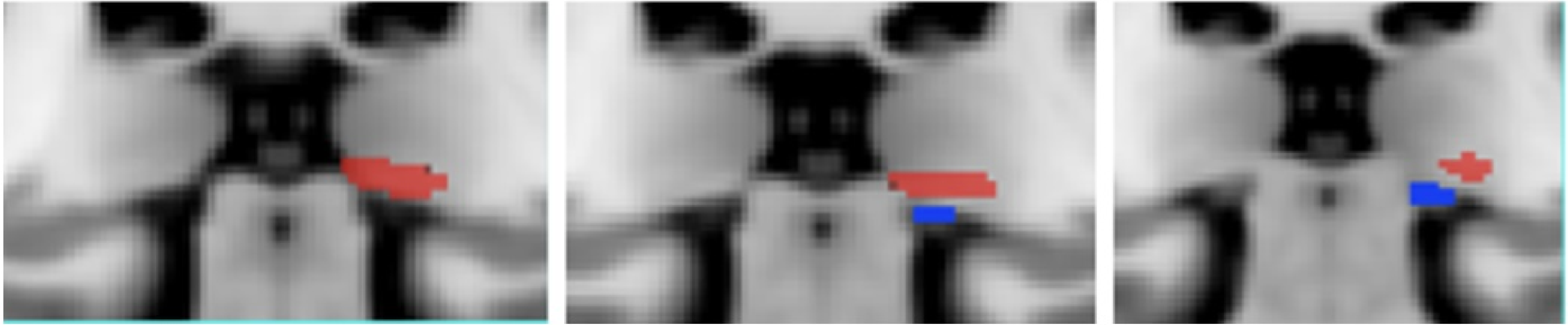
B.



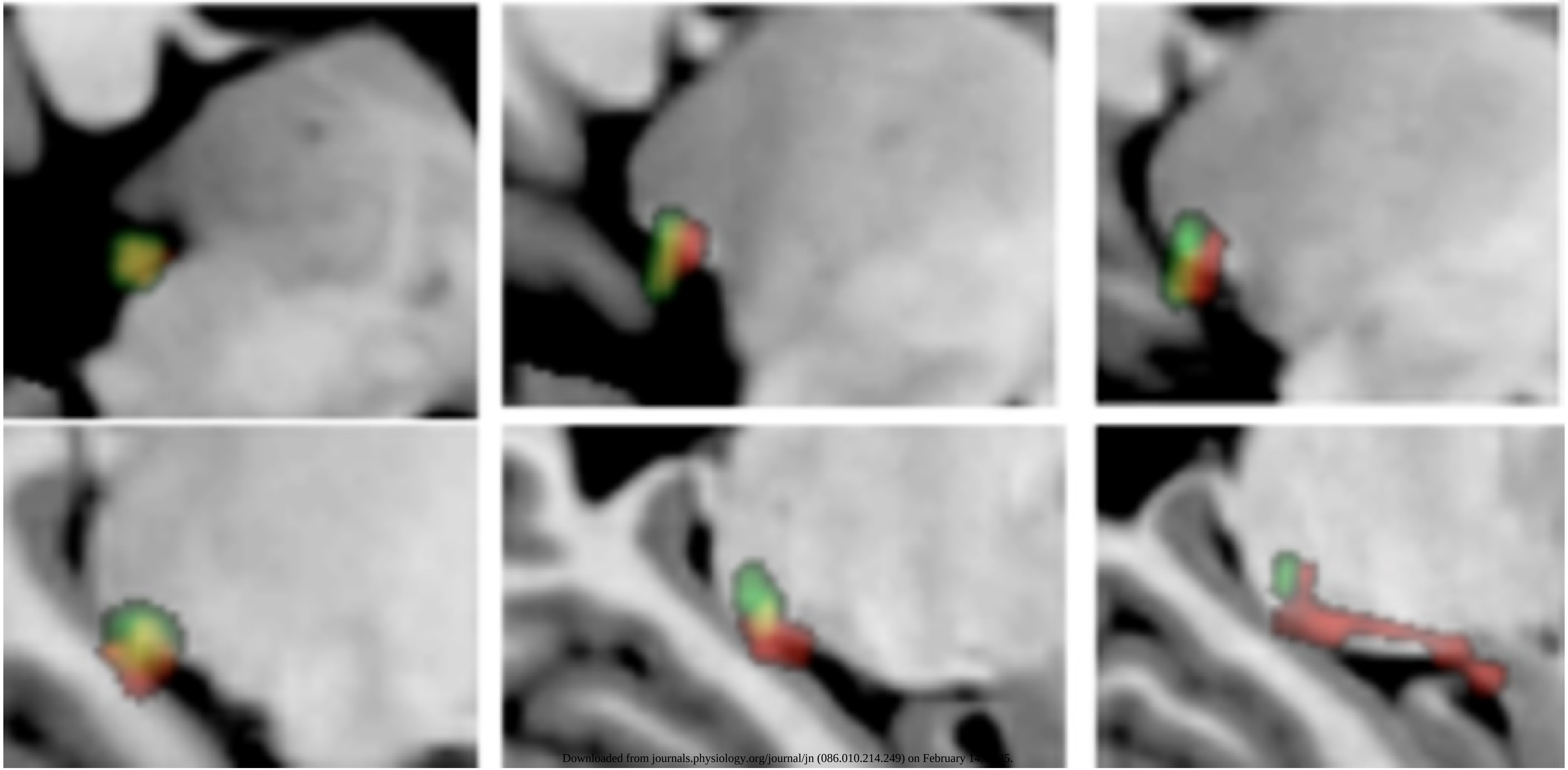
C.

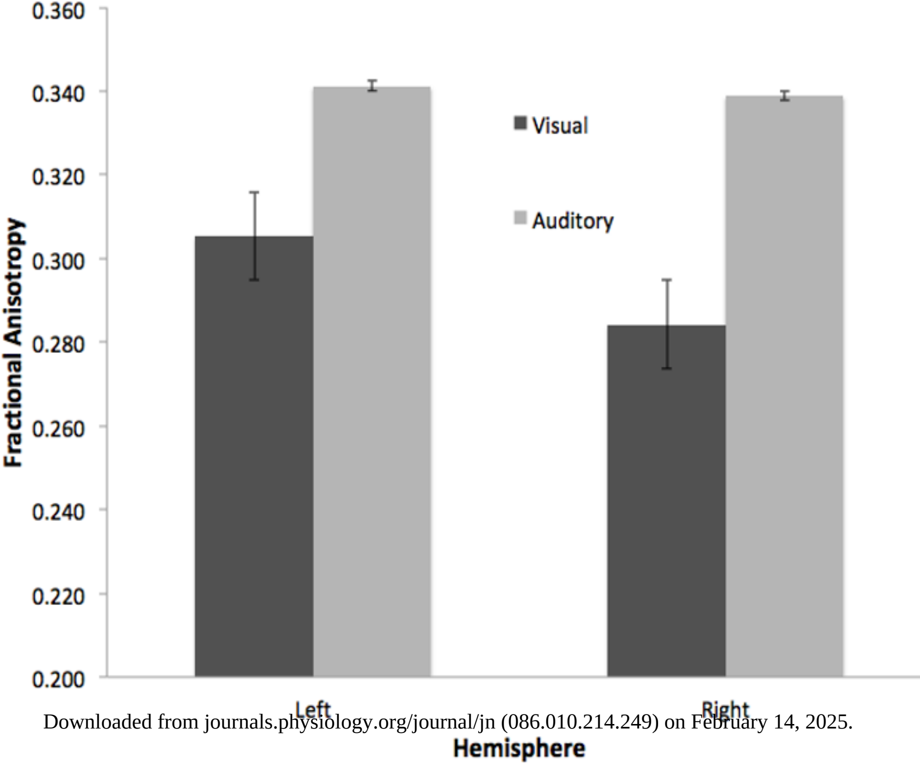


A.

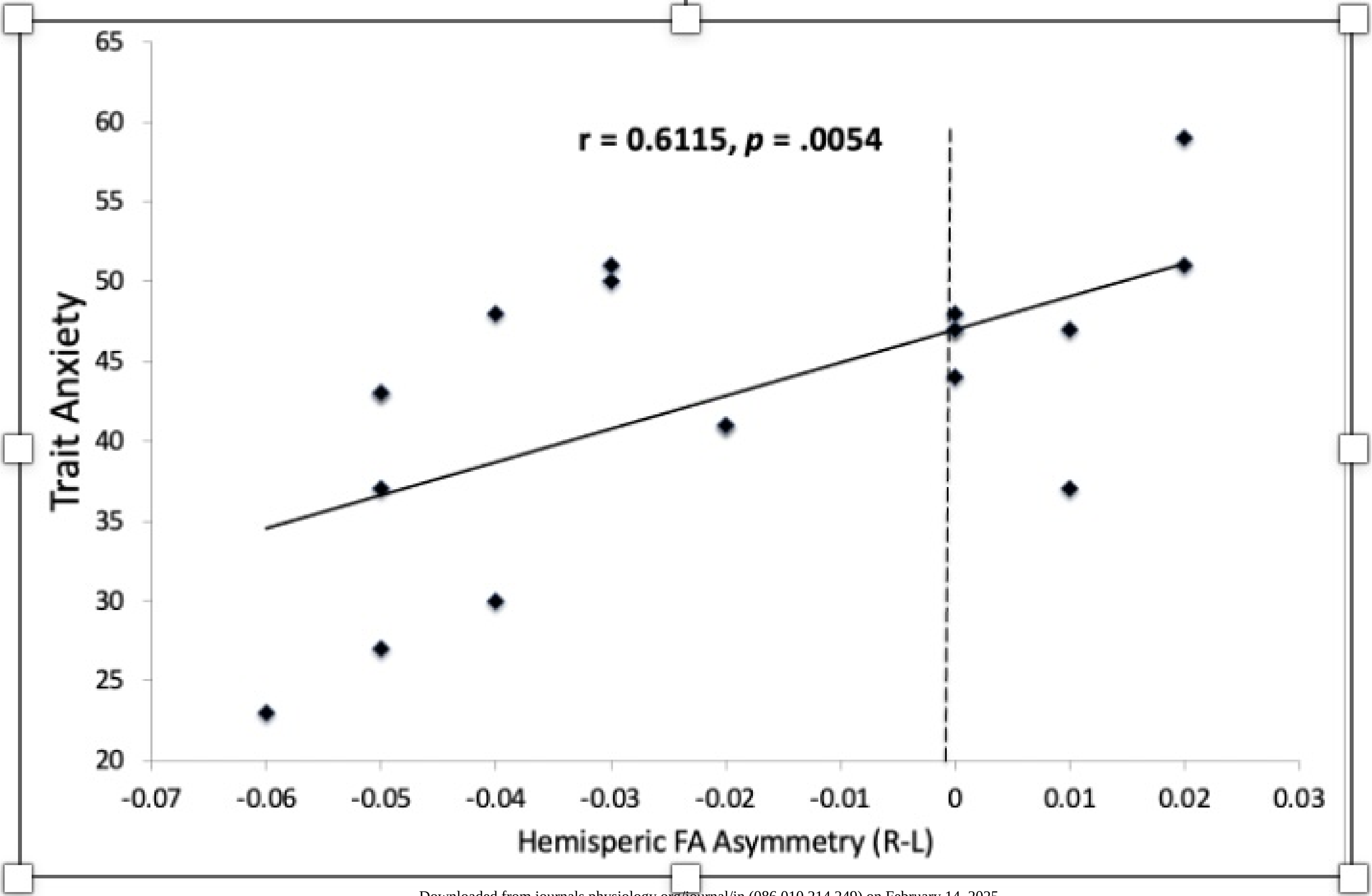


B.

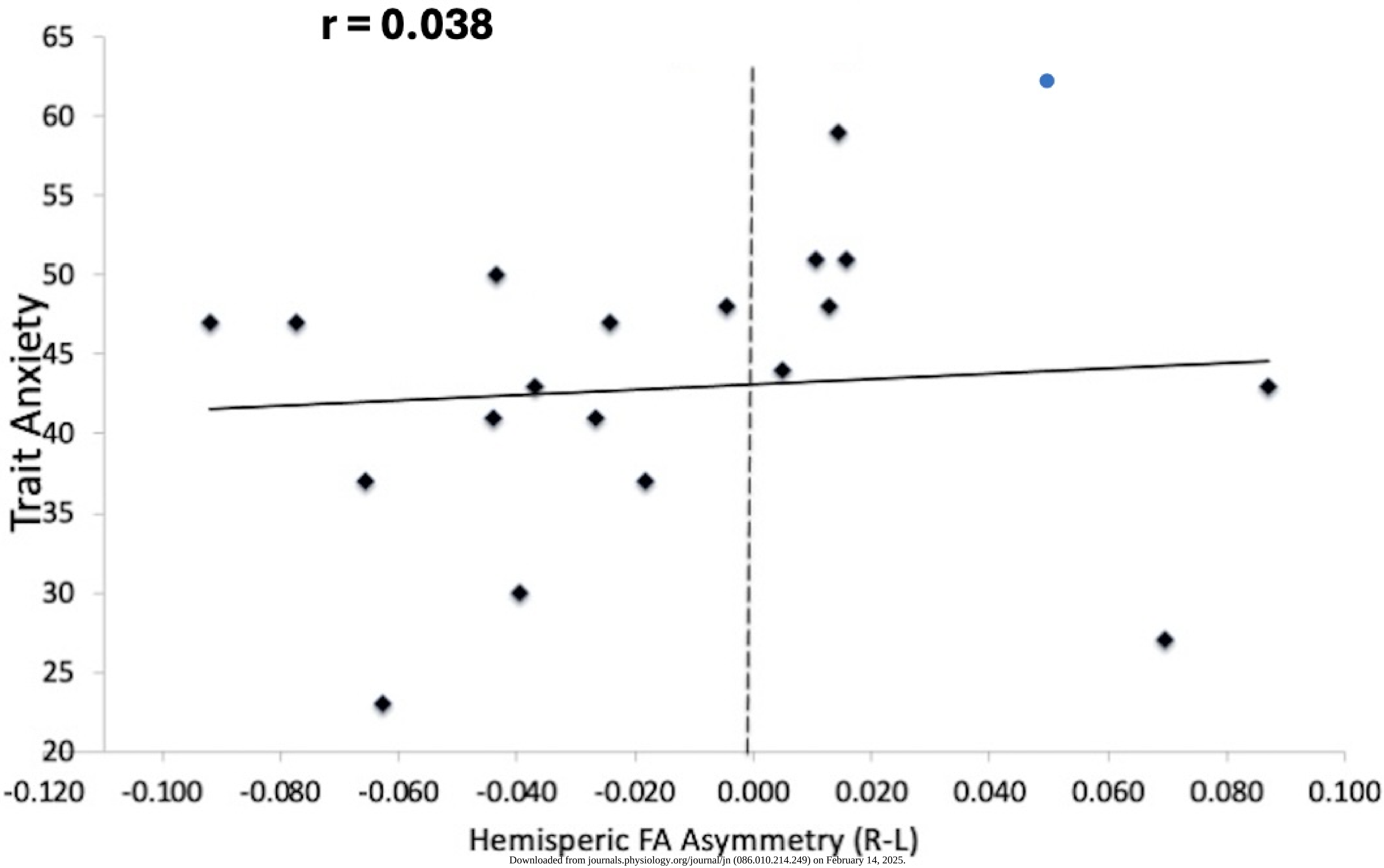


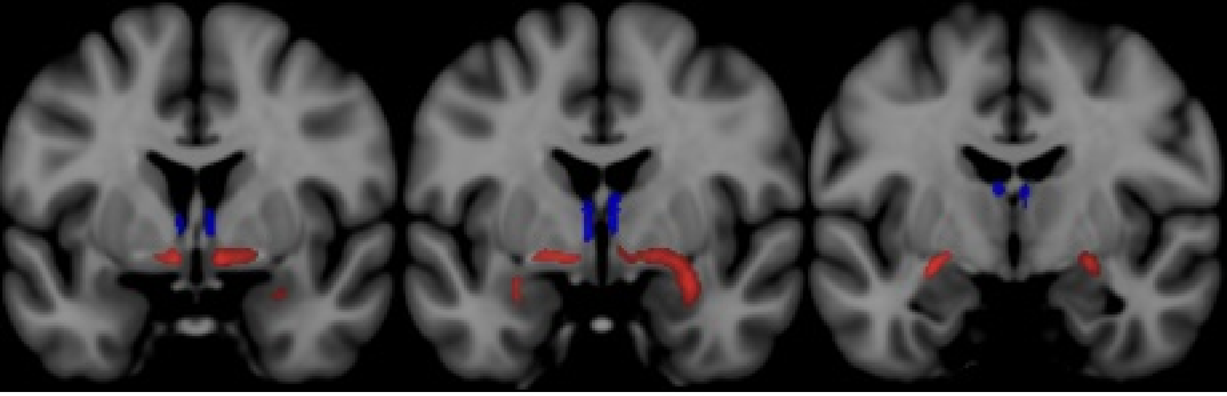

0.360
0.340
0.320
0.300
0.280
0.260
0.240
0.220
0.200
Fractional Anisotropy
■ Visual
■ Auditory
Left
Right
Hemisphere

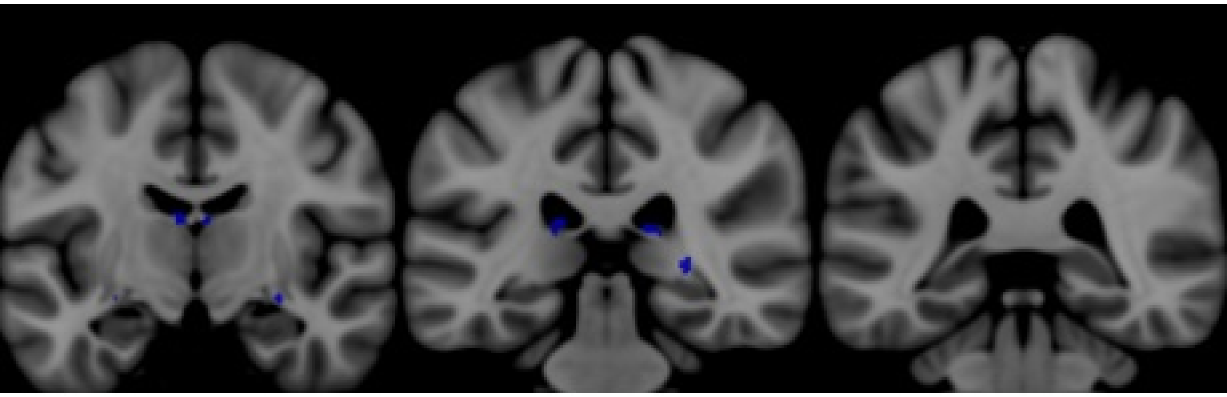
A. Visual (SC-posterior thalamus-amy dala) streamline FA asymmetry

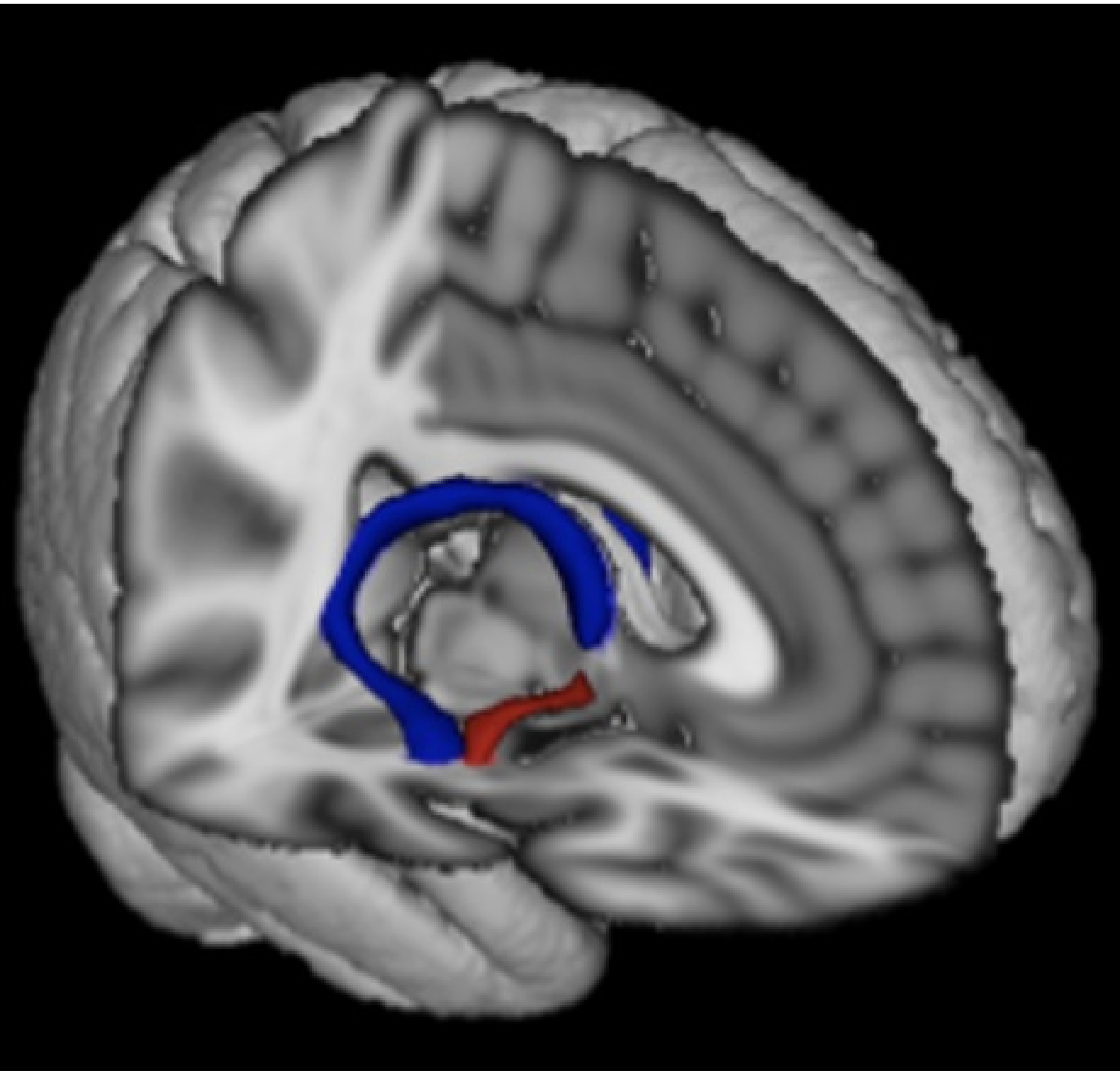


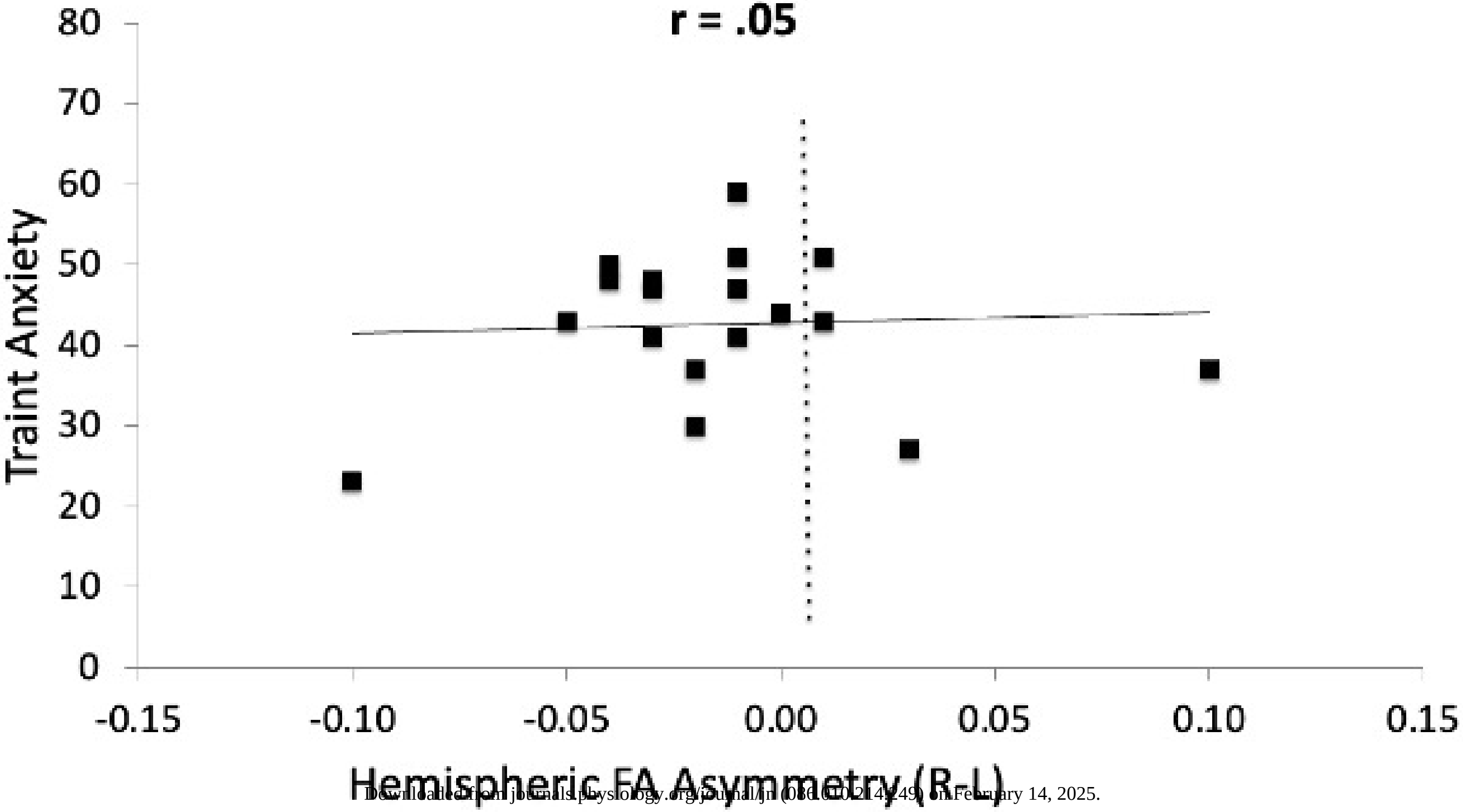
B. Auditory (MGn-amygdala) streamline FA asymmetry

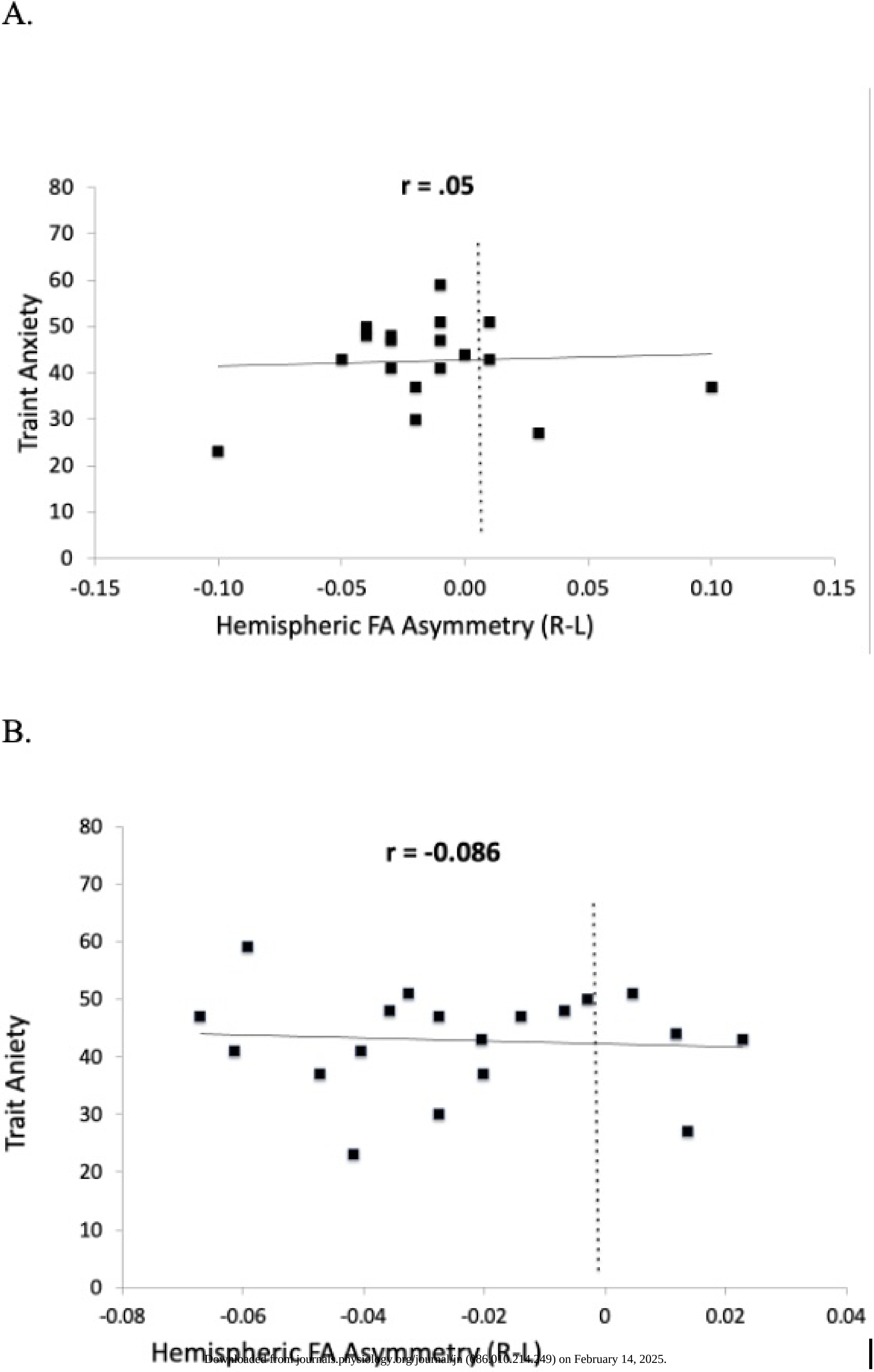


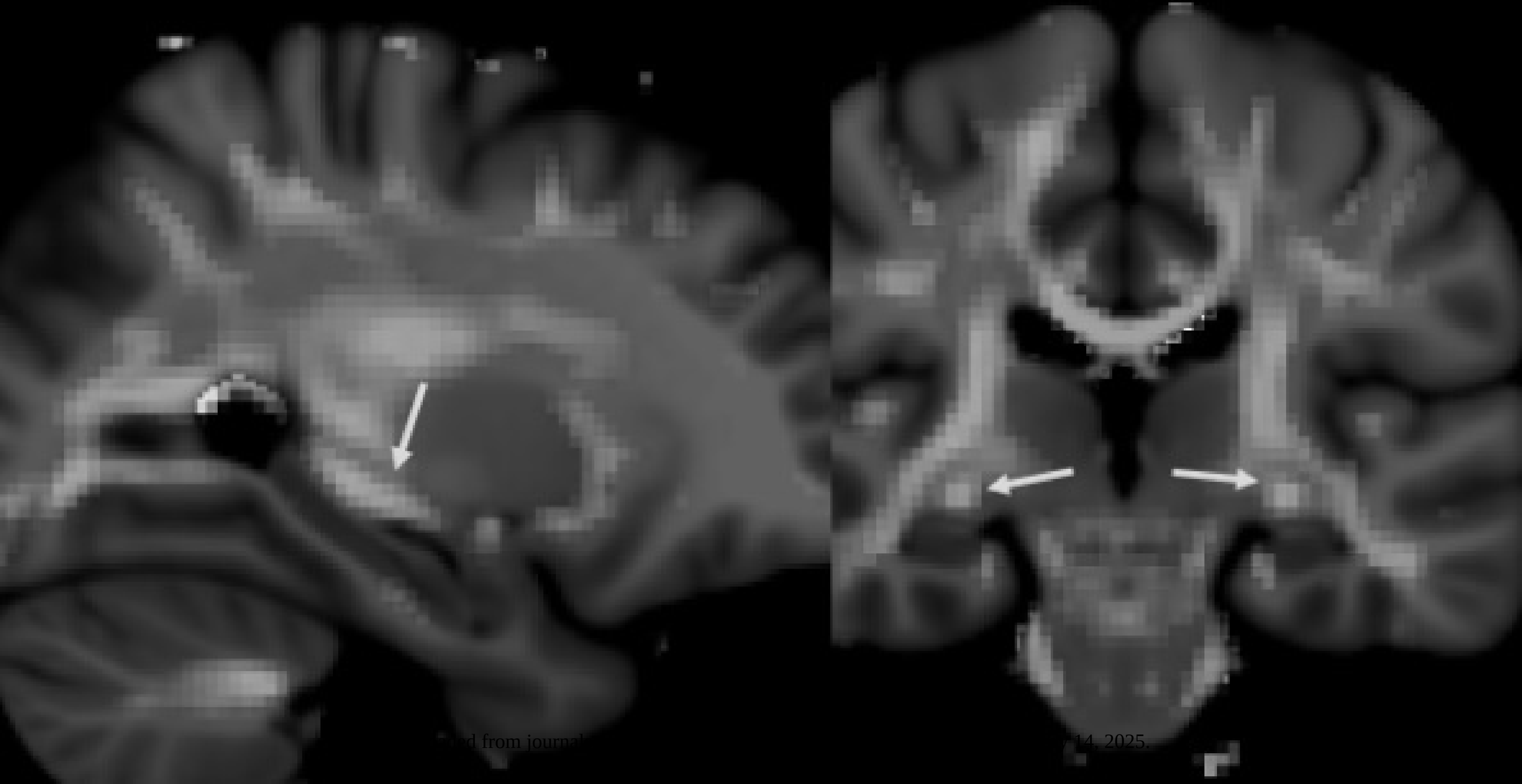







r = .05
80
70
60
50
40
30
20
10
0
Traint Anxiety
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
Hemispheric FA Asymmetry (R-L)


A.
r = .05
Traint Anxiety
80
70
60
50
40
30
20
10
0
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
Hemispheric FA Asymmetry (R-L)
B.
r = -0.086
Trait Aniety
80
70
60
50
40
30
20
10
0
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
Hemispheric FA Asymmetry (R-L)



Streamline	r	p
SC- thalamus-amygdala		
Right hemisphere	0.349	n.s.
Left hemisphere	-0.148	n.s.
MGn – amygdala		
Right hemisphere	0.009	n.s.
Left hemisphere	0.068	n.s
Stria terminalis		
Right hemisphere	0.086	n.s.
Left hemisphere	0.037	n.s.
Ventral amygdalofugal pathway		
Right hemisphere	0.1659	n.s.
Left hemisphere	0.1383.	n.s.

Table 1: Pearson correlation coefficients between the mean FA of each streamline and trait anxiety.

	Mean FA predicts visual threat orienting bias	Hemispheric FA asymmetry	Hemispheric FA asymmetry predicts trait anxiety
SC-thalamus-amygdala		Yes	Yes
Right	Yes		
Left	No		
MGn-amygdala		No	No
Right	No		
Left	No		
Stria terminalis		No	No
Right	Yes		
Left	Yes		
Ventral amygdalofugal		Yes	No
Right	No		
Left	No		

Table 2: Summary of key findings for amygdalo-petal and amygdalo-fugal pathways.