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AMYGDALAR AND HIPPOCAMPAL CONNECTIONS WITH BRAINSTEM AND SPINAL CORD: A DIFFUSION MRI STUDY IN HUMAN BRAIN

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Abstract—The limbic system has a central role for the integration of several cognitive and visceral functions through an extended network of connections involving the hippocampus and the amygdala. A number of studies performed in humans have been dedicated to the investigation of supratentorial limbic pathways by means of non-invasive MRI approaches, such as DTI. However, detection of possible limbic connections involving the brainstem and the spinal cord is still missing. Subtentorial limbic pathways have been previously studied in animals by means of invasive approaches, including viral tracing. The detection of limbic connections with the brainstem and the spinal cord has raised several new hypotheses regarding the interaction between the central nervous system and the periphery of the body. We investigated subtentorial limbic connections in twenty-one healthy humans by means of probabilistic constrained spherical deconvolution tractography. Our connectivity analysis showed, for both the hippocampus and the amygdala, a high probability of connections with the midbrain, pons, and bulb. Moreover, hippocampal and amygdalar pathways reaching the cervical spinal cord were also detected. Quantitative evaluation of diffusion parameters was also performed. Findings of the present study are in agreement with the literature and provide the first report of possible limbic connections between the brainstem and the spinal cord in human brain. Since these pathways might also have important implications both in physiological and pathological contexts, further studies should be conducted in order to confirm our data as well as to define functional features of these brain connections.

Key words: brainstem, diffusion MRI, limbic system, probabilistic tractography, spinal cord.

INTRODUCTION

The limbic system includes a wide interconnected network of cortical and subcortical structures integrating visceral inputs and emotional states to cognition and behavior. Subcortical components of the limbic system include: the amygdaloid complex, substantia innominata, hippocampal complex, olfactory nuclei, mammillary bodies, ventral striatum (nucleus accumbens), as well as some thalamic nuclei such as anterior, intralaminar, and medial dorsal. Those subcortical components are interconnected through well-known white matter pathways, such as fornix, anterior thalamic projections, uncinate fasciculus, mammillo-thalamic tract, and cingulum (Catani et al., 2013; Arrigo et al., 2014). In addition, a possible cerebellar direct connection with the hippocampus was recently shown (Arrigo et al., 2014).

A possible limbic system functional division includes three distinct networks: (i) hippocampal-diencephalic and parahippocampal-retrosplenial network, (ii) temporal-amygdala-orbitofrontal network, and (iii) medial default network (Catani et al., 2013). The hippocampus is conventionally related to the elaboration of inputs for subsequent memory storage (Dickerson and Eichenbaum, 2010), while the amygdala is mainly associated with emotional processing and motivation (Morris et al., 1996; Cardinal et al., 2002). Both the hippocampus and the amygdala perform their activities through functional interactions with other supratentorial brain structures. Interestingly, studies performed in animals have suggested the existence of a parallel sub-tentorial circuitry also involving direct spinal projections, the pathways of which were indeed proposed to be involved in pain transmission (Willis, 2007). Moreover, limbic connections with brainstem regions were also shown. In a tracing study performed in rats, Castle and colleagues (2005) demonstrated that the hippocampus is connected with the nucleus tractus solitaries (NTS). These latter connections were proposed to conduct visceral information to the limbic system through a complex multi-synaptic network including fibers coming from other regions, e.g. locus coeruleus (Loy et al., 1980). Interestingly, those studies performed on rats provided the anatomical basis to support the hypothesis that visceral activity may influence memory and learning processes (Berntson et al., 2003).

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Abbreviations: COV, coefficient of variation; DTI, diffusion tensor imaging; FA, Fractional Anisotropy; LTP, long term potentiation phenomena; MNI, Montreal Neurological Institute.

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In humans, a fMRI study showed involvement of brainstem–limbic pathways in the establishment of an alarm system addressed to fear signal transmission (Liddell et al., 2005). Although the above-mentioned studies have pointed out a close hippocampal and amygdalar relationships with the brainstem, to the best of our knowledge, a clear structural substrate for the brainstem–limbic interaction has not been revealed yet in humans.

The main purpose of the present study was to investigate, on a cohort of healthy subjects, the hippocampal and amygdalar connections with the brainstem and the spinal cord by means of probabilistic constrained spherical deconvolution (CSD) diffusion model (Tournier et al., 2007). Diffusion MRI-based tractography was indeed shown to allow a non-invasive virtual dissection of human brain white matter *in vivo* (Catani et al., 2002), thus providing a powerful tool for investigating white matter pathways in the human brain. CSD diffusion model is known to overcome some of the pitfalls characterizing diffusion tensor imaging (DTI) output (Farquharson et al., 2013). All neurophysiological implications are extensively discussed.

EXPERIMENTAL PROCEDURES

Twenty-two healthy subjects (11 males, 11 females; age range 30–45 years; mean age 36.4 ± 3.7) without any neurological disease were recruited. All subjects read and signed an informed consent before examination. The entire study was approved by Institutional Review Board of IRCCS Bonino Pulejo – Messina – Italy (Scientific Institute for Research, Hospitalization and Health Care). One female subject was excluded for the persistence of image artifacts in her MR acquisition; thus, analyses were performed on twenty-one subjects (11 males, 10 females; age range 30–45 years; mean age 36.1 ± 3.8). Participants were excluded based on the following criteria: any history of neurological disease, traumatic brain injuries, systemic diseases (e.g. diabetes and hypertension).

MRI protocol and preprocessing

The entire MRI protocol was performed on a 3 T MR scanner (Achieva, Philips healthcare, Best, The Netherlands), with a 32-channel SENSE head coil.

For each subject MRI protocol included:

- An anatomical volume (T1-weighted 3D high-resolution Fast Field Echo sequence): TR 25 ms, TE 4.6 ms, flip angle 30 degrees, FOV 240 × 240 mm^2, voxel size 1 × 1 × 1 mm;
- DWI volume (dual phase encoded pulsed gradient spin echo sequence): b-value 1500 s/mm^2, 64 gradient diffusion directions, 1 diffusion unweighted b0 volume, TR 11884 ms, TE 54 ms, FOV 240 × 240 mm^2, voxel size 2 × 2 × 2 mm.

DWI motion and susceptibility distortion artifacts were corrected using a diffusion toolkit available in SPM8 software package (www.fil.ion.ucl.ac.uk/spm); to this end, a full affine transformation was carried out. At the end of pre-processing, gradient directions were updated to account for rotational part of transformations. T1 images were then co-registered to preprocessed DWIs following a scheme outlined in (Besson et al., 2014): first of all b0 and T1 images were segmented in order to extract their CSF masks using SPM8 "New Segment" toolbox; CSF mask coming from b0 segmentation output were then up-sampled at the same resolution of T1-based ones; FLIRT and FNIRT FSL commands (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) were subsequently used to warp T1-based CSF components to CSF b0-based ones; estimated warping fields were eventually applied to structural scans.

It is known that, even after pre-processing, residual distortions may cause a suboptimal alignment between diffusion and structural spaces, especially at air–tissue boundaries. Therefore a non-linear registration procedure was preferred in order to provide a high alignment between T1 images and DWIs.

Prior to running tractography, we detected the right and left hippocampi and amygdalae, midbrain, pons, bulb and cervical portion of spinal cord (C1–C2) in each subject. For this step we adopted Jülich histological atlas (Eickhoff et al., 2005), following this procedure: co-registered T1s were normalized to Montreal Neurological Institute (MNI) stereotactic space using FLIRT and FNIRT utilities; normalized hippocampi and amygdalae were mapped back to native spaces by inverting warping previously estimated; ROIs were refined by removing voxels classified as being part of WM or CSF. These latter masks were obtained by means of New Segment SPM8 tool; an expert radiologist (M.G.) inspected all ROIs at the end of the automatic procedure to further minimize possible misleading ROI depiction; the brainstem and spinal ROIs were instead manually segmented by the same radiologist.

Tractographic analysis

Probabilistic CSD-based tractography was performed by means of MRtrix software package (Tournier et al., 2012) (http://jdtournier.github.io/mrtrix-0.2/index.html). The following reconstruction parameters were used: degree of spherical harmonics eight, radius of curvature 1 mm, step size 0.2 mm, minimal fODF amplitude 0.15, the latter approximately corresponding to a Fractional Anisotropy (FA) threshold of 0.3. The choice of such cutoff was driven by a study comparing *in vivo* DTI tractography with histological analysis (Dauguet et al., 2007), where a FA value of 0.25 was demonstrated to robustly depict WM pathways and minimize false-positive tracts. For each subject, whole-brain tractography has been performed by generating ten millions streamlines and using WM masks both as seed and mask regions. A dilatation step was performed on WM masks to allow streamlines to reach WM–GM interface and thus making possible their assignments to a specific path connecting any two target GM regions.

Connection density calculation and quantification

Connectivity measures were obtained using in-house scripts built with MATLAB software package