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Diffusion tensor imaging segments the human amygdala *in vivo*

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ABSTRACT

The amygdala plays an important role in emotion, learning, and memory. It would be highly advantageous to understand more precisely its internal structure and connectivity for individual human subjects *in vivo*. Earlier cytoarchitectural research in post-mortem human and animal brains has revealed multiple subdivisions and connectivity patterns, probably related to different functions. With standard magnetic resonance imaging (MRI) techniques, however, the amygdala appears as an undifferentiated area of grey matter. Using high-quality diffusion tensor imaging (DTI) at 3 Tesla, we show diffusion anisotropy in this grey matter area. Such data allowed us to subdivide the amygdala for the first time *in vivo*. In 15 living subjects, we applied a spectral clustering algorithm to the principal diffusion direction in each amygdala voxel and found a consistent subdivision of the amygdala into a medial and a lateral region. The topography of these regions is in good agreement with the fibre architecture visible in myelin-stained sections through the amygdala of a human post-mortem brain. From these *in vivo* results we derived a probabilistic map of amygdalar fibre orientations. This segmentation technique has important implications for functional studies in the processing of emotions, cognitive function, and psychiatric disorders and in studying morphometry and volumetry of amygdala subdivisions.

Introduction

The amygdala is a large grey matter complex in the dorsomedial sector of the temporal lobe, where it forms part of the rostromedial and rostrodorsal walls of the temporal horn of the lateral ventricle. Experimental findings from laboratory animals and clinical data from humans show that the amygdala plays a key role in emotion, motivation, learning, and memory (Aggleton, 1992). The amygdaloid complex determines the emotional, motivational, and social significance of complex sensory inputs and initiates appropriate neuroendocrine, autonomic, and behavioural responses (Nieuwenhuys et al., 2008; Schirmer et al., 2008). This considerable functional diversity is also reflected on a structural basis. Initially parcellated into more than 30 different cyto- and myeloarchitectonic entities (Brockhaus 1938), there is now widespread agreement that the amygdaloid complex is subdivided into three groups of nuclei (in medio-lateral orientation): (1) the corticomedial group with the cortical and medial nuclei, (2) the central nucleus, and (3) the basolateral group with the accessory basal, basal, and lateral nuclei (Amaral et al., 1992; McDonald 1992; de Olmos 2004; Mai et al., 2008; Nieuwenhuys et al., 2008). These subdivisions are specific elements of various distinct input–output loops. The basolateral group receives visual, auditory, somatosensory, and gustatory inputs, whereas olfactory afferents are mainly restricted to the cortical nucleus. Via the extensive intrinsic connections within the amygdaloid complex (running preferentially in a basolateral-to-dorsomedial direction), these sensory stimuli reach the central and medial nuclei. The extended amygdala (central and medial nuclei, bed nucleus of the stria terminalis, and substantia innominata; cf. Alheid and Heimer 1988) is the main output channel of the amygdaloid complex and projects to many hypothalamic and brain stem areas thus generating endocrine, autonomic, and somatomotor aspects of emotional and motivational states (Heimer et al., 1997; Nieuwenhuys et al., 2008).

Considering the ever-increasing spatial resolution of functional imaging studies, it would be a challenge to disentangle the specific functions of the individual amygdaloid nuclei, as suggested by the input–output loops described above. Unfortunately, with standard magnetic resonance imaging (MRI) sequences, the amygdala appears as a relatively uniform grey matter region. The individual nuclei can only be delineated by differences in their microanatomical properties (cytoarchitectonics, fibrearchitectonics, chemoarchitectonics; cf. de Olmos 2004) which are still beyond the spatial resolution of MRI.

One way to overcome this dilemma is the generation of cytoarchitectonic probabilistic maps in standard anatomical space. Specific amygdaloid nuclear groups are defined cytoarchitectonically in cell-stained whole brain sections. Their topography is then reconstructed in 3-D and warped to the reference space of the

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Montreal Neurological Institute (MNI) single subject brain. Superimposing the normalized data from ten brains generates a probabilistic map, i.e., a probabilistic description of the spatial variability of each nuclear group in standard MNI space (Amunts et al., 2005). In this format, the maps can then be matched with co-registered functional imaging data (probabilistic microstructural–functional correlation). However, due to the small size of each nuclear group, their close spatial proximity within the amygdala, and inter-individual variability among the ten brains, the population maps overlap to a considerable degree. Only after extensive thresholding (i.e., considering only those voxels of each population map in which four or more out of the ten brains overlap)–and thus discarding of structural information–is it possible to unequivocally assign a given voxel in standard space to a particular population map (Amunts et al., 2005). Furthermore, the invasive and irreversible nature of conventional anatomical studies virtually precludes microstructure and function to be studied in the same brains—such correlations can only be probabilistic in nature.

A new impetus towards mapping microstructure and function in identical brains has come from diffusion tensor imaging (DTI). Providing information about the magnitude and direction of the diffusion of water molecules in the brain and using the orientation of the principal axis of the diffusion tensor (commonly taken as the underlying main fibre direction), this technique has widely been used to map fibre tracts in the white matter both in health and disease (for recent reviews and/or atlases see, e.g., Jellison et al., 2004; Wakana et al., 2004; Mori et al., 2005; Nucifora et al., 2007; Lawes et al., 2008; Assaf and Pasternak 2008; Mori et al., 2008; Van Hecke et al., 2008; Oishi et al., 2008). Finding internal structures within the grey matter is a more challenging task due to its poor directional diffusion properties (i.e., low anisotropy; cf. Jaermann et al., 2008). Higher magnetic field strengths and sophisticated clustering algorithms, however, have, in recent years, set the stage for reproducible and anatomically valid parcellations of the grey matter of the cerebral cortex (Johansen-Berg et al., 2004; Klein et al., 2007; Anwander et al., 2007; Tomassini et al., 2007; Beckmann et al., 2009) and subcortical nuclei, such as the thalamus (Wiegell et al., 2003; Behrens et al., 2003; Johansen-Berg et al., 2005; Devlin et al., 2006) and, more recently, the basal ganglia (Draganski et al., 2008). The amygdala has not been studied so far.

The structural and functional diversity of the amygdaloid complex has prompted us to attempt a DTI-based mapping study of this nuclear region. Specifically we wanted to find out whether the amygdala can be parcellated into subregions which (1) are reproducible across subjects and (2) represent anatomically meaningful entities. If successful, this should be another step forward towards correlating microstructure and function in the same brain.

Materials and methods

Data acquisition

We used data from 15 healthy subjects (7 females and 8 males between the ages of 22–35 years) selected at random from our large in-house MRI database. Subjects were given diffusion-weighted MRI (Turner et al., 1991) and T1-weighted structural scanning on a whole-body 3 Tesla Trio scanner (Siemens, Erlangen, Germany) equipped with an 8-channel head array coil.

Written informed consent was obtained from all participants in accordance with ethical approval from the University of Leipzig. The T1-weighted structural scans were used for skull-stripping, and the brain images were then co-registered into Talairach space (Talairach and Tournoux 1988). Diffusion-weighted images (DWI) were acquired with a twice-refocused spin-echo echo-planar imaging sequence (TE = 100 ms, TR = 12 s, 128 × 128 image matrix, FOV = 220 × 220 mm^2) providing 60 diffusion-encoding gradient directions with a *b*-value of 1000 s/mm^2 (Reese et al., 2003; Weiskopf et al., 2007). Seven images without any diffusion weighting (*b*-value = 0) were obtained at the beginning of the scanning sequence and again after each block of 10 diffusion-weighted images as an anatomical reference for offline motion correction. The interleaved measurement of 72 axial slices with 1.7 mm thickness (no gap) covered the entire brain. Each DWI scan took 13 min. Random noise in the data was reduced by averaging 3 acquisitions, resulting in an total acquisition time of 42 min. Cardiac gating was not utilized, in order to limit the acquisition time. Additionally, fat saturation was employed, together with 6/8 partial Fourier imaging, Hanning window filtering, and parallel generalized autocalibrating partially parallel acquisition (GRAPPA, reduction factor = 2).

The 21 images without diffusion weighting distributed in the whole sequence were used to estimate motion correction parameters using rigid-body transformations (Jenkinson et al., 2002), implemented in FSL (FMRIB Software Library, University of Oxford, 2006, http://www.fmrib.ox.ac.uk/fsl). Motion correction for the 180 diffusion-weighted images was combined with a global registration to the T1 anatomy computed with the same method. The gradient direction for each volume was corrected using the rotation parameters. The registered images were interpolated to the new reference frame with an isotropic voxel resolution of 1 mm and the three corresponding acquisitions and gradient directions were averaged. A diffusion tensor and the fractional anisotropy (FA) were computed from the DWI data of each voxel.

Amygdala segmentation

Automatic segmentation tools, while under active development in several laboratories, are still not fully reliable. The amygdaloid complex is a subcortical structure located deep within the temporal lobe, and several of its boundaries are subtly defined in MR images. We decided to use a more robust manual delineation of the amygdala (Horinek et al., 2007) to exclude extraneous tissue, as far as possible. Individual masks were created for each brain, guided by the 2-mm-thick anatomical sections of human cadaver brains shown in the Duvernoy (1999) atlas. The resulting masks were conservatively small.

Coronal limits were defined from rostral to caudal sections by the gyrus ambiens, lateral ventricle, entorhinal area, semilunar gyrus, anterior perforated substance, and hippocampal formation. The sagittal boundaries were defined, from lateral to medial, by the peduncle of the lentiform nucleus, hippocampus, anterior commissure, temporal gyrus, and optic tract. Finally, boundaries in axial sections from ventral to dorsal were defined by the hippocampus, parahippocampal gyrus, piriform lobe, entorhinal area, gyrus ambiens, temporal horn of the lateral ventricle, and semilunar gyrus.

Amygdala parcellation

All 15 anatomical and DWI data sets were subsequently non-linearly aligned (Thirion 1998) with a single subject template brain, based on the FA contrast of all images, so that the amygdala regions were located in approximately the same position in all transformed data sets. The masks were transformed likewise. We then computed an intersection across all masks so that a single mask resulted which was common to all data sets.

For each voxel, a diffusion tensor was fitted to the spatially normalized DWI data. This tensor characterizes the water mobility for each voxel (Basser 1993). The preferential fibre direction in each voxel is characterized by the principal eigenvector of the diffusion tensor. The angular direction of the diffusion tensors can be conveniently visualized using an RGB colour coding, so that a specific colour represents a particular fibre orientation (Douek et al., 1991).

To group voxels with comparable tissue orientation within the mask of the amygdala, the similarities between two voxels were