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High-resolution magnetic resonance imaging reveals nuclei of the human amygdala: manual segmentation to automatic atlas

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ABSTRACT

The amygdala is composed of multiple nuclei with unique functions and connections in the limbic system and to the rest of the brain. However, standard *in vivo* neuroimaging tools to automatically delineate the amygdala into its multiple nuclei are still rare. By scanning postmortem specimens at high resolution (100–150 μm) at 7 T field strength (n = 10), we were able to visualize and label nine amygdala nuclei (anterior amygdaloid, cortico-amygdaloid transition area; basal, lateral, accessory basal, central, cortical medial, paralaminar nuclei). We created an atlas from these labels using a recently developed atlas building algorithm based on Bayesian inference. This atlas, which will be released as part of FreeSurfer, can be used to automatically segment nine amygdala nuclei from a standard resolution structural MR image. We applied this atlas to two publicly available datasets (ADNI and ABIDE) with standard resolution T1 data, used individual volumetric data of the amygdala nuclei as the measure and found that our atlas i) discriminates between Alzheimer's disease participants and age-matched control participants with 84% accuracy (AUC=0.915), and ii) discriminates between individuals with autism and age-, sex- and IQ-matched neurotypically developed control participants with 59.5% accuracy (AUC=0.59). For both datasets, the new *ex vivo* atlas significantly outperformed (all $p < .05$) estimations of the whole amygdala derived from the segmentation in FreeSurfer 5.1 (ADNI: 75%, ABIDE: 54% accuracy), as well as classification based on whole amygdala volume (using the sum of all amygdala nuclei volumes; ADNI: 81%, ABIDE: 55% accuracy). This new atlas and the segmentation tools that utilize it will provide neuroimaging researchers with the ability to explore the function and connectivity of the human amygdala nuclei with unprecedented detail in healthy adults as well as those with neurodevelopmental and neurodegenerative disorders.

Introduction

The amygdala is composed of heterogeneous nuclei, defined primarily by their distinct cytoarchitecture, neurotransmitters, and connectivity patterns (Alheid, 2003; Freese and Amaral, 2005, 2006, 2009; Price et al., 1987; Aggleton, 2000; Gloor, 1972, 1978, 1997; McDonald, 1998; LeDoux, 1998, De Olmos, 2004; De Olmos and Heimer, 1999). Studies on rodents and non-human primates have advanced our understanding

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of the functions of the individual nuclei. For example, the lateral (La) and basal (Ba) nuclei are engaged in updating current stimulus value associations, primarily through connections with orbitofrontal regions (Baxter and Murray, 2002); the central nucleus (Ce) is believed to mediate behavioral responses to potentially harmful stimuli and fear perception through its connectivity with hypothalamus, basal forebrain, and the brainstem (Kalin et al., 2004; Phillips and LeDoux, 1992). In humans, the amygdala as a whole is thought to play a key role in emotional and social cognitive processes (e.g. Adolphs et al., 2005, Kliemann et al. 2012, Hortensius et al., 2016), and accordingly, its dysfunction is implicated in psychopathologies, such as mood disorders (Phillips et al., 2003; Siegle et al., 2002), anxiety disorders (Birbaumer et al., 1998; Rauch et al., 2003), and developmental disorders (Baron-Cohen et al., 2000; Dziobek et al., 2010). Additionally, several post mortem studies have shown that the amygdala is a common site for neurofibrillary tangles and senile plaques in mild cognitive impairment (Markesbery, 2010) and Alzheimer's disease (Yilmazer-Hanke, 1998) as well as Lewy bodies (Kotzbauer et al., 2011, Fujishiro et al., 2002).

However, the relationship between the structure and function of the distinct nuclei in humans remain largely unknown, both in health and disease. The small size of the amygdala's nuclei has made it difficult to study this structure noninvasively in the living brain using standard neuroimaging resolution. Previous segmentation studies of the amygdala have used either (i) visual approximation based on a single-subject histological atlas (Etkin et al., 2004; dorsal vs. ventral amygdala Dolan, 2002, 2007); (ii) manual segmentations based on *in vivo* neuroimaging; (iii) normalization and application of a probabilistic atlas (Amunts et al., 2005, Solano-Castiella et al., 2011); or (iv) segmentations based on diffusion-weighted imaging. The first two approaches are labor intensive and susceptible to human error. Using the reference space of the MNI single subject, has limited applicability in segmentation for two reasons: first, spatial normalization can lead to inaccuracies due to the fact that the annotations were made on histology, which leads to blurry probability maps; and second, the direct warping of such probability maps to obtain segmentations greatly suffers from registration errors. Additionally, these previous approaches have segmented the amygdala into 2-4 nucleus groups. The use of diffusion-weighted imaging to segment the amygdala has been attractive due to the possibility of automation and within-subject segmentation (rather than normalization to a template). Fiber orientations within the amygdala have been used to divide the structure into two subregions, centromedial and basolateral (Solano-Castiella et al., 2010). However, this method, like others before it, performed analyses on images normalized to a template brain, and were restricted to only two subdivisions. Diffusion connectivity patterns have also been used to delineate each individual's amygdala into four nucleic groups, using nucleus-specific connectivity patterns based on previous animal literature (Saygin et al., 2011; Saygin et al., 2015). While this method offered more nucleic groups (parcellated into 4 groups), the nuclei were dependent on each individual's connectivity patterns, which may be compromised in some patient populations. Thus, a segmentation method independent of connectivity and with finer detail (i.e. nuclei instead of subregions) offers a better understanding of the individual nuclei of the amygdala.

Without an easily accessible technique with which to parcellate the amygdala, it is difficult to elucidate the separate roles of the human amygdala nuclei, as well as the impact of individual differences in nucleus structure and function. Moreover, progress towards mechanistic theories of dysfunction and abnormal development will remain hindered until these structures can be explored *in vivo*.

Here, we use *ex vivo* MRI data from autopsy brains to delineate the amygdala nuclei and build a probabilistic atlas of amygdala anatomy, using a novel algorithm, which will be distributed as part of the FreeSurfer software. Our *ex vivo* imaging protocol yields images with extremely high resolution and signal-to-noise ratio, dramatically higher than is possible *in vivo*, which allows us to accurately identify more nuclei with a segmentation protocol specifically designed for this study. We define nine amygdala nuclei that are major subdivisions in human and animal histology literature (e.g. De Olmos 2004; De Olmos et al., 1999; Gloor et al., 1997; Brockhaus, 1938; Sims and Williams, 1990; Freese and Amaral, 2009; Whalen and Phelps, 2009; LeDoux 1998), and whose boundaries are clearly visible in the *ex vivo* images (see also *Methods*). This segmentation focuses on the main amygdala nuclei in the medial temporal lobe and not the extended amygdala. Our previous work - the *ex vivo* hippocampal atlas (Iglesias et al., 2015) - uses a generative modeling framework to directly segment individual subject *in vivo* MRI data in target space; the resulting segmentation algorithm can be used to analyze standard *in vivo* MRI scans with varying overall image contrast properties and intensity distributions, while producing sharper and more accurate label posterior probabilities than direct registration to a reference space. Here, we use this approach and extend it to the amygdala. We also apply this atlas to two publicly available datasets with standard resolution T1 data, and evaluate how well the resulting amygdala nucleus segmentation volumes can classify i) individuals with Alzheimer's disease and older adult controls and ii) individuals with autism and age-matched controls.

Materials and methods

Autopsy brain samples and ex vivo MRI acquisition

The dataset of *ex vivo* scans comprised 10 autopsied brain hemispheres from the Massachusetts General Hospital Autopsy Service (Massachusetts General Hospital, Boston, MA) and from the Framingham Heart Study and Boston University Alzheimer's Disease Center (Veterans Administration Medical Center, Bedford, MA). Samples consisted of 5 right and 5 left hemispheres (or blocks encompassing the amygdala) of 10 cases (9 without any neurological conditions, 1 with mild AD). Table 1 lists the subject-specific demographic information. In short, subjects were on average 68 years old at

Table 1
Basic demographics and diagnostic information about brain samples used in this study. *Abbreviations*: AD, Alzheimer's disease; h, hours, m, male; f, female; *PMI*, post-mortem interval; *n/a*, data not available.

Case #	Sex	Age	Laterality	Isotropic Resolution (μm)	Clinical Diagnosis	Neuropathology Diagnosis	PMI
1	n/a	n/a	left	150	control	control	< 24 h
2	m	60	right	100	control	control	< 24 h
3	f	86	left	100	mild AD	mild AD	18 h
4	m	68	right	100	control	control	< 24 h
5	m	n/a	left	120	control	control	< 24 h
6	f	83	left	120	control	control	6 h
7	m	63	left	120	control	control	< 24 h
8	m	60	right	100	control	control	14 h
9	m	68	right	100	control	control	< 24 h
10	m	58	right	100	control	control	< 24 h