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Resting state connectivity of the human habenula at ultra-high field

Salvatore Torrisi[a,*], Camilla L. Nord[b], Nicholas L. Balderston[a], Jonathan P. Roiser[b], Christian Grillon[a], Monique Ernst[a]

[a] Section on the Neurobiology of Fear and Anxiety, National Institute of Mental Health, Bethesda, MD, United States
[b] Neuroscience and Cognitive Neuropsychiatry group, University of College, London, UK

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ABSTRACT

The habenula, a portion of the epithalamus, is implicated in the pathophysiology of depression, anxiety and addiction disorders. Its small size and connection to other small regions prevent standard human imaging from delineating its structure and connectivity with confidence. Resting state functional connectivity is an established method for mapping connections across the brain from a seed region of interest. The present study takes advantage of 7 T fMRI to map, for the first time, the habenula resting state network with very high spatial resolution in 32 healthy human participants. Results show novel functional connections in humans, including functional connectivity with the septum and bed nucleus of the stria terminalis (BNST). Results also show many habenula connections previously described only in animal research, such as with the nucleus basalis of Meynert, dorsal raphe, ventral tegmental area (VTA), and periaqueductal grey (PAG). Connectivity with caudate, thalamus and cortical regions such as the anterior cingulate, retrosplenial cortex and auditory cortex are also reported. This work, which demonstrates the power of ultra-high field for mapping human functional connections, is a valuable step toward elucidating subcortical and cortical regions of the habenula network.

Introduction

The habenula is an evolutionarily-conserved brain structure important for emotion and reward modulation (Aizawa et al., 2011). It has attracted attention from researchers for its putative role in negative affective states, including depression (Lawson et al., 2016), anxiety (Hikosaka, 2010), addiction (Velasquez et al., 2014), and pain (Shelton et al., 2012). Because of its small size, however, it is difficult to examine this structure in humans. The current study is the first to address this limitation through the use of ultra-high resolution 7 T imaging and resting state functional connectivity. The sensitivity of this approach makes it possible to build an accurate connectivity map of this structure. Such a map can provide a benchmark that permits comparison with animal work, drawing hypotheses about evolutionary changes across species, and a clearer understanding of putative functions of the habenula.

The habenula, a part of the epithalamus near the posterior commissure, is a connecting link among basal forebrain, striatal and midbrain regions (Hikosaka, 2010). It comprises a medial and a lateral portion with overlapping but distinct projections. Two afferent paths have been identified in the medial portion in rodents. One originates from septal nuclei and the nucleus basalis of Meynert via the stria medullaris. The second originates from the periaqueductal gray and raphe nuclei via the fasciculus retroflexus. The major efferent connections from the medial habenula project to much the same targets, as well as the lateral hypothalamus and ventral tegmental areas (Sutherland, 1982). The lateral habenula receives a number of forebrain afferents, including the diagonal band of Broca, globus pallidus and hypothalamus, and projects to multiple regions including the rostromedial tegmental nucleus (RMTg), ventral tegmental area, ventral striatum, substantia inominata, dorsomedial nucleus of the thalamus, raphe nuclei and periaqueductal gray (Sutherland, 1982).

The aforementioned subcortical connectivity is consistent with the notion that the habenula plays a role in multiple processes, including reward and stress (Hikosaka, 2010). For example, single cell recording studies in rodents and primates reveal that the lateral habenula responds to prediction error and negative reward (Pobbe and Zangrossi, 2008; Proulx et al., 2014). Likewise, the lateral habenula's connection with the raphe nuclei suggests a role of this structure in modulating the serotoninergic system (Roiser et al., 2009; Shabel et al., 2012; Zhao et al., 2015).

Due to its influence on monoaminergic systems, the habenula has been implicated in two major classes of psychiatric disorders: depression and anxiety. Indirect evidence for the role of the habenula in depression comes from studies that implicate this structure in models of learned helplessness in animals (Gass et al., 2014; Mirrione, 2014), and during encoding of negative motivational values in humans (Lawson et al., 2014). Recent work in humans has demonstrated

* Corresponding author.

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abnormal habenula responses during primary aversive conditioning in depression (Lawson et al., 2016), strengthening a previous case report describing remission from intractable depression following deep brain stimulation of the habenula (Sartorius et al., 2010). Anatomically, the habenula has also been found to be larger in individuals with more severe depression (Schmidt et al., 2016). Evidence from animal research suggests that the habenula is an important component of stress and anxiety circuits as well (Mathuru and Jesuthasan, 2013; Pobbe and Zangrossi, 2008; Viswanath et al., 2013; Yamaguchi et al., 2013).

Collectively, these data highlight the potential importance of the habenula in psychopathology, but this notion requires a better understanding of its functional circuitry. One strategy to address this question in humans is through the functional connectivity of endogenous, low-frequency fluctuations of fMRI blood-oxygen-level dependent (BOLD) signal, an established method of mapping functional networks (Fox and Raichle, 2007). Other researchers have already begun mapping the habenula network in this manner, using standard 3 T field strength, which suffers from limited signal to noise ratio. These studies have revealed a number of connections. For example, one study reported resting state habenula connectivity with the ventral tegmental area (VTA), thalamus and sensorimotor cortex, as well as connectivity differences between subjects with low versus high subclinical depression (Ely et al., 2016). Another study acquired cardiac-gated functional images, revealing connectivity to subcortical areas such as the VTA and periaqueductal grey (PAG) (Hétu et al., 2016). The authors of these studies suggest that some of these findings should be considered with caution, particularly considering the small size of the habenula and some of its identified targets. Nonetheless, these studies represent an important starting point for the evaluation of habenula connectivity in humans, which can be more clearly identified using ultra-high field fMRI.

Ultra-high field (≥7 T [7 T]) fMRI has a greatly improved BOLD signal-to-noise ratio compared to 3 T scanners (van der Zwaag et al., 2009), and enables the acquisition of finer spatial resolution. As with our previous study of the bed nucleus of the stria terminalis (BNST) at 7 T (Torrisi et al., 2015), we hypothesized that we would recover much of the habenula functional network known from animal studies, and further uncover connectivity unique to humans. These mappings will pave the way for more targeted testing of habenula function by using cognitive and emotional tasks.

Materials and methods

Subjects

Thirty-four right-handed, healthy volunteers from a mixed urban and suburban population were recruited through internet advertisements, flyers, and print advertisements, and were compensated for their time. This sample was an extension of a previous study (Torrisi et al., 2015). Exclusion criteria included: (a) current or past Axis I psychiatric disorder as assessed by SCID-I/NP (First et al., 2007), (b) first degree relative with a known psychotic disorder, (c) brain abnormality on MRI as assessed by a radiologist, (**d**) positive toxicology screen, (**e**) MRI contraindication, or (**f**) excessive head motion during the functional scans. Depressive symptoms were assessed with the Beck Depression Inventory (Beck et al., 1961). Excessive head motion was defined as more than 15% of a subject's images censored, where the criterion for censoring was a Euclidean norm motion derivative greater than 0.3 mm for temporally adjacent time points. Two subjects were removed for this reason, yielding an N=32 for the study (16 females, mean (SD) age = 27.57 (5.8)). Subjects showed no evidence of depressive symptoms (mean (SD) Beck Depression Inventory scores = 0.9 (1.3)). Written informed consent was obtained from subjects, approved by the National Institute of Mental Health (NIMH) Combined Neuroscience Institutional Review Board.

Functional image acquisition

Data acquisition was identical to our previous work on the BNST (Torrisi et al., 2015) and is described here briefly. Images were acquired on a 7 T Siemens Magnetom MRI with a 32-channel head coil. Third-order shimming was implemented to correct for magnetic inhomogeneities (Pan et al., 2011). We collected a high-resolution, 0.7mm isotropic, T1-weighted MPRAGE anatomical image. The functional images had 1.3 mm isotropic voxels, an interleaved TR of 2.5 s, and 240 images collected over a 10-min acquisition. Our EPI field of view (FOV) covered ~2/3 of the brain (Supplemental Fig. 1). Participants were instructed to keep their eyes open and look at a white fixation cross on a black background.

Physiological measures

To clean the fMRI data for physiological signals of non-interest, respiration was measured with a pneumatic belt placed around the stomach and cardiac rhythm with a pulse oximeter around the index finger. Data were sampled at 500 hz using a BioPac MP150 system (www.biopac.com).

Habenula definition

One rater (author CN) separately drew left and right habenulae on the subjects' anatomical images in AC-PC-aligned native space, as outlined in a validated protocol (Lawson et al., 2013). The drawing process, performed in AFNI (Cox, 1996), was also informed by verifying anatomical landmarks using a detailed atlas (Mai et al., 2015). Briefly, the protocol involved distinguishing the habenula from adjacent cerebral spinal fluid, posterior commissure, medial thalamus and stria medullaris. Following manual tracing of the habenula, the volumes of the left and right habenulae for each subject were computed separately. Because of reports of structural and functional laterality differences (Ahumada-Galleguillos et al., 2016; Bianco and Wilson, 2009; Hétu et al., 2016), left and right habenula volumes were compared using a paired *t*-test.

Preprocessing

Preprocessing and analysis proceeded identically to our previous work with the BNST (Torrisi et al., 2015) and is described briefly. Tissues were segmented for each individual with FreeSurfer (Fischl et al., 2002). Resting state preprocessing and analyses were then performed within AFNI. Subjects' first three functional volumes were removed to allow for scanner equilibrium and the remaining functional volumes were slice-time and motion corrected, and coregistered to their structural image. Subjects' anatomy was non-linearly normalized to a skull-stripped *ICBM 2009b Nonlinear Asymmetric* template in MNI space using *3dQwarp* (Cox and Glen, 2013). The resulting transformation parameters were then applied to the hand-drawn habenulae, FreeSurfer segmentations and functional data. A group average of the normalized habenulae was created to check alignment (supplemental Fig. 2). Functional images were smoothed with a 2.6 mm FWHM Gaussian kernel.

A number of time series were then modeled as covariates of non-interest and regressed out to leave residuals with which voxel-wise correlations were performed. Regressors of no interest included: 0.01–0.1 hz bandpass filter regressors; 6 head motion parameters and their 6 derivatives; 13 slice-based cardiac (RETROICOR) and respiration volume per unit time (RVT) measures (Birn et al., 2008; Glover et al., 2000) and two time series from lateral and 3rd+4th ventricle masks. Finally, the regressions were performed at each gray matter voxel within a 13 mm radius sphere which took into account local white matter (ANATICOR method), which controls for both signal heterogeneity and hardware-related artifacts (Jo et al., 2010).