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Technical Note

Defining the habenula in human neuroimaging studies

Rebecca P. Lawson [a,*], Wayne C. Drevets [b], Jonathan P. Roiser [a]

[a] UCL Institute of Cognitive Neuroscience, 17 Queen Square, London, UK
[b] Laureate Institute for Brain Research and Department of Psychiatry, University of Oklahoma College of Medicine, Tulsa, OK, USA

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ABSTRACT

Recently there has been renewed interest in the habenula; a pair of small, highly evolutionarily conserved epithalamic nuclei adjacent to the medial dorsal (MD) nucleus of the thalamus. The habenula has been implicated in a range of behaviours including sleep, stress and pain, and studies in non-human primates have suggested a potentially important role in reinforcement processing, putatively via its effects on monoaminergic neurotransmission. Over the last decade, an increasing number of neuroimaging studies have reported functional responses in the human habenula using functional magnetic resonance imaging (fMRI). However, standard fMRI analysis approaches face several challenges in isolating signal from this structure because of its relatively small size, around 30 mm^3 in volume. In this paper we offer a set of guidelines for locating and manually tracing the habenula in humans using high-resolution T1-weighted structural images. We also offer recommendations for appropriate pre-processing and analysis of high-resolution functional magnetic resonance imaging (fMRI) data such that signal from the habenula can be accurately resolved from that in surrounding structures.

Introduction

The habenula comprises a pair of small nuclei adjacent to the posterior end of the medial dorsal (MD) thalamus and in many vertebrates can be divided into medial (MHb) and lateral (LHb) portions (Andres et al., 1999). The dorsal conduction stream provides largely distinct pathways relayed by the MHb and LHb (Bianco and Wilson, 2009). The MHb primarily receives inputs from the septum and projects to the interpeduncular nucleus (IPN). The globus pallidus internus, lateral septo-hypothalamic continuum and suprachiasmatic nucleus primarily, but not exclusively, provide the subcortical inputs to the LHb (Bianco and Wilson, 2009) whilst its efferent projections include inhibitory neurons in the rostromedial tegmental nucleus (rMTG), which synapse onto dopamine neurons in the ventral tegmental area and the substantia nigra (Jhou et al., 2009). The LHb also has substantial reciprocal anatomical connections with serotonergic neurons in the median and dorsal raphe nuclei (Herkenham, 1979) and in rats receives a direct cortical projection from prelimbic frontal cortex (Beckstead, 1979).

This unique position, as a hub between corticolimbic and midbrain monoaminergic regions, could allow positively or negatively valenced states or stimuli to modulate motor output, consistent with the hypothesis that the habenula plays a critical role in the motivational aspects of reinforcement learning and decision-making, as extensively reviewed elsewhere (Hikosaka et al., 2008; Hikosaka, 2010). The habenula responds to primary aversive stimuli (Matsumoto and Hikosaka, 2009), cues that predict aversive stimuli (Matsumoto and Hikosaka, 2007), and appetitive stimuli that are less rewarding than predicted (Matsumoto and Hikosaka, 2009). Its ability to inhibit dopamine neuron firing, via the rMTG, has been established in rodents (Ji and Shepard, 2007) and non-human primates (Matsumoto and Hikosaka, 2007). Furthermore, evidence from rodent models of depression suggests a role for the habenula in learned helplessness behaviour (Caldecott-Hazard et al., 1988; Shumake and Gonzalez-Lima, 2003), leading to the hypothesis that habenula dysfunction may play an important role in major depression (Morris et al., 1999; Sartorius et al., 2010).

Studies of the habenula in humans

Advances in high-resolution T1-weighted image acquisition have been exploited in two recent structural MRI studies of the habenula in humans. One study comparing habenula volume between groups of healthy volunteers, unipolar and bipolar depressed populations acquired T1-weighted structural images with a resolution of 550 μm isotropic, and demonstrated that habenula volume is decreased in unmedicated, depressed bipolar patients and unipolar females, relative to controls (Savitz et al., 2011a). Another high-resolution structural study of habenula volume found no significant difference between healthy controls and individuals with post-traumatic stress disorder (Savitz et al., 2011b), consistent with post-mortem data suggesting that altered habenula volume may be relatively specific to depression (Ranft et al., 2010).

* Corresponding author at: Institute of Cognitive Neuroscience, University College London, 17 Queen Square, London, WC1N 3AR, UK. Fax: +44 20 7813 2835.
E-mail address: rebecca.lawson@ucl.ac.uk (R.P. Lawson).

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A handful of fMRI studies have reported habenula blood oxygen-level-dependent (BOLD) responses to making errors and receiving negative feedback on a range of different tasks (Ide and Li, 2011; Li et al., 2008; Noonan et al., 2011; Ullsperger and von Cramon, 2003) and also recently to noxious heat stimulation (Shelton et al., 2012). These studies used standard fMRI acquisition protocols, with images of slice thickness 3–5 mm and pre-processing steps that included spatial normalisation to the Montreal Neurological Institute (MNI) brain template and the application of smoothing kernels between 5 and 10 mm full-width at half-maximum (FWHM) prior to group-level voxel-wise analysis. One BOLD fMRI study used a different approach (Salas et al., 2010), acquiring higher resolution images (2 mm isotropic) and manually co-registering functional and structural images without applying smoothing. A cubic 6 mm region of interest (ROI) was then placed manually in the vicinity of the left and right habenula for each subject, and for subsequent analysis habenula voxels were defined as those correlating negatively with BOLD signal in the ventral striatum during a separate fMRI time series. Using a fixed-effects analysis this study reported habenula responses to omission of expected reward. Finally, one study used arterial spin labelling to measure habenula perfusion during the resting-state in remitted depressed patients and controls following acute tryptophan depletion (Roiser et al., 2009). This study acquired images of 4 mm slice thickness, outlining the habenula manually for each subject on T1-weighted structural images prior to automated co-registration between structural and functional images following global normalisation.

Challenges to analysing the habenula in human fMRI

There exist a number of challenges in identifying responses from the human habenula using fMRI. Post-mortem estimates of total (medial plus lateral) habenula volume in humans are ~31 mm^3 on the left and ~33 mm^3 on the right (Ranft et al., 2010) (though these estimates do not account for the expected post-mortem brain shrinkage: Stockmeier et al., 2004). This volume approximates the size of a single image voxel acquired in standard fMRI protocols. Moreover, existing data from structural MRI scans reported combined habenula volumes ~30–36 mm^3 (Savitz et al., 2011a, 2011b) suggesting that in each hemisphere the habenula may be even smaller than the standard functional MRI voxel size.

Voxel-wise approaches to fMRI analysis across subjects typically employ several pre-processing steps to account for inter-individual variability in brain structure, including spatial normalisation to a standard stereotaxic space and substantial smoothing. Although these steps are advantageous for locating signal from larger structures, typically-employed smoothing kernels are larger than the habenula itself, raising the possibility that signal measured over the stereotaxic atlas coordinates for the habenula may include substantial components that emanate instead from adjacent structures, such as the MD thalamus or the epithalamic paraventricular nucleus.

Recent advances in high-resolution fMRI sequence development permit the acquisition of 1.5 mm isotropic *functional* images on 3 T MRI scanners and have permitted the study of fine grained microstructure and small nuclei such as the periaqueductal grey (PAG) (Mobbs et al., 2007). Here we outline a detailed approach to anatomically define the habenula for human high-resolution MRI studies in conjunction with a stereotactic atlas of the human brain (Mai et al., 2008). Our procedure involves manually reorienting each structural image into close alignment with the atlas to permit accurate habenula definition according to clear anatomical landmarks without normalisation. When the tracings had been made, inter-rater reliability correlation coefficients for volume were calculated for left, right and combined habenula volume. We also provide estimates of habenula position and volume in Montreal Neurological Institute (MNI) space, as well as accompanying reliability statistics. Our analyses suggest that even with the best available spatial normalisation (DARTEL), achieving excellent registration between subjects, variation across individuals in post-normalisation habenula location would still necessitate the employment of smoothing kernels of a similar size to that of the habenula itself for voxel-wise group-level analyses, running the risk of contaminating signal ostensibly measured in this structure from surrounding regions.

Materials and methods

Participants

Twenty-four healthy adults (15 females, mean age = 26 years, standard deviation (SD) = 4.7, range 20–37) participated in this study. All reported no history of neurological or psychiatric illness and were screened for standard MRI contraindications in advance of testing. Participants were compensated for their time and provided written informed consent, as approved by the London Queen Square Research Ethics Committee.

Imaging parameters

High-resolution T1-weighted anatomical images were acquired with a Siemens Magnetom Trio 3 T MRI scanner using a 32-channel phased-array head coil and an optimised 3D MDEFT imaging sequence with correction for B1 inhomogeneities at 3 T (Deichmann, 2006). Image resolution was 770 μm isotropic (matrix size = 304 × 288 × 224, TR = 7.92 ms, TE = 2.48 ms, excitation flip angle = 16°). A single 17-minute scan was acquired for each participant.

Manual reorienting

Anatomical images were viewed and reoriented using Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Each subject's anatomical image was manually reoriented such that the co-ordinates [x = 0, y = 0, z = 0] occupied the midpoint of the anterior commissure (AC) with deviations from the origin now defined relative to the AC, the image was rotated and translated as necessary until the x and z co-ordinates of the midpoint of the posterior commissure (PC) were the same as for the AC (i.e. x = 0, z = 0); in other words the brain was oriented along the AC–PC line passing through the centres of each commissure, after Mai et al., 2008). Finally, the image was viewed in coronal section and rotated as necessary to ensure symmetry across a sagittal plane that included the AC–PC line. With the anatomical images now aligned (but not spatially transformed) to the same reference frame as our stereotactic atlas (Mai et al., 2008), the habenula was defined using anatomical landmarks. We note that MNI space is oriented slightly differently to the Mai atlas (the z co-ordinate of the PC is approximately −4.5 in MNI space) and as such the procedure we describe below may not be accurate for images oriented to MNI space.

Anatomical delineation

The habenula lies immediately dorsal to the PC and anterior to the pineal gland, and occupies approximately the same z-coordinate as the latter structure, around 1–5 mm dorsal to the AC–PC line. Moving anterior approximately 1 mm from the most anterior extent of the pineal stalk in coronal sections, the posterior aspect of the habenula can be seen protruding into the third ventricle on either side of the midsaggital plane (Fig. 1). Due to the extensive white matter plexuses contained within the habenula, this structure's density appears brighter than the adjacent thalamic grey matter on T1-weighted images, aiding its delineation from surrounding grey matter and cerebrospinal fluid (CSF).

For each participant the left and right habenula were segmented using MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/)