NeuroImage 116 (2015) 187–195

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Functional connectivity of the dorsal and median raphe nuclei at rest

Vincent Beliveau [a,b], Claus Svarer [a], Vibe G. Frokjaer [a], Gitte M. Knudsen [a,b], Douglas N. Greve [c,d,1], Patrick M. Fisher [a,*,1]

[a] Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, Rigshospitalet, Copenhagen, Denmark
[b] Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[c] Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Boston, MA, USA
[d] Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Article history:
Received 9 January 2015
Accepted 28 April 2015
Available online 9 May 2015

Keywords:
Serotonin
Resting-state
Functional connectivity
Dorsal raphe
Median raphe

ABSTRACT

Serotonin (5-HT) is a neurotransmitter critically involved in a broad range of brain functions and implicated in the pathophysiology of neuropsychiatric illnesses including major depression, anxiety and sleep disorders. Despite being widely distributed throughout the brain, there is limited knowledge on the contribution of 5-HT to intrinsic brain activity. The dorsal raphe (DR) and median raphe (MR) nuclei are the source of most serotonergic neurons projecting throughout the brain and thus provide a compelling target for a seed-based probe of resting-state activity related to 5-HT. Here we implemented a novel multimodal neuroimaging approach for investigating resting-state functional connectivity (FC) between DR and MR and cortical, subcortical and cerebellar target areas. Using [^{11}C]DASB positron emission tomography (PET) images of the brain serotonin transporter (5-HTT) combined with structural MRI from 49 healthy volunteers, we delineated DR and MR and performed a seed-based resting-state FC analysis. The DR and MR seeds produced largely similar FC maps: significant positive FC with brain regions involved in cognitive and emotion processing including anterior cingulate, amygdala, insula, hippocampus, thalamus, basal ganglia and cerebellum. Significant negative FC was observed within pre- and postcentral gyri for the DR but not for the MR seed. We observed a significant association between DR and MR FC and regional 5-HTT binding. Our results provide evidence for a resting-state network related to DR and MR and comprising regions receiving serotonergic innervation and centrally involved in 5-HT related behaviors including emotion, cognition and reward processing. These findings provide a novel advance in estimating resting-state FC related to 5-HT signaling, which can benefit our understanding of its role in behavior and neuropsychiatric illnesses.

Introduction

The serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system is a critical component in the healthy functioning of the human brain and is involved in many functions such as sleep–wake cycle (Portas et al., 2000), reward (Liu et al., 2014), appetite (Curzon, 1990), emotion (Meneses and Liy-Salmeron, 2012), motor function (Di Matteo et al., 2008) and cognition (Meneses, 1999). Disruptions in the serotonin system have been implicated in a wide spectrum of neuropsychiatric disorders, including major depression disorder (Paul-Savoie et al., 2011), anxiety (Sullivan et al., 2005), bipolar disorder (Mahmood and Silverstone, 2001), chronic stress (Jovanovic et al., 2011), and drug addiction (Müller and Homberg, 2014).

Serotonergic innervation of cerebral cortex, subcortical structures and cerebellum originates for the greater part from the dorsal (DR) and median (MR) raphe nuclei (Dorocic et al., 2014; Hornung, 2003; Jacobs and Azmitia, 1992; Vertes and Linley, 2008). Thus, effects of serotonin signaling on brain function and behavior critically depend on appropriate communication with these nuclei. Despite substantial focus and clear relevance to delineating neurobiological mechanisms associated with various neuropsychiatric illnesses, the effects of serotonin signaling on brain function are not fully understood. Recent studies have reported that serotonin signaling modulates resting-state networks (RSNs) including the commonly studied default mode network (DMN) (Hahn et al., 2012; McCabe and Mishor, 2011). However, these studies have focused on networks modulated by serotonergic input rather than more directly modeling serotonin-related connectivity based on raphe nuclei intrinsic connectivity. The evaluation of the functional connectivity (FC) with DR or MR at rest would provide yet

* Corresponding author at: Copenhagen University Hospital Rigshospitalet, Neurobiology Research Unit, NRU 6931, Rigshospitalet, Blegdamsvej 9, Copenhagen O DK-2100, Denmark. Fax: +45 3545 6713.
E-mail address: patrick@nru.dk (P.M. Fisher).
[1] Both authors share senior authorship equally.

http://dx.doi.org/10.1016/j.neuroimage.2015.04.065
1053-8119/

unreported novel insight into how serotonin signaling shapes intrinsic brain connectivity.

The purpose of this study was to elucidate FC of the DR and MR in the healthy human brain at rest. We used high resolution imaging of the serotonin transporter (5-HTT) with [^{11}C]DASB positron emission tomography (PET), an effective probe of 5-HTT binding in receptor-rich regions (Frankle and Slifstein, 2006), combined with anatomical landmarks from structural magnetic resonance imaging (MRI) to determine subject-specific DR and MR regions of interests (ROIs). These ROIs were then transferred to functional MRI (fMRI) space where we performed seed-based FC to identify areas showing significant resting-state FC with DR and MR. Finally we correlated regional DR and MR FC with regional 5-HTT binding to assess the association between the identified FC maps and serotonin signaling.

Methods

Participants

Data from 63 healthy women were collected at baseline as part of a broader randomized, placebo-controlled and double-blind intervention study. Subjects were scanned before (baseline) and after an intervention. In the current study, the baseline data was used for the main analysis and the placebo intervention data was included only for the test–retest evaluation. The placebo intervention consisted of a single subcutaneous injection of saline into the abdomen approximately 4 weeks prior to rescan. Additional details regarding the overall study design can be found elsewhere (Frokjaer et al., in press). Importantly, all fMRI and PET data were acquired at a fixed time relative to their menstrual cycle phase (follicular), as determined by ovarian ultrasound. The study was registered and approved by the local ethics committee under the protocol number H-2-2010-108. After complete description of the study, written informed consent was obtained from all participants.

Of the 63 participants, 14 were excluded due to excessive motion (detailed below in the fMRI analysis section) during the rs-fMRI scans at baseline. Thus rs-fMRI data from 49 participants (age 24.2 ± 4.7) was available for our analyses. To evaluate the reproducibility of the FC results, we repeated the FC analysis on data from the 20 participants that received placebo (age 25.6 ± 6.2) but were without excessive motion at baseline and rescan, using the seeds defined on the baseline PET images. DR and MR delineation was also performed on baseline and rescan PET images for these 20 participants and the overlap were evaluated.

Data acquisition

Magnetic resonance imaging (MRI)

Participants completed a 10-minute rs-fMRI scan (280 volumes) acquired on a Siemens (Erlangen, Germany) 3 T Verio MR scanner. During rs-fMRI scans, participants were instructed to close their eyes, but not to fall asleep. The participants were asked after the scan whether they fell asleep during the scan; all participants reported not falling asleep. Scans were acquired using a T2*-weighted gradient echo-planar imaging (EPI) sequence sensitive to blood-oxygen level dependent (BOLD) signal (TR = 2.15 s, TE = 26 ms, flip-angle = 78°, in-plane matrix 64 × 64, number of slices = 42, voxel size = 3 × 3 × 3 mm, GRAPPA acceleration factor 2, no gap, interleaved slice order). Pulse and respiratory data were sampled at 50 Hz using the Siemens' Physiological Monitoring Unit.

A high-resolution 3D T1-weighted structural image was acquired using a sagittal, magnetization prepared rapid gradient echo (MP-RAGE) sequence (TE/TR/TI = 2.32/1900/900 ms, flip angle = 9°, in-plane matrix 256 × 256, number of slices = 224, voxel size = 0.9 × 0.9 × 0.9 mm, GRAPPA acceleration factor 2, no gap, acquisition time = 8 min 30 s). A high-resolution 3D T2-weighted image was acquired using a sagittal, Turbo Spin Echo (TSE) scan of the whole head (TE/TR = 409/3200 ms, flip angle = 120°, in-plane matrix 256 × 256, number of slices = 176, voxel size = 1 × 1 × 1 mm, GRAPPA acceleration factor 2, acquisition time = 4 min 43 s).

[^{11}C]DASB positron emission tomography (PET) imaging

[^{11}C]DASB PET list-mode data were acquired with a Siemens ECAT HRRT scanner operating in 3D-acquisition mode, with an approximate in-plane resolution of 2 mm. Scan duration was 90 min and started immediately after bolus injection of 585 ± 34 MBq [^{11}C]DASB. Thirty-six dynamic PET frames (6 × 10 s, 3 × 20 s, 6 × 30 s, 5 × 60 s, 5 × 120 s, 8 × 300 s, 3 × 600 s) were reconstructed using a 3D-OSEM-PSF algorithm (Comtat et al., 2008; Hong et al., 2007; Sureau et al., 2008). Realignment of PET frames was performed using AIR 5.2.5 (Woods et al., 1992) to account for within-scan motion.

5-HTT binding was quantified as [^{11}C]DASB nondisplaceable binding potential (BP_{ND}) values determined with the Multilinear Reference Tissue Model 2 (Ichise et al., 2003) as previously described (Frokjaer et al., 2014). The kinetic modeling was performed using Freesurfer (Greve et al., 2013) with cerebellum gray matter segmentation as reference region and a combined thalamus, caudate, putamen and pallidum region as the high binding region for determining k2′. Surface and volume [^{11}C]DASB BP_{ND} maps were smoothed by 10 and 6 mm full width half maximum (FWHM) Gaussian 2D and 3D filters, respectively.

Anatomical MRI analysis

Structural images was analyzed in FreeSurfer (FS, surfer.nmr.mgh.harvard.edu, version 5.3) (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999a, 1999b; Greve and Fischl, 2009; Ségonne et al., 2007, 2004). The T2-weighted structural images were used to refine the delineation of the pial surfaces. This process creates mesh models of the cortical surfaces and labels cortical and subcortical ROIs customized to each subject. Some of these ROIs were used to help create search spaces for the DR and MR. The cortical surfaces were aligned with a cortical surface atlas using nonlinear surface-based registration (Fischl et al., 1999a). This atlas is the surface-based equivalent to Talairach or MNI space and serves as a space in which voxel-wise group analysis can be performed on the surface. The anatomical volume was also registered to the MNI305 atlas which serves as the group analysis space for volume-based analysis of subcortical structures.

Delineation of DR and MR

Histological studies performed by Baker et al. (1991a, 1991b, 1990) have provided in-depth knowledge of the morphology and location of the DR and the MR in the ex vivo human brain. However, to perform seed-based FC, accurate in vivo segmentation of DR and MR are needed (Kalbitzer and Svarer, 2009). This presents a challenge (Kranz and Hahn, 2012), as the raphe nuclei are composed of sparse neurons surrounded by white matter and they have no well-defined boundaries visible in MRI (Baker et al., 1996, 1991a, 1990).

We have adopted a method similar to Schain et al. (2013) in which liberal search volumes were defined on the structural MRI and then refined using the PET image. The DR lies on the midline of the brainstem and extends from the oculomotor nucleus to the middle of the pons (Baker et al., 1990). It can be subdivided at the level of the isthmus into two groups, a midbrain (B7) group and a pontine (B6) group (Dahlström and Fuxe, 1964) which meet near the inferior opening of the cerebral aqueduct (CA). The B7 group is adjacent to the CA. The B6 group is only about 0.5 mm in radius, well below current scanner resolution for fMRI. For this reason, we focused on the B7 group as the seed region for our analysis. The search volume for the DR was defined from the inferior to the superior limit of the CA and from the anterior boundary of the CA to approximately 6 mm (5 voxels) anterior to that