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Functional connectivity hubs of the mouse brain

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ABSTRACT

Recent advances in functional connectivity methods have made it possible to identify brain hubs — a set of highly connected regions serving as integrators of distributed neuronal activity. The integrative role of hub nodes makes these area points of high vulnerability to dysfunction in brain disorders, and abnormal hub connectivity profiles have been described for several neuropsychiatric disorders. The identification of analogous functional connectivity hubs in preclinical species like the mouse may provide critical insight into the elusive biological underpinnings of these connectional alterations. To spatially locate functional connectivity hubs in the mouse brain, here we applied a fully-weighted network analysis to map whole-brain intrinsic functional connectivity (i.e., the functional connectome) at a high-resolution voxel-scale. Analysis of a large resting-state functional magnetic resonance imaging (rsfMRI) dataset revealed the presence of six distinct functional modules related to known large-scale functional partitions of the brain, including a default-mode network (DMN). Consistent with human studies, highly-connected functional hubs were identified in several sub-regions of the DMN, including the anterior and posterior cingulate and prefrontal cortices, in the thalamus, and in small foci within well-known integrative cortical structures such as the insular and temporal association cortices. According to their integrative role, the identified hubs exhibited mutual preferential interconnections. These findings highlight the presence of evolutionarily-conserved, mutually-interconnected functional hubs in the mouse brain, and may guide future investigations of the biological foundations of aberrant rsfMRI hub connectivity associated with brain pathological states.

Introduction

Resting-state BOLD functional magnetic resonance imaging (rsfMRI) has been widely employed to investigate the intrinsic functional organization of the human brain (Bullmore and Sporns, 2009). Graph theory representations of rsfMRI networks, whereby brain connectivity is conceptualized as a set of nodes (neuronal elements) and edges (their interconnections), have demonstrated that the human brain has topological features recapitulating the defining characteristics of complex networks (Watts and Strogatz, 1998), including the presence of functionally specialised modules encompassing well-characterised neurofunctional systems (Fair et al., 2009; Meunier et al., 2009; Power et al., 2011). In order to account for the brain's ability to simultaneously coordinate multiple network systems and ensure efficient communication, the presence of functional hub nodes serving as integrators of distinct neuronal systems has been hypothesized. Numerous rsfMRI studies have indicated the presence of highly-connected cortical regions as putative functional hubs for the human brain, most of which appear to exhibit overlap with sub-regions of the default mode network (DMN) (Cole et al., 2010; Tomasi and Volkow, 2011; Zuo et al., 2012). Importantly, the integrative role of these hub regions renders them points of potential vulnerability to dysfunction in brain disorders. Consistent with this notion, aberrant rsfMRI connectivity profiles have been described for several hub regions in pathological conditions such as autism, schizophrenia and neurodegenerative disorders (Buckner et al., 2009; van den Heuvel and Sporns, 2013). However, fundamental issues related to the etiopathological and biological foundations of these alterations remain to be addressed. For one, the neurophysiological cellular underpinnings of functional hub derangement observed in neuropsychiatric disorders remain largely unknown. It is also unclear whether these alterations are patho-physiologically relevant, or just epiphenomenal to underlying brain disorders. (See Table 1.)

Functional hub identification in preclinical species like the mouse, where genetic, cellular and molecular underpinnings of several brain disorders can be reproduced in controlled conditions and manipulated with cellular specificity (Deisseroth, 2011), may offer new critical insight into the above-mentioned issues. Initial attempts to unravel the rodent's brain functional topology have been carried out in rats (D'Souza et al., 2014; Liang et al., 2011, 2012) and more recently in mice (Mechling et al., 2014; Stafford et al., 2014). By using independent-component analysis (ICA) decomposition of rsfMRI signals in awake rats, Liang et al. (2011) reported the presence of three large modules, covering cortical areas, prefrontal and limbic hippocampal regions and basal forebrain

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Table 1
List of abbreviations.

Abbreviation	Description
Acb	Nucleus accumbens
Amy	Amygdala
AO	Anterior olfactory nucleus
AON	Anterior olfactory nucleus
BF	Basal forebrain module
CA1/3	CA1/3 fields of hippocampus
Cg	Cingulate cortex
CM	Central medial nucleus
dHc	Dorsal hippocampus
DMN	Default mode network
FrA	Frontal association cortex
Hc	Hippocampus/hippocampal module
Hypo	Hypothalamus
Ins	Insular cortex
LCN	Lateral cortical network
M1/2	Primary/secondary motor cortex
M2	Secondary motor cortex
mPFc	Medial prefrontal cortex
MS	Medial septal nucleus
OFc	Orbitofrontal cortex
P	Pons
PtA	Parietal association cortex
Rs	Retrosplenial cortex
S1/2	Primary/secondary somatosensory cortex
TeA	Temporal association cortex
Thal	Thalamus module
Th	Thalamus
vHc	Ventral hippocampus
VM	Ventral midbrain module
vSub	Ventral subiculum
VTA	Ventral tegmental area

structures, respectively. Using anatomically-defined labels, D'Souza et al. (2014) identified six communities in medetomidine sedates rats, including two purely cortical systems (i.e., frontal and somatosensory) together with four mixed communities involving hippocampal and perihippocampal cortices, basal ganglia, thalamic nuclei and pons. ICA-based decomposition has also been recently applied to mouse rsfMRI datasets acquired under isoflurane anaesthesia (Mechling et al., 2014), leading to the identification of a basal ganglia module plus four other composite communities which included complex combinations of cortical and subcortical systems. Two of the above studies also report attempts to identify inter-connecting hub regions. D'Souza (2014) attributed a putative integrative function to the hippocampus, striatum plus all cortical subdivision, with the sole exception of visual, primary motor and parietal cortices. These latter regions are part of a set of eleven putative hub regions described by Mechling in the mouse brain (2014), which also included somatosensory, frontal as well as subcortical diencephalic structures and the striatum. Collectively, while these initial studies led to the identification of seemingly stable functional partitions, substantial heterogeneity exists in their anatomical composition, as well as in the location of integrative structures, a finding that may reflect discrepant experimental procedures (e.g., anaesthesia, preprocessing procedures) and is probably exacerbated by heterogeneity in the regional parcellation schemes (coarse ICA-based, or anatomical volumes) and network thresholding strategies employed. Moreover, none of the functional partitions described so far can be straightforwardly related to known distributed human networks (e.g., DMN), which is a limiting factor in the translation of preclinical research to human condition.

Employing rigorous control of motion and potential physiological confounds (Ferrari et al., 2012), we recently demonstrated the presence of robust distributed rsfMRI networks in the mouse brain (Zhan et al., 2014), including functional precursors of the human salience and default mode networks (Sforazzini et al., 2014a,b), an observation recently replicated by an independent group (Stafford et al., 2014). Our datasets offer the opportunity to spatially locate functional hubs in the mouse brain and relate them to known network system of the human brain, which greatly enhances the translational value of this approach. To this purpose, here we applied a computationally unbiased, fully-weighted network analysis of rsfMRI connectivity at a voxel scale in a large cohort of adult mice. We show the presence of six large-scale functional partitions, and anatomically localise mutually inter-connected hubs in several sub-regions of the DMN as well as in several cortical association areas of the mouse brain. These bear a strong resemblance to findings in the human brain, suggesting the presence of evolutionarily conserved cortical regions serving as integrators of segregated brain systems in the mouse, and supporting the use of this species to investigate aberrant rsfMRI hub connectivity associated to brain pathological states.

Materials and methods

All in vivo studies were conducted in accordance with the Italian law (DL 116, 1992 Ministero della Sanità, Roma) and the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal research protocols were also reviewed and consented to by the animal care committee of the Istituto Italiano di Tecnologia (permit 07-2012). All surgical procedures were performed under anaesthesia.

Animal preparation

MRI experiments were performed on male 20–24 week old C57BL/6J (B6) mice (n = 41, Charles River, Como, Italy). The animal preparation protocol was recently described in detail (Ferrari et al., 2012; Sforazzini et al., 2014a,b; Zhan et al., 2014). Briefly, mice were anaesthetised with isoflurane (5% induction), intubated and artificially ventilated (2% maintenance). The left femoral artery was cannulated for continuous blood pressure monitoring and blood sampling. At the end of surgery, isoflurane was discontinued and substituted with halothane (0.75%). Functional data acquisition commenced 45 min after isoflurane cessation. Mean arterial blood pressure was recorded throughout the imaging sessions. Arterial blood gases (p_aCO_2 and p_aO_2) were measured at the end of the functional time series to exclude non-physiological conditions. Mean p_aCO_2 and p_aO_2 levels recorded were 20 ± 5 and 257 ± 33 mm Hg, respectively, well within the physiological range.

Image data acquisition

All in vivo experiments were performed using a 7.0 T MRI scanner (Bruker Biospin, Milan). Transmission and reception were achieved using a 72 mm birdcage transmit coil and a custom-built saddle-shaped four-channel solenoid coil for signal reception. Shimming was performed on a 6 mm × 6 mm × 6 mm region, using a FASTMAP protocol. For each session, high-resolution anatomical images were acquired with a fast spin echo sequence (RARE, Hennig et al., 1986) with the following parameters: repetition time (TR)/echo time (TE) 5500/60 ms, matrix 192 × 192, field of view 2 × 2 cm^2, 24 coronal slices, and slice thickness 0.50 mm. Co-centred single-shot BOLD rsfMRI time series were acquired using an echo planar imaging (EPI) sequence with the following parameters: TR/TE 1200/15 ms, flip angle 30°, matrix 100 × 100, field of view 2 × 2 cm^2, 24 coronal slices, slice thickness 0.50 mm, 300 volumes and a total rsfMRI acquisition time of 6 min.

Image data preprocessing

Image preprocessing was carried out using tools from FMRIB Software Library (FSL, v5.0.6; http://fsl.fmrib.ox.ac.uk/fsl/) (Jenkinson et al., 2012) and AFNI (v2011_12_21_1014; http://afni.nimh.nih.gov/afni/). RsfMRI time series were despiked (AFNI/3dDespike), corrected for motion (AFNI/3dvolreg), and spatially normalised to an in-house C57Bl/6J mouse brain template (Sforazzini et al., 2014b) (FSL/FLIRT, 12

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