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THE LOW-FREQUENCY BLOOD OXYGENATION LEVEL-DEPENDENT FUNCTIONAL CONNECTIVITY SIGNATURE OF THE HIPPOCAMPAL–PREFRONTAL NETWORK IN THE RAT BRAIN

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Abstract—Interactions between the hippocampus and the prefrontal cortex (PFC) are of major interest in the neurobiology of psychiatric and neurodegenerative disorders and are central to many experimental rodent models. Non-invasive imaging techniques offer a translatable approach to probing this system if homologous features can be identified across species. The objective of the present study was to systematically characterize the rat brain connectivity signature derived from low-frequency resting blood oxygenation level-dependent (BOLD) oscillations associated with and within the hippocampal–prefrontal network, using an array of small seed locations within the relatively large anatomical structures comprising this system. A heterogeneous structure of functional connectivity, both between and within the hippocampal–prefrontal brain structures, was observed. In the hippocampal formation, the posterior (subiculum) region correlated more strongly than the anterior dorsal hippocampus with the PFC. A homologous relationship was found in the human hippocampus, with differential functional connectivity between hippocampal locations proximal to the fornix body relative to locations more distal being localized to the medial prefrontal regions in both species. The orbitofrontal cortex correlated more strongly with sensory cortices and a heterogeneous dependence of functional coupling on seed location was observed along the midline cingulate and retrosplenial cortices. These findings are all convergent with known anatomical connectivity, with stronger BOLD correlations corresponding to known monosynaptic connections. These functional connectivity relationships may provide a useful translatable probe of the hippocampal–prefrontal system for the further study of rodent models of disease and potential treatments, and inform electrode placement in electrophysiology to yield more precise descriptors of the circuits at risk in psychiatric disease.

Key words: hippocampus, prefrontal cortex, resting state, fMRI, network, functional connectivity.

INTRODUCTION

Interactions between the hippocampus and the prefrontal cortex (PFC) are of major interest in the neurobiology of psychiatric and neurodegenerative disorders. Both regions have individually been implicated in the pathophysiology of these diseases (Weinberger, 1999; Weinberger et al., 2001; Neary et al., 2005; Artigas, 2010; Goghari et al., 2010; Heckers and Konradi, 2010; Marlatt and Lucassen, 2010; Meyer, 2012) and convergent evidence of dysfunctional prefrontal–temporal interactions has been identified in human disease populations (Heckers et al., 1998; Meyer-Lindenberg et al., 2005; Zhou et al., 2008; Goveas et al., 2011) and in subjects with an at-risk mental state (Benetti et al., 2009) or carrying genetic risk factors (Esslinger et al., 2009; Rasetti et al., 2011). In schizophrenia, these observed disturbances are consistent with the dysconnection hypothesis of the disorder (Wernicke, 1906; Friston, 1998; Stephan et al., 2009), a concept implicit even in the etymology of the term (Bleuler, 1911; Stephan et al., 2009). Recent rodent models provide a mechanistic approach to understanding possible biological underpinnings of human disease (Rocher et al., 2004; Agid et al., 2007;

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Abbreviations: AFNI, analysis of functional neuroimages; BOLD, blood oxygenation level dependent; CA1, field CA1 (cornus ammonis 1) of the hippocampus; CA2, field CA2 of the hippocampus; CA3, field CA3 of the hippocampus; Cg1, primary cingulate cortex; Cg2, secondary cingulate cortex; DMN, default mode network; EEG, electroencephalography; EPI, echo-planar imaging; fMRI, functional magnetic resonance imaging; FMRIB, functional magnetic resonance imaging of the brain (Oxford); FSL, FMRIB's software library; LTP, long-term potentiation; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PFC, prefrontal cortex; PrL, prelimbic cortex; rsfMRI, ''resting state'' fMRI; S, subiculum; SPM, statistical parametric mapping; TR, repetition time.

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Sigurdsson et al., 2010; Colgin, 2011), and often exhibit neurophysiological phenotypes involving this circuit (Sigurdsson et al., 2010; Hradetzky et al., 2012). Therefore, it has been proposed that hippocampal–prefrontal dysconnectivity is an ideal systems-level phenotype that can be used for back-translation to animal models of psychiatric diseases such as schizophrenia (Meyer-Lindenberg, 2010).

Accumulating evidence of hippocampal–prefrontal dysfunction in disease and models of disease in different species, established anatomical projections (Jay and Witter, 1991; Hoover and Vertes, 2007) and functional linkage (Jay et al., 1996) between these structures, has led to hippocampal–prefrontal interactions being central to many experimental investigations in rodent models (Colgin, 2011; Gordon, 2011). However, to date these have mostly involved invasive probe-based electrophysiological measures that are difficult to directly translate into human studies. In contrast, non-invasive imaging techniques offer a more translatable approach if homologous features can be identified across species. In humans, the study of low-frequency blood oxygenation level-dependent (BOLD) signal oscillations via task-free (“resting state”) functional magnetic resonance imaging (fMRI) (rsfMRI) is now established as a means of probing functional connectivity between brain regions and appears to correlate with both low-frequency modulation of conventional electroencephalography (EEG) components and slow cortical potentials per se (Raichle, 2011). In healthy subjects, convergent evidence from both task-based and task-free fMRI studies has identified that low-frequency BOLD oscillations in the hippocampus are coupled to posterior cingulate (which itself is coupled to dorsolateral PFC), parietal and medial prefrontal regions considered to subserve memory consolidation and overlapping with the default-mode network (Vincent et al., 2006). More recently, rsfMRI has begun to be back-translated into rodents (Pawela et al., 2008; Becerra et al., 2011; Jonckers et al., 2011), opening the possibility of more closely aligned physiological measures in the disease state and in preclinical models. Functional connectivity within sensory pathways (Pawela et al., 2008, 2010) and other prominent networks has been reported in both rat and mouse (Zhang et al., 2010; Becerra et al., 2011; Jonckers et al., 2011; Liang et al., 2012). Depending on the study and the analysis, certain networks variously included features located in the anterior cingulate, medial prefrontal cortex (mPFC) and hippocampus. Anatomical tracing studies in the rodent and detailed rsfMRI studies in humans have revealed differential connectivity within networks and even within anatomical brain regions. However, the extent and detailed nature of the low-frequency BOLD interactions between these functionally heterogeneous brain structures in the rat remains to be fully elucidated.

The objective of the present study was to systematically characterize the rat brain connectivity signature derived from low-frequency resting BOLD oscillations associated with the hippocampal–prefrontal network, considered to include the hippocampal formation, medial prefrontal and orbitofrontal cortices and also the midline cingulate and retrosplenial cortices. In particular, we sought to characterize the dependence of connectivity patterns as a function of different locations within the relatively large anatomical structures comprising this system.

EXPERIMENTAL PROCEDURES

Animals and experimental procedures

MRI experiments were performed on $N = 16$ Sprague–Dawley male rats (weight 340–392 g, age 2–3 months; Janvier, France). All procedures complied with the regulations covering animal experimentation within the European Union (European Communities Council Directive 86/609/EEC) and Germany (Deutsches Tierschutzgesetz). Experiments were approved by the German animal welfare authorities (Regierungspräsidium Karlsruhe) and performed at the Central Institute of Mental Health in Mannheim, Germany.

Rats were initially anesthetized with 4% isoflurane in a mixture of 70% N_2/30% O_2. After positioning in the scanner (head first, prone), anesthesia was maintained at ~2.5% for adjustments. After completion of these, a bolus of 0.5 ml (0.07 mg/kg) medetomidine solution (Domitor®, Janssen-Cilag, Neuss) was injected subcutaneously. Isoflurane was slowly discontinued within the next 10 min, and a continuous infusion of 1 ml/h (0.14 mg/kg/h) medetomidine solution was initiated. Functional data acquisition commenced approximately 20 min after isoflurane cessation. This protocol was adapted from (Kalthoff et al., 2011) and optimized for physiological stability within our experimental setup in pilot studies.

After completion of each experiment, Atipamezol (Antisedan®, Janssen-Cilag, Neuss; 1 mg/kg bodyweight) was injected subcutaneously together with ~2 ml of saline to reverse the sedative effect and compensate the fluid loss during the experiment.

Breathing and cardiac rates were monitored using a respiration pad placed beneath the chest (Small Animal Instruments Inc., NY, USA) and a pulse oximeter attached to the hindpaw. Signals were recorded (10-ms resolution) using a signal breakout module (Small Animal Instruments Inc., NY, USA) and a 4-channel recorder (Velleman® N.V., Gavere, Belgium).

Image data acquisition

All experiments were performed using a 94/20 Bruker Biospec MRI scanner (9.4 T; Bruker BioSpec, Ettlingen, Germany) with Avance III hardware, BGA12S gradients system with maximum strength 30,042 Hz/mm. Transmission and reception were achieved using a linear transmitter coil combined with rat brain linear receive-only coil array. Functional imaging data were acquired using an echo-planar imaging (EPI) sequence with the following parameters: repetition time (TR)/echo time (TE) 1700/17.5 ms, flip angle 60°, 1 segment, 1 average, 29 coronal slices, 96×96 matrix, field of view (35×35) mm^2, slice thickness 0.5 mm with 0.2-mm inter-slice gap, 300 volumes and a total rsfMRI acquisition time of 8.5 min. In-plane linear voxel dimension was thus 0.36 mm. The data were recorded using Paravision 5.1 software.

Image data post-processing

The image time series data were post-processed with tools from statistical parametric mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) (Frackowiak et al., 1997; Ashburner, 2012), functional magnetic resonance imaging of the brain (Oxford) (FMRIB) Software Library (FSL, v.4.1; http://