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Intrinsic connectivity of neural networks in the awake rabbit

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ABSTRACT

The way in which the brain is functionally connected into different networks has emerged as an important research topic in order to understand normal neural processing and signaling. Since some experimental manipulations are difficult or unethical to perform in humans, animal models are better suited to investigate this topic. Rabbits are a species that can undergo MRI scanning in an awake and conscious state with minimal preparation and habituation. In this study, we characterized the intrinsic functional networks of the resting New Zealand White rabbit brain using BOLD fMRI data. Group independent component analysis revealed seven networks similar to those previously found in humans, non-human primates and/or rodents including the hippocampus, default mode, cerebellum, thalamus, and visual, somatosensory, and parietal cortices. For the first time, the intrinsic functional networks of the resting rabbit brain have been elucidated demonstrating the rabbit's applicability as a translational animal model. Without the confounding effects of anesthetics or sedatives, future experiments may employ rabbits to understand changes in neural connectivity and brain functioning as a result of experimental manipulation (e.g., temporary or permanent network disruption, learning-related changes, and drug administration).

Introduction

The brain constantly transmits neural signals among various regions whether during idle wakefulness (i.e., "at rest") or different behavioral states like cognitively-demanding tasks (Baldassarre et al., 2012; Hampson et al., 2006; Tambini et al., 2010). Studies using functional magnetic resonance imaging (fMRI) in humans, non-human primates, and rodents have consistently observed neural networks of coherent activity within and between brain structures subserving some functional purpose or neuronal processing (Beckmann et al., 2005; Belcher et al., 2013; Hutchison et al., 2011; Lu et al., 2012; Mantini et al., 2013; Power et al., 2011; Shirer et al., 2012). These neural networks appear malleable as a function of development (Betzel et al., 2014; Greene et al., 2014; Pizoli et al., 2011; Power et al., 2010) or cognitive training (Lewis et al., 2009; Mackey et al., 2013).

The ability to collect imaging data during a resting, wakeful state eliminates the potential confounds associated with task-related performance in clinical populations. Intrinsic network connectivity measures have the potential to determine the potential efficacy of treatment (Fox et al., 2012) and might provide biomarkers for the identification of specific abnormal brain function related to psychiatric disease (Fox and Greicius, 2010). Robust differences in intrinsic network connectivity have been seen between healthy controls and individuals with ADHD (Fair et al., 2012; McLeod et al., 2014), neurodegenerative and Alzheimer's disease (Damoiseaux et al., 2012; Greicius et al., 2004; Lehmann et al., 2013; Lustig et al., 2003; Seeley et al., 2009; Supekar et al., 2008), schizophrenia (Yu et al., 2012), Tourette's (Church et al., 2009), or Fragile X syndrome (Hall et al., 2013).

Animal models serve a useful purpose to study the phenomena of intrinsic connectivity as some experimental manipulations are difficult or unethical to perform in humans (e.g., temporary or permanent lesioning of neural hubs). However, many animal models require sedation or anesthesia to be imaged which can significantly alter functional networks (Boveroux et al., 2010; Brevard et al., 2003; J.V. Liu et al., 2013; X. Liu et al., 2013). Identifying animal models that can be imaged in an awake and conscious state in order to preserve intrinsically active neural networks allows for greater translatability to humans.

The rabbit is an ideal and unique animal model for the study of intrinsic connectivity due to their ability to be imaged while in a docile awake state without the need for any sedation or anesthetic agents, their tolerance for restraint (Li et al., 2003; Wyrwicz et al., 2000), and their adaptations to living in narrow underground burrows. A relatively simple surgery to implant an atraumatic restraining headpost assembly allows the rabbit to remain in a standard stereotaxic orientation thus minimizing movement of the head and brain and preventing image

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artifacts and distortion. A single day of habituation to the MRI and gradient sequence provides sufficient acclimation to the environment (Wyrwicz et al., 2000).

In this study, we characterize the intrinsic connectivity networks of the rabbit brain for the first time. Group independent component analysis revealed seven networks related to the hippocampus, default mode, cerebellum, thalamus, and visual, somatosensory, and parietal cortices that are similar to previously observed networks in humans, non-human primates and/or rodents. Understanding the neural networks of the rabbit brain will provide an additional translational animal model to probe alterations in functional connectivity as a consequence of experimental manipulation, drug administration or disease states/agents without the confounding factors of anesthesia or sedation.

Methods

Subjects and surgery

Twelve female, New Zealand White rabbits (2–4 kg) were used in the current study. Surgery was performed under NIH and Northwestern University IACUC approved protocols to implant a restraining bolt assembly onto the rabbit's skull in order to fix the head in our custom-built MR cradle. Anesthesia was induced with ketamine (60 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.). Buprenex (0.03 mg/kg, s.c.) was administered to minimize discomfort during and after the procedure and ophthalmic ointment was applied to keep the eyes moist. After rabbits were placed into a stereotaxic apparatus, the scalp was incised and the skull was positioned with lambda 1.5 mm below bregma. In order to secure the headpost onto the skull in the stereotaxic plane (Girgis and Shih-Chang, 1981; Sawyer et al., 1954), six holes (four rostral to bregma and two lateral to lambda) were drilled into (but not through) the skull. Nylon machine screws were turned into holes threaded with a 2–56 bottoming tap. After Grip cement (Dentsply) was placed on the skull and machine screws, a custom-built headpost assembly (four upright nylon bolts (6–32 × 3/4″)) encased in Grip cement) was lowered onto the cement-covered skull. Additional cement was added as necessary to secure the headpost assembly and cover the skull. Metacam (0.2 mg/kg, s.c.) was administered once the rabbits were sternal and again 24 h later to provide analgesia. Grip cement did not induce any susceptibility artifacts in EPI images (Supplemental Fig. 1).

Animal restraint for resting-state fMRI

After one week of post-operative recovery (i.e., to insure that normal eating, drinking, and activity returned), rabbits underwent a one-day habituation protocol to the MRI scanner environment. For habituation and all subsequent scanning, earplugs were inserted and rabbits were placed in a prone position inside a cotton wrap and a canvas bag (Lomir) secured with Velcro. A single-channel, receive-only RF surface coil was secured to the underside of a Plexiglas crossbar and secured onto the rabbit's headpost with four nylon nuts. The crossbar was fastened to the custom-built cradle to stabilize the rabbit's head and prevent movement. With the headposted rabbit fixed inside the cradle, it was placed in the MR scanner. A 1 h EPI sequence was performed to fulfill habituation training. Repositioning of the same animal was achieved in all three directions (X, Y, and Z) with great accuracy (<500 μm) across sessions. The configuration of the custom-built cradle with the single-channel, receive-only RF surface coil is presented in Supplemental Fig. 2.

Criterion for habituation was achieved after a single session. By visualizing the EPI images in real-time, no signs of excessive movement (i.e., >0.3 mm) lasting longer than 2 repetition times (i.e., 5 s) occurred during the habituation protocol. Although we did not collect any measurement to ascertain the stress of the rabbit (e.g., corticosterone levels), rabbits did not display any signs of overt stress (i.e., struggling) and direct monitoring of the digital waveform generated by a respiration pillow revealed consistent and maintained breathing patterns not interrupted by any excessive movement throughout the duration of scanning.

MRI data acquisition

MR scanning was conducted in a Bruker 7 T/30 cm wide horizontal magnet (ClinScan, Bruker Biospin, Ettlingen, Germany) using Syngo VB15 platform from Siemens. Transmission was achieved with a two channel volume coil fixed inside the magnet with a single-channel, receive-only RF surface coil with an inner diameter of 30 mm. Single anatomical and functional scans were acquired once per day for seven consecutive days. An anatomical reference image was first acquired using a gradient echo sequence with the following geometrical and MR parameters: 1.0 mm slice thickness (40 slices), 0.5 × 0.5 mm in-plane resolution, FOV = 64 × 64 mm, matrix size = 128 × 128 × 40, TR = 500 ms, TE = 2.09 ms, flip angle = 90°. Blood-oxygen-level dependent (BOLD) contrast-sensitive T_2^*-weighted gradient-echo echo-planar images (EPI) covering the entire rabbit brain were acquired for intrinsic connectivity scans (200 volumes, 20 coronal slices, repetition time (TR) = 2.5 s, echo time (TE) = 25 ms, total bandwidth = 367 kHz, flip angle = 90°, 2.0 mm slice thickness, 0.5 × 0.5 mm in-plane resolution, FOV = 35 × 26 mm; matrix size = 70 × 52 × 20, 200 volumes, total time = 8:20). Adjustments to optimize shimming, reduce air-tissue artifacts and produce a uniform magnetic field were performed on a manually selected region (centered on, but not exceeding the size of, the rabbit brain). First and second order shimming was performed using an automated field map algorithm included in the Syngo platform. Shim tables showing the resulting x, y, z and higher order shim values confirmed no major variability in shim values across subjects and sessions.

fMRI data analysis

Data analysis was performed with AFNI (Cox, 1996) and FSL (Beckmann and Smith, 2004). The first three volumes of each dataset were discarded to account for eddy currents and NMR equilibrium. After performing slice-timing and motion correction, displacement of each brain volume relative to the previous volume was calculated as the Euclidian norm of the translational (x, y, z) and rotational (α, β, γ) rigid-body motion correction parameters (displacement = square root of $[(\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2 + (\Delta\alpha)^2 + (\Delta\beta)^2 + (\Delta\gamma)^2]$ (Belcher et al., 2013). Rotational displacements were converted from radians to millimeters by calculating displacement on the surface of a sphere of radius 14 mm (about the mean distance from the cerebral cortex to the center of the head). Since rotational or translational displacement did not exceed our criterion of 0.3 mm, no data points were eliminated due to excessive motion. The average maximum displacement across the entire subject population was 0.11 mm (s.d. = 0.07 mm). EPI images from each rabbit were co-aligned with the anatomical reference scan collected during the same session. Anatomical scans were then spatially aligned to a separate, previously collected, high-resolution rabbit brain (0.2 mm^3 resolution). Output from coregistration procedures demonstrated no significant issues and based on our semi-quantitative estimates, we are confident of the spatial accuracy in coregistered images. The same transformation was applied to the EPI images and the original voxel resolution (2.0 × 0.5 × 0.5 mm) was kept. Additional preprocessing steps included regression of motion parameters, temporal filtering (0.005–0.1 Hz), and spatial smoothing (FWHM = 0.71 mm).

For each of the rabbit's seven intrinsic connectivity scans, voxels were divided by their mean signal intensity and concatenated. Group-level independent component analysis was performed using the FSL program MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components). MELODIC uses independent component analysis to linearly decompose multiple 4D data sets into a set of spatial maps (i.e., independent components) without the need

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