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Mapping thalamocortical networks in rat brain using resting-state functional connectivity

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ABSTRACT

Thalamocortical connectivity plays a vital role in brain function. The anatomy and function of thalamocortical networks have been extensively studied in animals by numerous invasive techniques. Non-invasively mapping thalamocortical networks in humans has also been demonstrated by utilizing resting-state functional magnetic resonance imaging (rsfMRI). However, success in simultaneously imaging multiple thalamocortical networks in animals is rather limited. This is largely due to the profound impact of anesthesia used in most animal experiments on functional connectivity measurement. Here we have employed an awake animal imaging approach to systematically map thalamocortical connectivity for multiple thalamic nuclei in rats. Seed-based correlational analysis demonstrated robust functional connectivity for each thalamic nucleus in the cortex, and the cortical connectivity profiles revealed were in excellent accordance with the known thalamocortical anatomical connections. In addition, partial correlation analysis was utilized to further improve the spatial specificity of thalamocortical connectivity. Taken together, these findings have provided important evidence supporting the validity of rsfMRI measurement in awake animals. More importantly, the present study has made it possible to non-invasively investigate the function, neuroplasticity and mutual interactions of thalamocortical networks in animal models.

Introduction

The thalamus and cerebral cortex are connected through topologically well-organized connections. Studies have long revealed that the thalamus acts as the "gateway" for almost all extrinsic and intrinsic information before they reach the cortex via thalamocortical connections (Guillery and Sherman, 2002). More recently, research has suggested that the thalamus plays many significant roles that extend beyond the relay function. For instance, it has been shown that the lateral geniculate (LG) nucleus—a thalamic nucleus that transmits visual information—is actively involved in information processing (Schmid et al., 2010), binocular rivalry (Haynes et al., 2005; Wunderlich et al., 2005), visual attention (O'Connor et al., 2002), perception and cognition (Saalmann and Kastner, 2009). In addition, the function of thalamus is critical to the states of wakefulness, sleep and consciousness (Alkire et al., 2008; Poulet et al., 2012). It has also been reported that thalamocortical connectivity is essential for the establishment of oscillatory brain waves (Jones, 2001). Importantly, abnormal thalamocortical connectivity has been observed in multiple brain disorders like schizophrenia (Welsh et al., 2010; Woodward et al., 2012), suggesting its vital psychopathological relevance.

Given the critical importance of thalamocortical networks, numerous studies have examined the anatomical and functional aspects of thalamocortical connectivity through a wide range of invasive techniques such as retrograde/anterograde tracing and electrophysiological methods (Krettek and Price, 1977; Van Groen and Wyss, 1995; Vertes and Hoover, 2008). These studies have identified characteristic connectivity patterns for separate thalamic nucleus groups (Swanson, 2004). Specifically, thalamocortical connections related to primary sensory and motor cortices are relatively simple and well organized. For instance, LG nucleus is predominantly connected to the visual system; medial geniculate (MG) nucleus is essentially connected to the auditory system; and ventral group of dorsal thalamus (VENT) is primarily linked to the sensorimotor system. Interestingly, these thalamocortical connectivity patterns are well preserved in multiple species from rodents to humans (Jones, 2007). In contrast, thalamic nuclei connected to higher-tier association cortices can have more complex connectivity patterns with possible overlapping cortical projections. For example, medial group of dorsal thalamus (MED) has connections to the prefrontal cortex and cingulate; midline group of dorsal thalamus (MTN) and anterior group of dorsal thalamus (ATN) both contain connections to the prefrontal cortex, cingulate and hippocampal formation; MTN is also connected to subcortical regions like lateral septal complex (LSX); and lateral group of dorsal thalamus (LAT) projects to association

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cortices in parietal, temporal and occipital regions. A summary of the thalamocortical connectional relationship in the rat brain was shown in a diagram in SI Fig. 1.

Most techniques for studying thalamocortical connectivity suffer from invasiveness and inability to systematically trace connectivity across multiple thalamocortical networks. Non-invasively and simultaneously examining functional connectivity between separate thalamic nuclei and their corresponding cortices has posed a significant challenge, albeit this ability can be extremely important in further understanding the characteristic functions of individual thalamocortical networks as well as their mutual interactions. To bridge this gap, Zhang et al. (2008); D. Zhang et al., 2010 have successfully demonstrated the feasibility of mapping the thalamocortical networks in humans by utilizing an emerging brain mapping technique of resting-state functional magnetic resonance imaging (rs-fMRI). rs-fMRI measures functional connectivity between brain regions based on synchronized spontaneous fluctuations of the rsfMRI signal (Biswal et al., 1995). With this technique, the thalamocortical connectivity patterns revealed in humans well agreed with the known anatomical connectivity relationship (Zhang et al., 2008; D. Zhang et al., 2010). However, success in this research topic in animals is rather limited (Pawela et al., 2008). This can largely be attributed to the profound impact of anesthesia used in most animal experiments on the measurement of resting-state functional connectivity (Liang et al., 2012b; Liu et al., 2011; Lu et al., 2007). Indeed, our recent study showed that thalamocortical connectivity and local network organizations of the rat brain can be significantly altered by using routine anesthetizing procedures (Liang et al., 2012a). Consequently, the confounding effects of anesthesia have significantly hindered the exploration of thalamocortical networks in animals using rsfMRI.

In the present study we have employed a previously established awake animal rsfMRI approach to map thalamocortical connectivity in rats. This imaging method can avoid the confounding effects of anesthesia on rsfMRI measurement. To examine whether specific thalamocortical connectional relationship in rodents can be reliably revealed by this approach, anatomically defined thalamic nucleus groups were used as separate seed regions of interest (ROIs) to generate the corresponding functional connectivity maps. Resulting functional connectivity in the cortex demonstrated high spatial specificity and was in excellent accordance with the known thalamocortical anatomical connectivity.

Materials and methods

Animal preparation and MR experiment

rsfMRI data collected at the identical condition from several previous studies (Liang et al., 2011, 2012a; N. Zhang et al., 2010) were pooled and re-analyzed for the purpose of the present study. Detailed descriptions of the experimental procedures can be found in aforementioned studies. Briefly, 42 adult male Long-Evans rats were acclimated to MRI restraint and noise for seven days to minimize imaging-induced stress and movement during imaging as described before (Liang et al., 2011, 2012a; N. Zhang et al., 2010). During the experimental setup, the rat was briefly anesthetized by isoflurane (2%) and the head was secured into a head restrainer with a build-in coil, and the body was fit into a body restrainer. After the setup was completed, isoflurane was removed and the whole system was positioned in magnet. Rats were all fully awake during imaging sessions. In order to compare the thalamocortical connectivity at the awake and anesthetized conditions, 16 of 42 rats underwent the imaging session at the anesthetized condition at a minimum of 7 days after they were imaged at the awake condition. In this experiment, the animal preparation procedure was the same as that in the awake imaging experiment. Isoflurane gas (2%) was then delivered to the animal through a nose cone in the magnet to maintain the anesthetized state. The body temperature of the animal was monitored and maintained at 37 °C ± 0.5 °C by using a feedback controlling heating pad. All studies were approved by IACUC of the University of Massachusetts Medical School.

All MRI experiments were conducted on a Bruker 4.7 T magnet. A dual ^{1}H radiofrequency (RF) coil configuration (Insight NeuroImaging Systems, Worcester, MA) consisting of a volume coil for exciting the water proton spins and a surface coil for receiving MRI signal was used; the volume and surface coils were actively tuned and detuned to prevent mutual coil coupling. This dual-coil configuration allows for sufficient RF field homogeneity in the rat brain for RF transmission, while preserving the advantage of higher signal-to-noise ratio (SNR) provided by the smaller reception coil. For each MRI session, RARE sequence was used to acquire anatomical images with the following parameters: TR = 2125 ms, TE = 50 ms, matrix size = 256 × 256, FOV = 3.2 × 3.2 cm^2, slice number = 18, slice thickness = 1 mm, and RARE factor = 8. Gradient-echo images were then acquired using the echo-planar imaging (EPI) sequence with the following parameters: TR = 1 s, TE = 30 ms, flip angle = 60°, matrix size = 64 × 64, FOV = 3.2 cm × 3.2 cm, slice number = 18, and slice thickness = 1 mm. Two hundred volumes were acquired for each scan, and six to nine scans were obtained for each session.

rsfMRI data analysis

rsfMRI images of all rats were first co-registered to a fully segmented rat atlas based on anatomical images by using Medical Image Visualization and Analysis (MIVA, http://ccni.wpi.edu/). Preprocessing steps included motion correction with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/), spatial smoothing (FWHM = 1 mm), regression of motion parameters and the signals of white matter and ventricles to eliminate the contributions of physiologic noise to the rsfMRI signal, and 0.002–0.1 Hz band-pass filtering. Scans with excessive motion (>0.25 mm) were discarded.

To accommodate the spatial resolution of rsMRI, the thalamus was partitioned into eight bilateral thalamic nuclei ROIs as seeds for functional connectivity analysis (Fig. 1). The total number of voxels in EPI images that fit into the ROI of each individual thalamic nucleus was LG: 15, MG: 17, VENT: 79, LAT: 34, MED: 26, MTN: 26, ATN: 30 and RT: 21. This calculation was based on our segmented atlas template that was resampled to the spatial resolution of EPI images (0.5 × 0.5 × 1 mm^3), which gave the volume of each individual thalamic nucleus (in mm^3): LG: 3.75, MG: 4.25, VENT: 19.75, LAT: 8.5, MED: 6.5, MTN: 6.5, ATN: 7.5 and RT: 5.25. Anatomical definitions were based on the Swanson atlas (Swanson, 2004). Detailed anatomical information of all seed ROIs can be found in SI Table 1.

Functional connectivity was evaluated using seed-based correlational analysis on a voxel-by-voxel basis (Liang et al., 2012a; N. Zhang et al., 2010). Time courses from all voxels within individual seed regions were averaged and used as reference time courses. Pearson cross-correlation coefficients between these reference time courses and the time course of each individual voxel were then calculated. Correlation coefficients (i.e. r values) were transformed to z scores using Fisher's z transformation. This correlational analysis was carried out for each scan.

To assess the reproducibility of functional connectivity maps, rats were randomly split into two subgroups for each (awake or anesthetized) condition. A thalamocortical connectivity map was generated for each seed in each subgroup. The correlation coefficient of z scores between individual corresponding voxels from two subgroups was then calculated. This process was repeated for 100 times. Averaged correlation coefficients from 100 repetitions provided a measure of reproducibility of functional connectivity maps.

Statistics

For each seed ROI, a linear mixed-effect model was calculated using the lme4 package in the *R* environment (http://www.r-project.org,