Published in final edited form as:

J Neurosci Methods. 2010 June 15; 189(2): 186–196. doi:10.1016/j.jneumeth.2010.04.001.

Mapping Resting-State Brain Networks in Conscious Animals

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Abstract

In the present study we mapped brain functional connectivity in the conscious rat at the “resting state” based on intrinsic blood-oxygenation-level dependent (BOLD) fluctuations. The conscious condition eliminated potential confounding effects of anesthetic agents on the connectivity between brain regions. Indeed, using correlational analysis we identified multiple cortical and subcortical regions that demonstrated temporally synchronous variation with anatomically well-defined regions that are crucial to cognitive and emotional information processing including the prefrontal cortex (PFC), thalamus and retrosplenial cortex. The functional connectivity maps created were stringently validated by controlling for false positive detection of correlation, the physiologic basis of the signal source, as well as quantitatively evaluating the reproducibility of maps. Taken together, the present study has demonstrated the feasibility of assessing functional connectivity in conscious animals using fMRI and thus provided a convenient and non-invasive tool to systematically investigate the connectional architecture of selected brain networks in multiple animal models.

Keywords

functional connectivity; conscious animals; functional magnetic resonance imaging; rat; prefrontal cortex; thalamus; retrosplenium

Introduction

The task of investigating brain functions and networks is conventionally explored with studies of brain responses to carefully controlled sensory, cognitive and motor events. These responses can be measured and mapped using neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) using the hemodynamic

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processes spatially associated with locally elevated neuronal activities. However, one major challenge of these methods is that it is difficult to determine intrinsic relations among distributed brain regions activated by designed external stimulations. This challenge has become a critical barrier to investigating selective brain networks sub-serving cognitive and emotional events.

Recently, a series of studies have demonstrated that patterned activities exist within various brain networks during resting and passive task states (Gusnard et al., 2001b; Fox et al., 2005). During these non-cognition-related states, distributed brain regions within functional-anatomic networks spontaneously increase and decrease their intrinsic activity together (Fox et al., 2005). An important implication of this synchrony of intrinsic brain activity is its potential utilization in studies pertaining to functional connectivity within and across separate brain networks since spontaneous fluctuations of intrinsic activity from functionally-connected brain regions should be temporally correlated. Indeed, Biswal et al. first reported the correlation in fMRI signal fluctuations between the left and right motor cortices in the absence of any motor task (Biswal et al., 1995). Subsequently, a large set of studies have found consistent connectivity in motor, auditory, visual and language areas (Biswal et al., 1995; Lowe et al., 1998; Hampson et al., 2002). This method was later utilized by several groups to identify the connections between several brain regions referred to as default-mode network, which consistently shows higher intrinsic activities at rest (Gusnard et al., 2001b; Greicius et al., 2003; Damoiseaux et al., 2006; De Luca et al., 2006).

Besides the potential discovery of novel brain circuitries and networks, the intrinsic brain activity is tightly linked to a number of neurobiological behaviors and thus should lead to better understanding of neuronal mechanisms underlying these behaviors (Fox and Raichle, 2007; Fox et al., 2007). A recent study found that the spontaneous activities recorded from the hippocampus in navigating rats during the stopped periods contain structured patterns that echoed the sequential patterns that occurred when the rats were actively navigating, only much faster and in reverse order (Foster and Wilson, 2006). This study indicates that resting-state intrinsic brain activity may be critical to understanding information gathering and processing (Buckner and Vincent, 2007) and may constitute a general mechanism of learning and memory (Foster and Wilson, 2006; Albert et al., 2009). Additionally, it has been found that alterations of resting-state brain activity and networks are tightly linked to degenerative disease processes like that observed in Alzheimer's disease (Lustig et al., 2003; Rombouts et al., 2005; Tian et al., 2006; Buckner and Vincent, 2007). Emergent data have further revealed differences in resting-state activity and networks in other disorders including autism (Kennedy et al., 2006), depression (Anand et al., 2005), multiple sclerosis (Lowe et al., 2002), and attention deficit hyperactivity disorder (Tian et al., 2006). Taken together, these results strongly support the notion that understanding intrinsic brain activity and functional connectivity at resting states has the potential to open a new avenue to uncover brain functions and brain networks in both normal and abnormal conditions.

To date, the vast majority of studies on intrinsic brain activity and resting-state functional connectivity are conducted on human subjects. Systematic investigations of this phenomenon in different animal models have been underexplored (Leopold et al., 2003; Lu et al., 2007; Vincent et al., 2007; Kannurpatti et al., 2008; Pawela et al., 2008; Zhao et al., 2008; Kojima et al., 2009; Majeed et al., 2009). This may be attributed largely to unknown effects of anesthetic agent used in most animal studies on functional connectivity between different brain regions. Although the anesthetized monkey study showed remarkable agreement with awake human studies (Vincent et al., 2007), results from a recent study suggest that the functional connectivity in "default-mode" network is reduced even during conscious sedation (Greicius et al., 2008). Moreover, Lu et al clearly demonstrated a dose-dependent decrease of cross-hemispheric functional connectivity in α -chloralose-anesthetized rats (Lu et al., 2007). This

result is in agreement with the study by Liu and colleagues who found that intrinsic BOLD fluctuations and functional connectivity in the resting rat were strongly dependent on anesthesia depth (Liu et al., 2009). In human subjects, functional connectivity can be detected with light anesthesia but is completely ablated with deep anesthesia (Peltier et al., 2005). Taken together, these results strongly suggest that anesthesia can be a potential confound in studying functional connectivity of animal models (Massimini et al., 2005).

The confounding effects of anesthetics limit the full potential of investigating intrinsic brain activity and resting-state functional connectivity in various animal models. However, such ability is extremely important particularly in behavioral and cognitive neuroscience because it not only can provide invaluable information regarding cognitive and emotional tasks in animal models, but also may provide a unique window to explore comparative functional anatomy between species (Buckner and Vincent, 2007) using translational models. Therefore, for the purpose of establishing functional connectivity studies in animal models, it is intriguing and vital to investigate intrinsic brain activity and functional connectivity in conscious animals. Technical challenges involved in imaging conscious animals in MR scanners include controlling for motion artifacts and minimizing stress induced by the scanning noise and environment. To resolve these issues, we have developed an animal model (Ferris et al., 2006) through which animal motion and stress during MRI scanning are substantially minimized by using an entirely noninvasive system (Lahti et al., 1998) and a routine acclimation procedure (King et al., 2005) (See **Methods and Materials**). This animal model allowed the brain activation in conscious animals to be reliably imaged using fMRI (Lahti et al., 1999; Ferris et al., 2001; Brevard et al., 2003; Sicard et al., 2003; Tenney et al., 2004).

In the present study we have created resting-state functional connectivity maps from seed regions that are crucial to cognitive and emotional processing including the prefrontal cortex (PFC), thalamus and retrosplenium cortex in the conscious rat. The results showed strong functional connectivity to cortical and subcortical areas from these seeds. The reliability of the functional connectivity maps obtained was validated by controlling for false positive detection of correlation, the physiologic basis of the signal source, as well as qualitatively and quantitatively evaluating the reproducibility of the maps.

Methods and Materials

Animals

Eight adult male Long-Evans (LE) rats (350 – 450 g for adult rats) were obtained from Charles River Laboratories. Animals were housed in Plexiglas cages (two to a cage) and maintained in ambient temperature (22–24°C) on a 12-h light : 12-h dark schedule. Food and water were provided ad libitum. All animals were acquired and cared for in accordance with the guideline published in the *NIH Guide for the Care and Use of Laboratory Animals* (#80-23, Revised 1996). These studies were approved by IACUC Committee of the University of Massachusetts Medical School.

Acclimation Procedure

The rats were briefly anesthetized with isoflurane before being secured in a Plexiglas stereotaxic head holder through plastic ear bars. Topical application of EMLA cream was used to relieve any pain associated with the head holder. The animal's forepaw and hindpaw were loosely taped to prevent any self injurious behavior. The body was then placed in a Plexiglas body tube. The entire unit was secured onto a firm base to prevent any motion. Rats restrained in the head holder and body tube were placed in a black opaque tube "mock scanner". A tape-recording of acoustic sounds from various imaging sessions was played during the acclimation. The animals were exposed to these conditions for 8 days before imaging. The time for exposure

was increased from 15 minutes on the first day to 90 minutes on days 6, 7 and 8 with an increment of 15 minutes per day.

Animal Preparation prior to MR Experiments

Under short-acting anesthetic (isoflurane gas) the animal was fitted into a head restrainer with a built-in saddle coil. A plastic semicircular headpiece with blunted projections that fit into the ear canals was positioned. The head was placed into the cylindrical head-holder with the canines secured over a bite bar, the nose secured with a noise clamp, and ears positioned inside the head-holder with adjustable screws fitted into lateral sleeves. The body of the animal was placed into a body restrainer that allowed unrestricted respiration. This novel design isolated all body movement from the head, thus minimizing motion artifact. Robustness of this system in controlling head movement has been demonstrated in our previous publications (Liu et al., 2009). After the animal was set up, the isoflurane gas was removed and the restraining system was positioned in the magnet. Animals were fully conscious within 10–15 min.

MR Experiments

All MR experiments were conducted on a 4.7T/40cm horizontal magnet (Oxford, UK) interfaced with a Biospec Bruker console (Bruker, Germany) and equipped with a 20G/cm magnetic field gradient. A dual ^1H 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.

At first, anatomical images were acquired using a multi-slice fast spin-echo sequence (RARE) with the parameters: repetition time (TR) = 2125 ms; RARE factor = 8; effective echo time (TE) = 50 ms; matrix size = 256×256; field of view (FOV) = 3.2×3.2 cm²; slice number = 18; slice thickness = 1 mm; n = 8. Based on the geometry of anatomical images, multi-slice gradient-echo images covering the whole brain were acquired using echo-planar imaging (EPI) with the parameters: TR = 1 s; Flip Angle = 60°; TE = 30 ms; matrix size = 64×64; FOV = 3.2×3.2 cm²; slice number = 18; slice thickness = 1 mm. Rats were at rest during image acquisition. 200 volumes were acquired for each run; 3–6 runs were obtained for each rat.

Data Analysis

All fMRI data analyses were performed on Medical Image Visualization and Analysis (MIVA) and Matlab (The Mathworks Inc., Natick, MA, USA). First, each subject was aligned and registered, based on anatomical images, to a fully segmented rat brain atlas with more than 200 built-in regions of interest (ROIs). The registration procedure provided the coordinates of each seed ROI in the image space. In this study we selected three seed regions: the PFC, thalamus and retrosplenium cortex, and examined the brain regions that are functionally connected to each of them. The alignment procedure also allowed for inter-subject analysis in the image space. After registration and alignment, fMRI time courses for individual pixels in a seed ROI were obtained according to their corresponding coordinates. A time course for each seed ROI was then created by averaging time courses of all pixels inside it. This time course was later used as the reference for this seed to calculate functional connectivity. Due to our stringent control of head movement as previously demonstrated (Lahti et al., 1998), head motion was minimal in the present study. Thus, motion correction was not conducted on all imaging data.

Functional connectivity was evaluated using the correlational analysis on a pixel-by-pixel basis. First, all raw fMRI images were spatially filtered using a symmetric 5×5 Gaussian low-pass filter with the standard deviation of 0.75 (FWHM = 1.88 mm). Almost identical results were

obtained using a 4×4 Gaussian low-pass filter with the standard deviation of 0.5 (FWHM = 1 mm). All time courses were then 0.01–0.1 Hz band-pass filtered and the first 10 images were discarded to ensure an equilibrium state. Since respiration and heart beat rates of rats are significantly higher than 0.1 Hz, this temporal filtering processing eliminated major artifact due to physiologic fluctuations as well as baseline drifting. After the spatial and temporal filtering processes, the cross-correlation (CC) coefficient between a reference time course and the time course of each individual pixel was calculated and used to quantify the strength of functional connectivity. A pixel's temporal relationship with the seed region was deemed significant if its CC coefficient > 0.22 (equivalent to p value < 0.005, uncorrected). A connectivity map for each seed region was created for each fMRI run and maps corresponding to the same seed across multiple runs were then averaged to create the connectivity map for each subject. At last, a composite connectivity map was generated by averaging connectivity maps corresponding to the same seed region across subjects using the aforementioned alignment procedure.

The reliability the functional connectivity maps created was examined through inter-subject reproducibility in a quantitative manner. Subjects were randomly divided into two groups. Functional connectivity maps were separately created for each group. Reproducibility of maps from the same seed between the two groups was statistically evaluated by comparing the CC coefficients (i.e. strength of connectivity) of the corresponding pixels from the two group maps.

Results

Functional Connectivity Maps from Different Seeds

Figure 1 shows the averaged functional connectivity map ($n = 8$) from the seed of the PFC of the adult rat brain. The map identifies very complex networks involving the PFC. Regions in these networks include olfactory bulb, hippocampus, subcortical regions such as thalamus, nucleus accumbens (NAcc) and caudate putamen (CPu), and a widely spread cortical areas including visual, motor, somatosensory, insular and cingulate cortices. These complex connections to other brain areas from the PFC reflect the intricate role in integrating brain functions at multiple levels.

Figure 2 shows the averaged functional connectivity map ($n = 8$) from the seed of thalamus of the adult rat brain. We observed a strong connection between thalamus and hippocampus, which has been well described anatomically as thalamo-hippocampal pathway (Herkenham, 1978; Yanagihara et al., 1987; Haase, 1990; Wouterlood et al., 1990; Dolleman-Van Der Weel and Witter, 1996). In addition, resting-state intrinsic brain activities also showed that thalamus is functionally connected to a number of cortical regions including visual, auditory, motor, somatosensory, retrosplenium, cingulate and prefrontal cortices. These connections have been systematically identified as thalamo-cortical network (Scannell et al., 1999). Moreover, we also observed a strong connection between thalamus and CPu which, together with connections in thalamo-hippocampal pathway, form hippocampal-basal ganglia-thalamo-hippocampal loop (Sil'kis, 2007).

The spatial pattern of functional connectivity to the retrosplenium in adult rats is remarkably different from the thalamus and PFC as shown in Figure 3. Brain regions connected to the retrosplenium at the resting state are predominantly located in the cortical ribbon covering the visual, motor, somatosensory, cingulate and prefrontal cortices. In subcortical areas, thalamus and hippocampus is functionally connected to the retrosplenial cortex at the resting state.

Evaluate the Reliability of Functional Connectivity Maps

In this section we aim to validate the capability of mapping whole-brain functional connectivity in adult rats shown above. Figure 4 shows the low-frequency BOLD fluctuations averaged from the regions of hippocampus and thalamus from four independent fMRI runs in one representative animal. It is evident that the temporal patterns of intrinsic BOLD activities in the two regions are extremely similar within each run, although their temporal evolutions are dramatically different across runs. The CC coefficient between the time courses from the two regions is 0.66, 0.83, 0.72 and 0.73 for run 1 to 4, respectively. As a control for false positive detection of correlation, we shuffled the BOLD time courses across runs and therefore broke the simultaneity within each run. Under this circumstance, the averaged CC coefficient considerably decreased to a statistically insignificant level (CC coefficient = 0.08; equivalent to p value = 0.27). This situation holds for all rats. The CC coefficient of synchronized intrinsic BOLD activities between hippocampus and thalamus was 0.62 when averaged from all adult rats (equivalent to p value $< 10^{-10}$), whereas it decreased to 0.07 (equivalent to p value = 0.33) if BOLD time courses were shuffled across runs within individual animals.

To evaluate the reproducibility of mapping functional connectivity in conscious animals, all adult subjects were randomly divided into two groups. Figure 5 shows the functional connectivity maps from the seed of retrosplenium from one subject group (Fig. 5a) and the other subject group (Fig. 5b). Both maps are remarkably similar to each other. We calculated the correlation between the strength of functional connectivity (as measured by the CC coefficient) of corresponding pixels from the two group maps in Fig. 5a and 5b. This correlation is statistically significant ($R = 0.39$, $p < 10^{-10}$), indicating a high reproducibility of the two maps. Mapping reproducibility was also statistically significant for the other two seeds ($p < 10^{-8}$ for PFC, $p < 10^{-10}$ for thalamus).

As a control for the physiologic basis of the signal source used to construct functional connectivity maps, Figure 6 shows the functional connectivity map in the brain from an expired rat from the seed region of the PFC. In contrast to the map in Fig. 1, no obvious anatomical structure was recognized showing any connection to the PFC in this map. This situation holds for all maps from other seed regions. These data indicate that the signal basis in this study is indeed from intrinsic brain activities. They can also rule out the possibility that connectivity maps obtained from these studies were biased by any systematic signal change of non-physiologic basis.

Figure 7 compares the functional connectivity maps from all three seed regions in a 3D view.

Discussion

In the present study we demonstrated the capability of mapping large-scale functional connectivity across the whole brain in the conscious rat at the resting state. Anatomically well-defined seed regions that are crucial for cognitive and emotional brain functions were selected including the thalamus, PFC and retrosplenial cortex. From these predefined seed regions, functional connectivity maps were obtained based on intrinsic BOLD fluctuations. The fundamental hypothesis underlying the present study is that intrinsic BOLD activities in functionally connected brain regions have similar temporal patterns (Biswal et al., 1995). This similarity was demonstrated through high CC coefficients between BOLD time courses from thalamus and hippocampus – the two regions that have been well documented to contain mutual connections using various techniques (Herkenham, 1978; Yanagihara et al., 1987; Haase, 1990; Wouterlood et al., 1990; Dolleman-Van Der Weel and Witter, 1996). The functional connectivity maps observed in the present study were validated from several perspectives: First, strong correlation between BOLD time courses from functionally connected regions disappeared once the synchrony was disrupted. Second, all regions showing significant

connectivity to seeds have unequivocal anatomical structure (e.g. regions connected to the retrosplenial cortex clearly follow cortical ribbons in Fig. 4). This is in sharp contrast to the connectivity map obtained from an expired brain where no anatomical structure could be identified (Fig. 6). Third, functional connectivity maps determined from two separate subject groups were highly reproducible. Taken together, the results validate the feasibility and reliability of mapping large-scale functional connectivity across the whole brain in conscious animals.

Functions and Circuitries involving the PFC

The PFC serves as the “cognitive center” of the brain with an integral role in a number of functions including working memory, attention, emotion, cognition, executive control and behavioral inhibition (Damasio et al., 1994; Dias et al., 1996; Goldman-Rakic, 1999; Varga et al., 2001; Yamasaki et al., 2002). The PFC functional connectivity map includes olfactory bulb, hippocampus, subcortical regions such as thalamus, NAcc and CPu, as well as visual, motor, somatosensory, insular and cingulate cortices. These connections are involved in multiple brain networks sub-serving numerous functions. For example, connections from the PFC to cingulate cortex and NAcc have been implicated in emotional processing (Hajos et al., 1998). While connections to the thalamus and cortical regions might be related to the modulatory function of the PFC on the thalamo-cortical input (Anderson and DeVito, 1987), connections to the olfactory bulb might be related to the finding that olfactory bulbectomy alters NMDA receptor levels in the rat PFC (Webster et al., 2000). In terms of the connections between the PFC and hippocampus, there are substantial evidences showing that the hippocampus projects densely to the medial PFC (Swanson, 1981; Ferino et al., 1987; Jay et al., 1989; Jay and Witter, 1991; Carr and Sesack, 1996; Ishikawa and Nakamura, 2003). Additionally, Vertes et. al. found that nucleus reuniens of the midline thalamus might serve as the link sending projection to the hippocampus from the medial PFC (Vertes et al., 2007). Such connections play a critical role in facilitating the well recognized role of PFC in memory processing (Baddeley, 1998). Taken together, these results demonstrate the capability of using intrinsic BOLD activities to simultaneously map multiple brain networks. This capability should be critical for studying the potential inter-relationships among different brain networks.

The Circuit of the Thalamus

The thalamus is one of the most important subcortical structures because it relays and coordinates almost all neural signals from the sensory and motor periphery and the cerebral cortex (Guillery, 1995). The thalamus is known to be connected to the visual, auditory, somatosensory and motor systems (Scannell et al., 1999). All these connections, which comprise the thalamo-cortical network, were reliably identified based on intrinsic fluctuations of the BOLD signal in the present study. Moreover, the thalamus connectivity map shows connections to medial PFC and cingulate cortices which are presumably part of the frontal-limbic network (Scannell et al., 1999). In addition to the thalamo-cortical network, a strong connection between thalamus and hippocampus was also found in the functional connectivity map. Inputs from the thalamus to CA1 of hippocampus have been well described anatomically in previous reports (Herkenham, 1978; Yanagihara et al., 1987; Haase, 1990; Wouterlood et al., 1990; Dolleman-Van Der Weel and Witter, 1996). Indeed it has been suggested in a recent study that this thalamo-hippocampal connection bypasses the trisynaptic/commissural pathway thought to be the exclusive excitatory drive to CA1, and have very different physiological effects on CA1 pyramidal cells (Bertram and Zhang, 1999). Conversely, the anterior thalamic nuclei are known to receive input from the hippocampal formation (Nauta, 1956; Aggleton et al., 1986). These two regions and their bi-directional connections are critical components of the anatomical system sub-serving spatial memory (Henry et al., 2004). Moreover, our data also suggest strong connections between thalamus and CPu which, together with connections in thalamo-hippocampal pathway, form the hippocampal-basal ganglia-thalamo-hippocampal

loop (Sil'kis, 2007). These results provide a composite view of the brain circuitry connected to the thalamus in the conscious rat. The ability to obtain this data in a convenient and noninvasive manner should have tremendous impact on research assessing brain functions involving arousal and the thalamus.

The Circuit of the Retrosplenium

Research on the function of the retrosplenial cortex has garner a lot of attention within the past decade. It is now known that the retrosplenium plays a key function in a range of cognitive functions including spatial memory (Sutherland et al., 1988; Whishaw et al., 2001; Vann and Aggleton, 2002; Vann et al., 2003; Harker and Whishaw, 2004; St-Laurent et al., 2009), simultaneous processing of multiple stimuli (Lukoyanov and Lukoyanova, 2006; Keene and Bucci, 2008a, 2008b), episodic memory (Maguire, 2001a; Svoboda et al., 2006), navigation (Maguire, 2001b; Epstein, 2008), imagination and thinking about the future (Vann et al., 2009). It is also evident that the retrosplenium is consistently compromised in the most common neurological disorders that impair memory (Vann et al., 2009). Axonal tracing studies in monkeys have revealed reciprocal connections between the retrosplenium and hippocampus and thalamus (Kobayashi and Amaral, 2007). Other notable connections include reciprocal retrosplenial pathways to the prefrontal cortex (Morris et al., 1999). All these connections, which can be described as the Papez circuit (Papez, 1995), are clearly mapped in the present study. More interestingly, it has been suggested that the retrosplenium is one of the main hubs of the default mode network involving medial frontal and medial temporal lobe regions, lateral and medial parietal areas and the retrosplenial cortex (Vann et al., 2009). These areas, which have been demonstrated in both human and monkey, are more active at the resting state (Gusnard et al., 2001b; Gusnard et al., 2001a; Raichle et al., 2001; Raichle and Snyder, 2007; Vincent et al., 2007). However, mapping the default network in conscious rats has never been attempted. Although we cannot conclude that the functional map from the retrosplenium in the rat shown in the present study is equivalent to the default-mode network found in the human and monkey, we did observe similar brain regions within this network such as the frontal, temporal and parietal areas. Therefore, the results in the present study will facilitate our understanding of functional homology across species.

In summary, in this study we have created resting-state functional connectivity maps from seed regions that are crucial to cognitive and emotional processing in conscious rats. The advantage of using anatomically well-defined seed regions is that functional connectivity identified is *specific* to the seed regions pre-selected. Multiple brain networks across the whole brain were revealed in this study. Compared to individual connection tracing studies focusing on the connections of a few brain structures in a few individuals, the results in the present study provide a convenient and non-invasive tool to systematically investigate the dynamic connectional architecture of brain networks in animal models.

Acknowledgments

We thank Dr Wei Chen for her technical assistance. This publication was made possible by the NIH Grant Number [1R01 MH067096-02](#) and [5R01DA021846-02](#) from the National Institute of Health. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Figure 1.

Functional connectivity map from the seed of the PFC in adult rats. Regions in the networks involving the PFC include olfactory blub, hippocampus, subcortical regions such as thalamus, NAcc and CPu, and a widely spread cortical areas including visual, motor, somatosensory, insular and cingulate cortices.

Figure 2. Functional connectivity map from the seed of the thalamus. Regions identified to be connected to the thalamus include visual, auditory, motor, somatosensory, retrosplenial, cingulate and prefrontal cortices, and CPu.

Figure 3.

Functional connectivity map from the seed of the retrosplenium. Brain regions connected to the retrosplenium at the resting state are predominantly located in the cortical ribbon covering the visual, motor, somatosensory, cingulate and prefrontal cortices. In subcortical areas, thalamus appears to be connected to the retrosplenial cortex.

Figure 4.

Low-frequency BOLD fluctuations (0.01–0.1Hz) averaged from the regions of hippocampus and thalamus from four independent fMRI runs in one representative rat. The temporal patterns of intrinsic BOLD activities in the two regions are extremely similar within each run, although their temporal evolutions are dramatically different across runs.

Figure 5.

Evaluate the reliability of mapping functional connectivity in conscious animals from the seed of the retrosplenium. The subjects were randomly divided into two groups. Functional connectivity maps were separately created from (a) one subject group and (b) the other subject group. Visual inspection manifests that the maps created are highly reproducible.

Figure 6.
Functional connectivity map from the seed of the PFC in an expired rat brain.

Figure 7.

A 3D view of functional connectivity maps from three seed regions of (a) PFC, (b) Retrosplenial cortex, and (c) thalamus.