Mapping Thalamocortical Networks in the Awake Rat Brain using Resting-State Functional Connectivity

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Introduction Thalamocortical connectivity plays a vital role in brain function. Anatomical and functional aspects of thalamocortical networks have been extensively studied in animals by numerous invasive techniques. Non-invasively and systematically mapping thalamocortical networks in humans has also been demonstrated by utilizing resting-state functional magnetic resonance imaging (rsfMRI). However, success in imaging multiple thalamocortical networks in animals is rather limited. This is largely due to profound impact of anesthesia used in most animal experiments on functional connectivity. In the present study we employed an awake animal imaging approach to systematically map thalamocortical connectivity for multiple thalamic nucleus groups in rats.

Methods Forty two adult male Long-Evans rats were acclimated to MRI procedures as described before[1,2] and were fully awake during MR imaging sessions conducted on a Bruker 4.7 T magnet. Functional images were acquired with a gradient-echo EPI sequence with TR=1s, TE=30ms, flip angle=60°, matrix size=64×64, FOV=3.2×3.2cm, 18 1mm thick slices. Standard preprocessing steps including co-registration, motion correction, spatial smoothing, regression of motion parameters and signals of white matter and ventricles, and 0.002-0.1Hz band-pass filtering were applied. Seven thalamic nucleus groups (Fig 1) were separately used as seeds region of interest (ROIs) in Pearson correlation analysis on a voxel-by-voxel basis[1,3]. Correlation coefficients were z-transformed and subject to linear mixed-effect analysis with subjects as the random effect and z scores as the fixed effect. Maps were thresholded at p value < 0.05, False Discovery Rate corrected.

Results Seed-based correlational analysis demonstrated robust functional connectivity for each thalamic nucleus group in the cortex. Multiple thalamocortical networks were simultaneously displayed using the whole-brain winner-take-all approach (Fig 2). The map clearly showed highly organized cortical connectivity patterns for individual thalamic nucleus groups that were in excellent accordance with the known anatomical connectivity relationship. For example, dominant LG connectivity was observed in the visual cortex (labeled in red); and MG connectivity was mostly in the auditory cortex (labeled in yellow). In addition, LAT showed robust connectivity in somatosensory cortex (labeled in blue). Furthermore, dominant MED cortical connectivity was seen in the prefrontal cortex and cingulate (labeled in green). To further improve the spatial specificity for three thalamic nucleus groups that had significant mutual connectivity (MTN, ATN and MED), partial correlation analysis was utilized. Resulting maps showed distinct connectivity patterns for each seed (Fig 3). MED retained the characteristic functional connectivity with prefrontal regions including infralimbic (IL), prelimic (PL), anterior cingulate (ACC) and orbital frontal cortex (ORB). Interestingly, its connectivity pattern obtained by partial correlation analysis was consistent with the pattern in the winner-take-all map (Figure 2, labeled in green). Also similar to the pattern in the winner-take-all map, MTN connectivity was dominant in subcortical areas including lateral septal complex (LSX, Fig 2, labeled in brown). In contrast, the ATN showed a distinct pattern with its specific connectivity in retrohippocampal regions—a more posterior part of the brain. To further examine the validity of connectivity profiles revealed by partial correlation analysis, we compared the functional connectivity results of the three nucleus groups with their anatomical connectivity pattern in existing tracing studies (Fig 3, tracing results adopted from[4-6] respectively). For each of all three nucleus groups, remarkable correspondence was observed between the functional connectivity pattern and the tracer destination from the injection site.

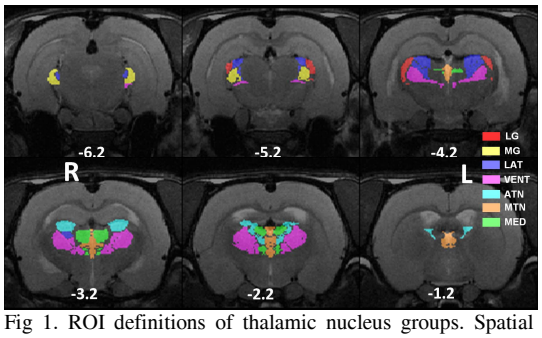

Fig 1. ROI definitions of thalamic nucleus groups. Spatial maps of seven thalamic nucleus groups were displayed in different colors overlaid on anatomical images. Distance to bregma (in mm) is labeled at the bottom of each slice. L, left, R, right.LG, lateral geniculate nucleus, MG, medical geniculate nucleus, LAT, lateral group of dorsal thalamus, VENT, ventral group of dorsal thalamus, ATN, anterior group of dorsal thalamus, MTN, midline group of dorsal thalamus, MED, medial group of dorsal thalamus.

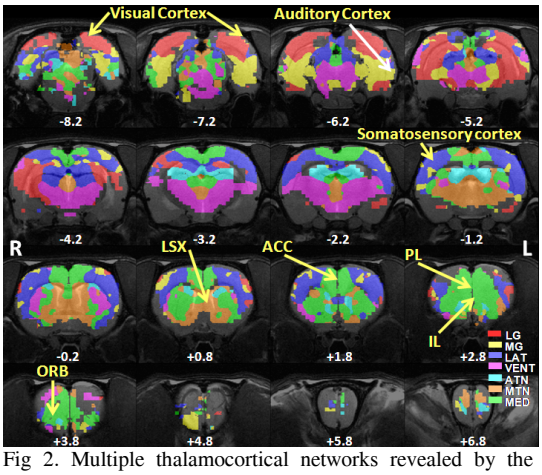

Fig 2. Multiple thalamocortical networks revealed by the winner-take-all approach. The color of each voxel was labeled as the color of winning thalamic nucleus seed. Distance to bregma (in mm) is labeled at the bottom of each slice.

Conclusion Taken together, these findings provided important evidence supporting the validity of rsfMRI in awake animals. More importantly, they have made it possible to non-invasively investigate the function, neuroplasticity and mutual interactions of thalamic nuclei and thalamocortical networks in animals.

References **1**. Zhang et al. J Neurosci Methods 189:186-196. **2**. Liang et al. J Neurosci 31:3776-83. **3**. Liang et al. Neuroimage 59:1190-9. **4**. Krettek et al. J Comp Neurol 171(2):157-91. **5**.Van Groen T et al. J Comp Neurol 358(4):584-604. **6**. Vertes RP et al. J Comp Neuron. 508(2):212-37.

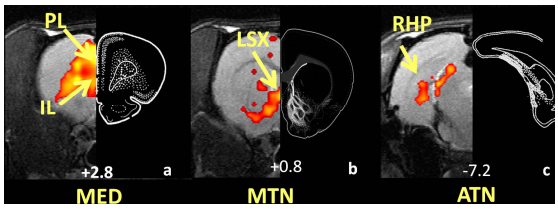

Fig 3. The spatial patterns of thalamocortical connectivity showed great agreement with the corresponding anatomical connectivity patterns identified in literature tracing studies. Left panels are functional connectivity map obtained by partial correlation analysis. Right panels are adopted from tracing studies with the injections sites originating from mediodorsal nucleus (part of MED, Panel a), paraventriclar nucleus (part of MTN, Panel b), anterodorsal nucleus (part of ATN, Panel c), respectively, in the rat[4,5,6]. White dots or lines indicated labeled neurons in destination regions. RHP, retrohippocampal region.