

Mapping the genomic influence of a single chromosome on the alterations of functional connectivity before and after somatosensory stimulation using fMRI and fcMRI

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Introduction: The genetic influence of specific gene variants on a behavioral phenotype and its underlying neural mechanisms is supported by a building body of preclinical and clinical work. However, the penetrance of individual chromosome on brain biology remain poorly understood. The field of Imaging genetics with noninvasive fMRI and fcMRI has provided a novel approach to investigate the genetic impact on normal brain function and disruption due to task or neurological disorder (1). Rat chromosome 13 is known to encode for many of the genes involved in cardiovascular regulation(2), we tested the hypothesis that the genomic influence of chromosome 13 contributes to the alterations of intrinsic functional connectivity before and after electrical forepaw stimulation using BOLD-fcMRI, in a Brown Norway (BN)/Dahl salt-sensitive (SS) consomic rat model.

Materials and Methods: Rat strains: For parental strains, 13 male BN and 10 male SS were included. For consomic strains, 9 male SS-13^{BN}/Mcow rats were chosen for this study. All studies and protocols were approved by the IACUC of the Medical College of Wisconsin. Rats were maintained on regular normal salt diet before BOLD-fMRI and BOLD-fcMRI experiments. **Anesthesia:** The animals were initially anesthetized with isoflurane (5% for induction and 2% for set-up). A MR-compatible needle electrode was inserted subcutaneously between the second and fourth digits of left forepaw for electrical stimulation. To minimize the confounding effects of surgical procedures on sensation (noxious stimulation), oral intubation was used for mechanical ventilation, and tail vein catheterization was used for continuous delivery of Medetomidine (0.05mg/kg/h) and Pancuronium bromide (2mg/kg/h) after isoflurane was tapered off for fMRI. **fcMRI data acquisition:** The animals were imaged in a Bruker 9.4T animal scanner equipped with a Bruker surface linear transmit coil and a Bruker surface receive coil. Gradient echo (single shot EPI, TE= 19.437 ms, TR=2 s. matrix size=96 x 96, FOV= 3.5 cm, number of repetitions = 110, 10 contiguous interleaved 1 mm slices.) were acquired with a total time of 3 min 40 s. Left forepaw was stimulated at 2 mA, 2 mS at 3, 5, 7 and 10 Hz frequencies in a standard fMRI boxcar sequence. Two sets of resting-state fcMRI data were acquired before and 5 minutes after stimulation for each rat (fig.A). Blood oxygen saturation, inspired/expired O₂ / CO₂, temperature, heart and respirations rate were maintained within physiological ranges. **fcMRI data processing and analysis:** The stimulation activated voxels detected in all strains within the right S1FL were used as a seed-region for resting-state fcMRI analysis of the right S1FL network. For both pre- and post-stimulation fcMRI data, a functional connectivity map was generated by cross-correlation between seed and every other BOLD temporal time series in the brain. A band-pass filter was applied to the pre-processed images to keep only low-frequency fluctuations within the frequency range of 0.01–0.1 Hz. For obtaining the network pattern in each strain, a voxel-wise one-sample t-test within group subjects was used against a null hypothesis of no connectivity (P<0.005 with correction). A one-way repeated measures ANOVA was performed (P<0.05 with correction) across the three groups, with rat body weight and age as covariates, to obtain the differential functional connectivity before and after stimulation respectively. The strength of connectivity within right S1FL network was examined by post hoc test in each two strains

Results: Left forepaw electric stimuli with different frequencies induced robust positive BOLD activations in right S1FL among SS, BN, and consomic SS-13^{BN} strains. A differential activation pattern was shown across three strains to left forepaw stimulation (1). Before stimulation, fcMRI data did not show significant functional connectivity differences within the right S1FL network across the three strains (fig.B and D). However, BN rats showed a more extensive resting-state network than both SS and SS-13^{BN} rats 5 minutes post stimulation (fig.C and D). The resting-state low frequency fluctuations were more synchronized and spatially distributed to the bilateral cortical and subcortical regions in SS and BN strains due to stimulation. The SS-13^{BN} strain only showed increased connectivity along the ipsilateral cortex (fig.C). When compared the post-stimulation fcMRI data with the same threshold among three strains, a region-specific differential connectivity was detected in bilateral cortical areas, which mainly located in motor and insular cortex (fig.D). Post hoc analysis revealed the source of group-paired difference (Bonferroni correction, p<0.05). As shown in fig.E, SS demonstrated significantly increased cortical (bilateral motor and insular cortex) connectivity compared to both BN and SS-13^{BN} strains due to stimulation. BN strain showed similar connectivity with SS-13^{BN} strain after stimulation.

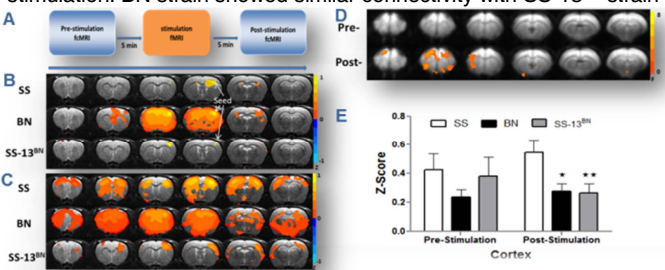


Figure (A) FcMRI with fMRI scanning paradigm. **(B)** Functional connectivity patterns of right S1FL network before left forepaw stimulations in SS, BN and consomic SS-13^{BN} rats. **(C)** Functional connectivity patterns of right S1FL network after left forepaw stimulations in SS, BN and consomic SS-13^{BN} rats. **(D)** Differential functional connectivity maps of right S1FL network among three strains before(pre-) and after(post-) stimulation ($p < 0.05$ after correction). **(E)** The quantitative strength of cortical connectivity within right S1FL network among three strains post stimulation. ($p < 0.05$: * BN vs. SS; ** SS-13^{BN} vs. SS). The color bar indicates Z-score in figure (B,C), and F-score in figure (D).

Discussion and Conclusion: FcMRI has been widely used to noninvasively map the brain functional connectivity in rats. Accumulating evidence shows that the resting-state functional connectivity is dynamically changing over time, and this connectivity status could be altered by specific biological events such as motor task, device training, pharmacological challenge, or nerve stimulation(3). The mechanism underlying this BOLD fcMRI signal alteration remains elusive. In a previous study, we have provided evidence suggesting the genomic influence of chromosome 13 on the thalamocortical networks associated with somatosensory stimulation. In the current study, by using the same inbred SS and BN rat strains combined with a consomic SS-13^{BN} strain, we demonstrated the genetic components of chromosome 13 that govern the acute alterations of contralateral S1FL functional network due to somatosensory stimulation. After regressing out the confounding effects of rat age and body weight, the introgression of entire chromosome 13 from BN strain into the SS genetic background significantly changed the dynamic properties of resting-state functional connectivity within the S1FL network. These alterations might disrupt the functional synchrony between primary somatosensory, motor as well as insular cortex. Our results not only suggest the genetic underpinnings of dynamic functional connectivity, but also provide a novel strategy to visualize the genetic effects of individual chromosome on the relevant brain networks.

Reference: 1.Li Z, et al. Neuroimage. 2013; Sep. 29. 2. Cowley AW, et al. Hypertension. 2001;37(2):456–461 3.Tung KC, et al. Neuroimage. 2012; 2013;78(9):316–241. **Acknowledgement:** This work was supported by Advancing A Healthier Wisconsin Program