Magnetic resonance imaging the genetic influence on intrinsic resting-state connectivity in Brown Norway and Dahl salt-sensitive rat brains

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Introduction: Genes are major contributors to many biological traits and susceptibility to specific diseases. However, the mechanisms of genotype action on central neural system have long been elusive (1).Recently, the spontaneous low-frequency fluctuations of the resting brain has been used to investigate functional connectivity at system-level, and the concept of neuroimaging-based intermediate phenotypes (endophenotypes) has emerged as a promising approach to map the genetic effects onto physiological processes in brain. In a parallel study, we have revealed distinct strain-specific activation in the brains of two genetically different inbred strains, Brown Norway(BN) and Dahl salt-sensitive(SS/Mcwi), by BOLD-fMRI under a well-established electrical stimulation paradigm. Here, we address for the first time the genetic influences on intrinsic neural connectivity in a resting-state fMRI study of same rat strains. Specifically, functional neural networks that are associated with electrical stimulation were analyzed using a seed-based voxel-wise approach (2).

Materials and Methods: Animal strains: Thirteen male BN, ten male SS were used for this study. All rats were maintained on regular dietary under the same environment before BOLD-fMRI 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. Oral intubation was used for mechanical ventilation, and tail vein catheterization was used for continuous delivery Medetomidine (0.05mg/kg/h) and Pancuronium bromide(2mg/kg/h) after isoflurane was tapered to zero for fMRI. **fMRI**: 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. Two initial 'resting-state" EPI scanned were carried out without any stimuli prior to stimulation procedures. Left forepaw was electrically stimulated at 2 mA D/C amplitude, 2 mS pulse-width at 7 Hz frequencies in a standard fMRI boxcar sequence using 40 sec off/20 sec on/40sec off for a total of three blocks. Blood oxygen saturation m inspired/expired O_2 and CO_2, temperature, heart and respirations rate were continually monitored through MR Sessions. All parameters were maintained within physiological ranges. **Data analysis**: AFNI software was used for BOLD-fMRI processing. Seed-based resting-state fMRI approach was employed to measure the intrinsic functional connectivity of somatosensory network (primary somatosensory cortex (S1FL) and caudate putamen (CPU). Seeds for S1FL and CPU were identified by comparing the activation maps of left forepaw electrical stimulation with 2 mA D/C amplitude, 2 mS pulse-width at 7Hz frequency between SS and BN groups (Figure d). Two sets of EPI scans were concatenated and coregistered to an ideal anatomy. The functional connectivity map was generated by cross-correlation between seed and other BOLD temporal time series in the brain. For the network pattern in each group, a voxel-wise one-sample *t*-test within group subjects was performed against a null hypothesis of no connectivity (P<0.005 with correction). To obtain the difference in connectivity strength between two groups, a two-sample *t*-test was used (P<0.05 with correction) with rat body weight and age as covariates.

Results: Analyses of stimulation activation patterns between two groups revealed two differential brain regions: ipsilateral S1FL and bilateral CPU (Figure d). In SS group, the functional connectivity patterns of rS1FL was observed in the bilateral somatosensory cortex and motor cortex (M1/M2) as well as right visual cortex, the functional network for both side CPU was mainly on right motor cortex (Figure a). In contrast to the SS group, BN rats displayed much more extensive connectivity within the brain. The differential brain regions between two groups in terms of right S1FL network were found in contralateral S1FL, S2, septal nuclei, and preoptic areas. For both CPU networks, the main regions were located in bilateral S1FL, insular cortex, right M1/M2, and right hippocampus area.

Discussion and Conclusion: It has long been recongnized that behavioral phenotypes are governed by genetic variants that affecting the structural and functional properties of neural circuits.(1). Recently, several neuroimaging methods have being used to explore the complex relationship between genes, neural circuits, behavior and found several intermediate phenotypes affecting neural network that are associated with some candidate genes of interests. (3-4). In the current study, after controlling the confounding effects of environmental variance, age and body weights, SS rat exhibited differential intrinsic functional connectivity patterns in primary somatosensory and caudate putamen network in comparison to BN rats. The distinct patterns of difference are mainly in sensorimotor, septal nuclei and insular cortex. These findings strongly implied a genetically driven influence on the intrinsic functional network properties at rest. These task-specific regional differences in neural network can speculatively suggest an "endophenotype" or imaging marker of genetic component of under specific biological events.

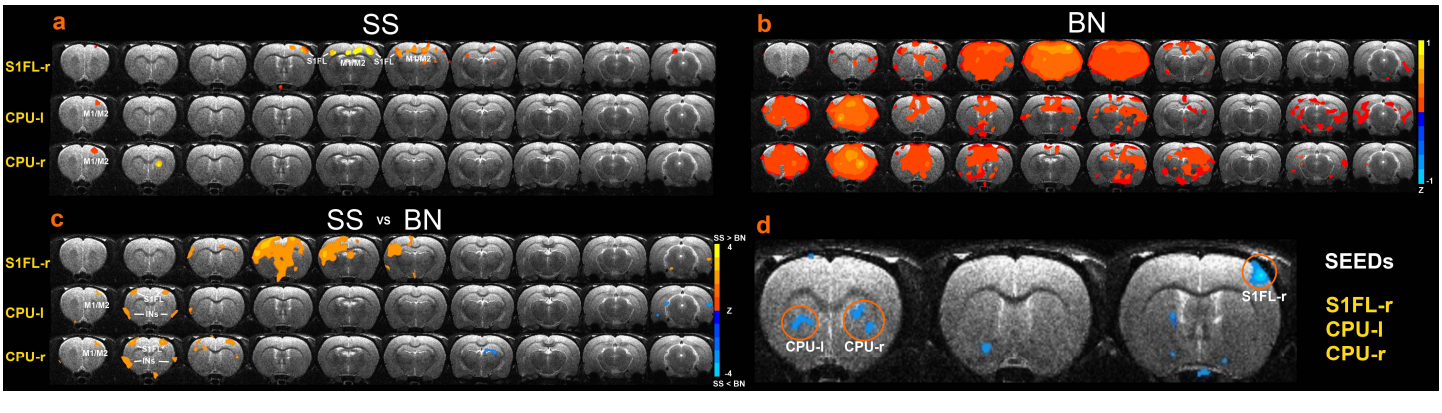

Figure . Resting-state connectivity networks of contralateral primary somatosensory and bilateral Caudate Putamen. (a) Network pattern in SS group. (b) Network pattern in BN group.(c) Differential connectivity between SS and BN groups. (d) Seed regions for both groups.

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