Neuroimaging measure as an endophenotype for genetic effects on electrical stimulation in Brown Norway and Dahl salt-sensitive rat strains

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Introduction: The association of a specific behavioral outcome with a specific gene variant has been weak and controversial. However, the penetrance of gene effects at the level of brain biology is more obvious and consistent than that at the level of behavioral phenotypes. Neuroimaging genetics techniques have provided a unique tool to explore and evaluate the phenotypic impact of brain-relevant genetic polymorphisms longitudinally and noninvasively. The goal of this study was to reveal the region-specific effects of genetic differences between two inbred rat strains, Brown Norway (BN) and Dahl salt-sensitive (SS/Mcwi), on a biologic measure in brain using BOLD-fMRI under a well-established task paradigm.

Materials and Methods: Animal Strains: Thirteen male BN, ten male SS and nine male SS-13^{BN}/Mcwi consomic rats were used for this study. All rats were maintained on regular dietary 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. 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 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 repetitioins = 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 D/C amplitude, 2 mS pulse-width at 3, 5, 7 and 10 Hz frequencies in a standard fMRI boxcar sequence using 40 sec off/20 sec on/40sec off for a total of three blocks. Two sets of EPI were acquired at each stimulus frequency for each rat. 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**: Two sets of EPI scans were averaged and coregistered to an ideal anatomy. Forepaw stimulation activated maps were created by an F-test with a P value of 0.005 after corrected for multiple comparisons. One-sample *t*-test was performed to generate the within-group activation patterns in each group (P<0.005 with correction), Two-sample *t*-test was used to determine the group-level difference patterns between groups (P<0.05 with correction). The numbers of activated voxels that survived the threshold and their BOLD percentage changes were further analyzed with rat body weight and age as covariates(1).

Results: While left forepaw stimuli with different frequencies induced robust positive activations in contralateral primary somatosensory cortex (S1FL) in both SS (Figure a) and BN(Figure b) groups, BN rats showed a significant increased activate volume(voxel numbers) in S1FL regions under different frequencies. The activation foci showed an increased spatial distribution within S1FL with the increased stimulus frequencies in both groups. In addition to the positive BOLD activation in contralateral S1FL in both groups, bilateral negative BOLD signals were detected in Caudate Putamen (CPu) in SS group at 3Hz, and contralateral positive BOLD signals were detected in secondary somatosensory cortex (S2) and Thalamus (TH) in BN group. When compared the group patterns, Differential BOLD activations were mainly detected in contralateral S1FL, S2, TH, and bilateral CPu regions. Within S1FL regions, although the BOLD percent changes were similar in two groups, the BOLD activated volumes in BN group were significantly increased at each frequencies compared to SS group ($p < 0.05$ with corrections).

Discussion and Conclusion: Inbred rat strains have been widely used in the development of physiological and pathophysiological models to study human disease because of the less interanimal variation. Striking differences among rat strains have been found in resistance to myocardial injury, sensitivity to painful stimuli, and neuroprotectivity to cerebral ischemia as well as other neurological disorders(2). In the current study, we demonstrated robust differences in response to a well-established electrical stimulus paradigm in two inbred rat stains by BOLD-fMRI. By minimizing the confounding environmental influences and controlling the effects of rat age and body weight, SS rats exhibited less activation volumes in somatosensory cortex at different frequencies compared to BN rats, although the BOLD percentage changes were not significantly different between two groups. Based on the comprehensive assessment of task-relevant BOLD-fMRI activation, we believe that there are likely to be genetic components responsible not only for the different sensitivity to stimulations, but also for the altered somatosensory neuropathway. These results suggest a new approach to visualize the genetic effects on the brain using neuroimaging measures.

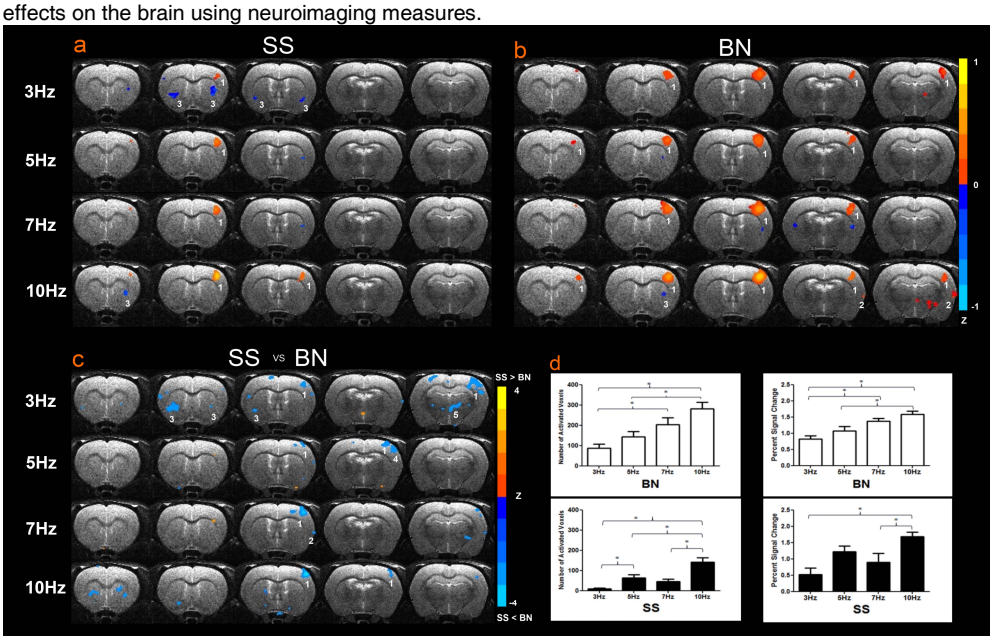

Figure. Left forepaw stimulation-induced BOLD activations at different frequencies. Activation maps are overlaid on ideal anatomical images. Positive BOLD responses are in warm color, and negative BOLD responses are in cold color. (a) Activation maps of **SS** group. (b) Activation maps of **BN** group. (C) Group-level differential activation maps between **SS** and **BN** groups(c). (d) Numerical presentation of activated volume (left) and percent changes of BOLD signals. (*p < 0.05). Primary somatosensory cortex (1), Secondary somatosensory cortex(2), Caudate Putamen(3), Primary somatosensory cortex-Barrel field(4), Thalamus(5).

Reference: 1. Pawela CP, et al. Neuroimage. 2009; 46(4):1137-47; 2. Baker JE, et al. Am J Physiol Heart Circ Physiol. 2000; 278(4):1395-400.

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