RODENT RESTING-STATE FMRI IN THE TRANSITION TO CHRONIC PAIN: RELATING FUNCTIONAL CONNECTIVITY TO RECEPTOR EXPRESSION CHANGES

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Purpose

Functional connectivity in nucleus of accumbus (NAc) is a potential biomarker for predicting chronic pain. Patients with chronic pain display distinct resting-state fMRI (rs-fMRI) compared to healthy subjects. Functional connectivity in NAc and prefrontal cortex (PFC) is enhanced in the chronic pain patients[1]. Furthermore, it has been shown that this enhanced functional connectivity in NAc is correlated with the pain intensity of chronic pain patients[2]. These findings suggest that functional connectivity in NAc may be critical in the transition to chronic pain.

Despite these advances, little has been known about the pre-existing conditions of the rs-fMRI in the chronic pain patients. This information is essential to understand the mechanisms necessary for the transition from the acute to chronic pain. rs-fMRI in the animal provides an opportunity to investigate the mechanisms for the transition to chronic pain. Moreover, it allows us to examine cellular and molecular mechanisms corresponding to changes in NAc-PFC functional connectivity, which further enable us to identify potential therapeutic treatments to prevent chronic pain. The purpose of this study is to evaluate rs-fMRI and receptor expression in NAc at different time points from a peripheral nerve injury (transition from acute to chronic pain) in a rodent model of neuropathic pain.

Methods

The experimental paradigm is summarized in Fig1A. All animals were scanned before injury (*b*). At 5 days (*d5*) and 30 days (*d30*) after injury, half of the animals were scanned and sacrificed for Q-RTPCR of dopamine receptors (D1, D2) and cannabinoid receptor (CB1) expression in the NAc. Tactile allodynia thresholds were tested on animals prior each scan. Spared nerve injury (SNI) is an animal model of persistent peripheral neuropathic pain. Sham was served as the control in which the same surgical procedures as in SNI were performed except there was no nerve lesion.

A 7 T Bruker ClinScan Scanner was used for imaging. Both high-resolution anatomical and functional resting-state scans were performed on each animal. Multislice T2-weighted images were acquired using a RARE sequence with 1.0 mm slice thickness, 0.273x0.273 mm^2 voxel size and used as anatomical reference. For the functional resting-state scans, a gradient-echo EPI sequence of 14 slices with 1.0 mm slice thickness, 0.5x0.5 mm^2 voxel size were acquired with repetition time (TR) of 1.3 s, echo time (TE) of 25 ms and 300 volumes. Anesthesia was induced and maintained with 1.75-2% isoflurane mixed with air. Physiological signals (respiratory rate, pulse oximeter and body temperature) were monitored and recorded during scanning.

The data analysis was carried out using FSL 5.1. Before statistical analysis, each functional scan was corrected for slice timing and for motion, spatially smoothed with a FWHM of 0.8 mm, and high pass filtered with a cutoff of 100 sec. The regression of motion parameters and global signal were carried out. NAc contralateral to the injury site was used as seed. The correlation coefficient for the functional connectivity of NAc was calculated using Fisher transformation. The correlation between functional connectivity and receptor expression in NAc was pooled from SNI animals of *d5* and *d30*.

Results

The tactile allodynia thresholds of all SNI animals decreased at *d5* and *d30*, but not in Sham animals (Fig 1B). We observed that functional connectivity of NAc enhanced with prefrontal regions at *d30* (Fig 1C and 1D). CB1 receptor was up-regulated at *d5*, while D1, D2, and CB1 receptors were down-regulated at *d30* (Fig 1E). Significant correlations between regional functional connectivity and receptors expression were observed in SNI (Fig1F). There were no correlations of connectivity or receptors expression to tactile allodynia.

Conclusions

This is the first demonstration of a link between human and animal fMRI for transition to chronic pain, and the first evidence relating changes in receptors expression and functional connectivity. Enhanced functional connectivity of NAc with prefrontal regions in SNI animal provided correspondence to increased PFC-NAc connectivity we observed in chronic back pain patients. CB1R and D2R showed opposite relationships to NAc functional connectivity, consistent with the evidence that dopamine and endocannabinoid systems exert a mutual control on each other.

References

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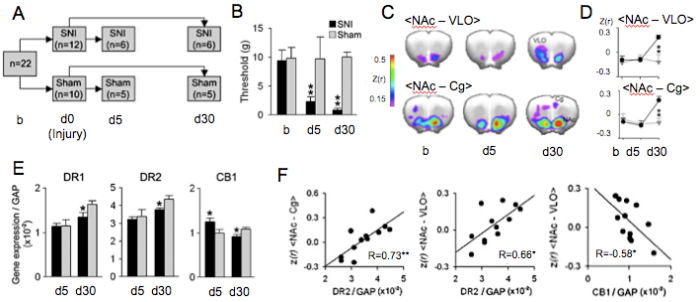

Figure1. rs-fMRI and receptors expression in the transition from injury to neuropathic pain. **A**. Experimental paradigm. **B.** Tactile allodynia threshold. **C.** Functional connectivity for a seed in the NAc. **D.** Interconnectivity changes of anterior cingulate (NAc-Gg), and prefrontal orbital cortex (NAc-VLO). **E.** D1, D2, CB1 receptors repression. **F.** Relationship between functional connectivity and receptors expression in NAc in SNI animal. *p< 0.05, **p<0.01