Diffuse noxious inhibitory controls and brain networks are modulated in a testosterone-dependent manner in Sprague Dawley rats

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Introduction

Diffuse noxious inhibitory control (DNIC), which involves endogenous pain modulation, has been investigated as a potential mechanism for the differences in pain modulation observed between men and women, though the literature shows contradictory findings. We used a capsaicin-induced DNIC behavioral assay and resting state functional magnetic resonance imaging (rsfMRI) to assess the effect of testosterone on pain modulation and related brain circuitry in rats. We hypothesized that testosterone is required for DNIC that leads to efficient pain inhibition by increasing descending pain modulation.

Methods

Animals

Age-matched adult male, female, and GDX male Sprague-Dawley rats.

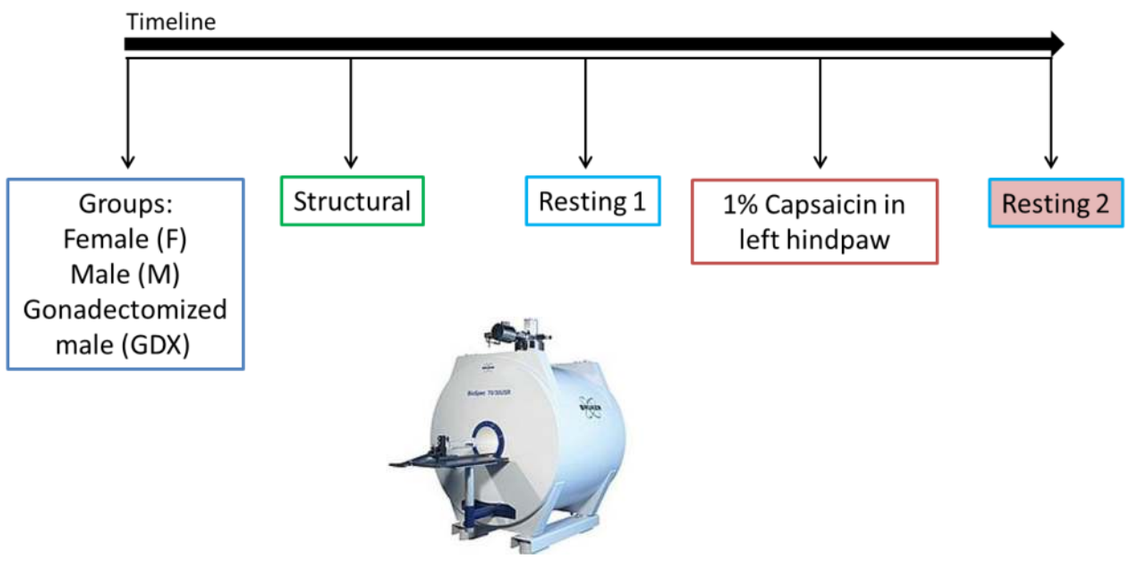
8 weeks old; 250–300 g for males and 225–260 g for females.

DNIC - nociceptive threshold

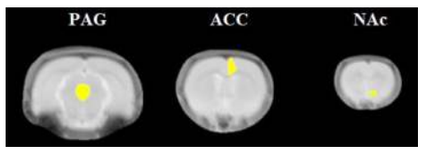
Capsaicin and mechanical stimulation

Hindpaw withdrawal thresholds to noxious mechanical stimulation were measured before and 15, 30, 45, 60 and 90 min after capsaicin injection (1% in 30 µl) into the left forepaw. PBS (30 µl) was injected in the control groups. Mechanical sensitivity of the left hindpaw was assessed with the Randall–Selitto test. Two-Way Repeated Measures ANOVA with Holm- Sidak method for correction of multiple comparisons were performed to determine significant group and time effects.

MRI design, data acquisition, preprocessing and statistical analyses



Rats were scanned on a Bruker 7T MRI scanner. During scanning, rats were anesthetized with ≤1.5% isoflurane. Functional scans (TR = 1500 ms, in plane resolution = 450 µm, slice thickness 1 mm) were acquired during 15.5 minutes. rsfMRI scans were done before and after capsaicin injection (1% in 30 µl) into the left hindpaw to analyze periaqueductal gray (PAG), anterior cingulate cortex (ACC) and nucleus accumbens (NAc) connectivity to the whole brain. We used seed-based analysis to assess how connectivity to these regions varies during DNIC. Group analysis was performed using a two-sample T-test. We examined group differences from male versus female, female versus GDX, and male versus GDX after capsaicin injection. For visualization, we extracted and plotted the averaged adjusted beta values from significant clusters for each animal and time-point. All the procedures were conducted in SPM12.



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Results

I) The analgesic effect of DNIC is attenuated in female and GDX male rats

Capsaicin treatment induced significantly greater DNIC responses in males compared to females and GDX males. GDX males and females exhibited similar extent and duration of DNIC responses (Fig. 1A). DNIC responses were not observed after PBS injection into the forepaw in any of the groups (Fig. 1B).

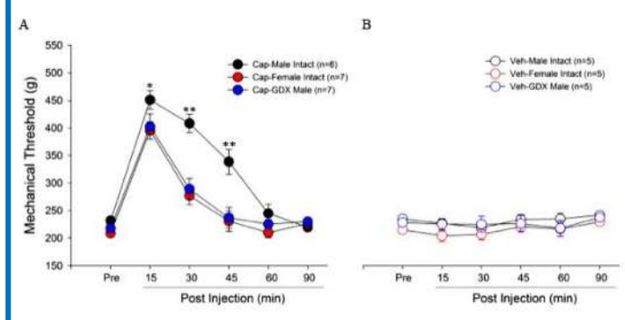
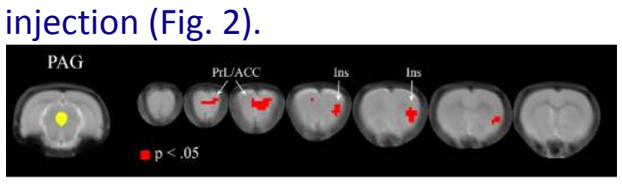


Figure 1. Testosterone significantly increases DNIC. Forepaw injection of Capsaicin (A), but not PBS (B), significantly increased mechanical thresholds (i.e., DNIC) of the hindpaw in intact male and female, and GDX male rats. (A) Males had significantly more DNIC than females and GDX males (*p ≤ .025 and **p < .001). Data are mean ± S.E.M., two-way ANOVA with Holm-Sidak method.

II) Testosterone-dependent changes in functional connectivity after DNIC induction

For PAG seed, connectivity in males was significantly increased with PrL, ACC and insula, compared to females and GDX males after capsaicin injection (Fig. 2).



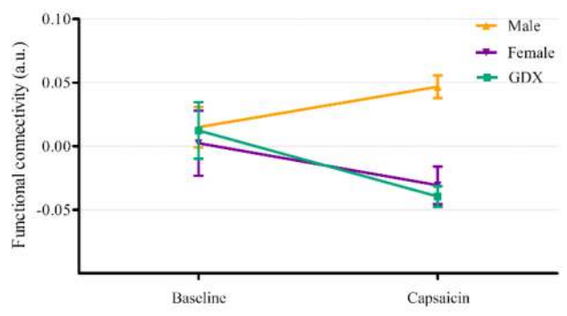
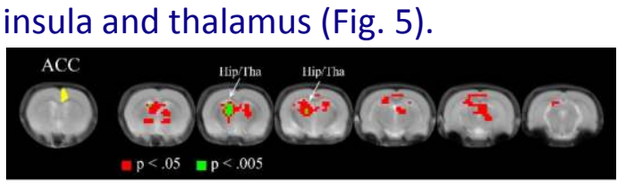


Figure 2. PAG seed region (yellow) and cluster with stronger connectivity with PrL, ACC and insula (red) in males, compared to females and GDX males. Plot shows extracted beta values (a.u.) from significant clusters (average ± S.E.M and p < .05) with cluster-forming thresholds at p < .05, .005, and .001 for each group and time-point. n = 4 per group. PrL: prelimbic cortex, ACC: anterior cingulate cortex, Ins: insula and a.u.: arbitrary units.

III) Females and GDX males had increased connectivity between the right ACC, hippocampus and thalamus (Fig. 3). GDX males also showed a stronger connectivity between right ACC and Nac (Fig. 4), and right NAc with PrL, ACC, insula and thalamus (Fig. 5).



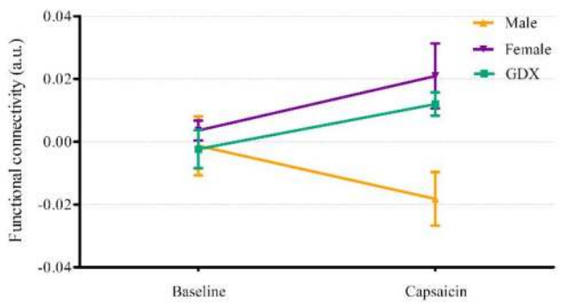
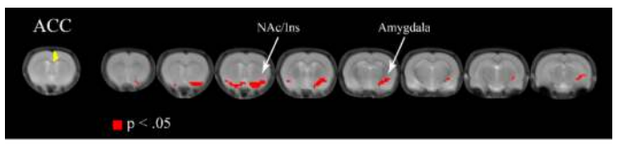


Figure 3. Right ACC seed region (yellow) with stronger connectivity with hippocampus and thalamus in females, compared to males and GDX males. Plot shows extracted beta values (a.u.) from significant clusters (average ± S.E.M and p < .05) with cluster-forming thresholds at p < .05, .005, and .001 for each group and time-point. n = 4 per group. Hip: hippocampus, Tha: thalamus and a.u.: arbitrary units.



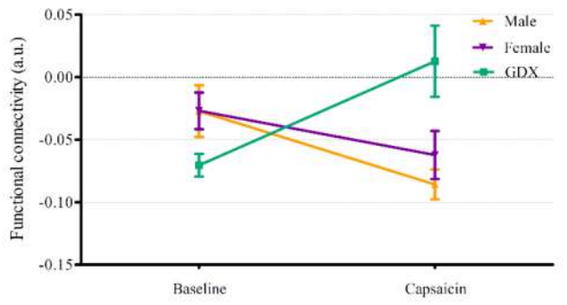
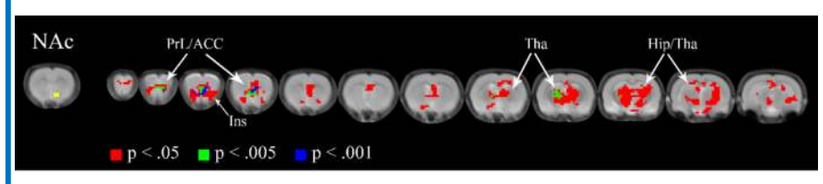


Figure 4. Right ACC seed region (yellow) and cluster with stronger connectivity with NAc, insula and amygdala (red) in GDX males, compared to females and males. Plot shows extracted beta values (a.u.) from significant clusters (average ± S.E.M and p < .05) with cluster-forming thresholds at p < .05, .005, and .001 for each group and time-point. n = 4 per group. NAc: nucleus accumbens, Ins: insula and a.u.: arbitrary units.



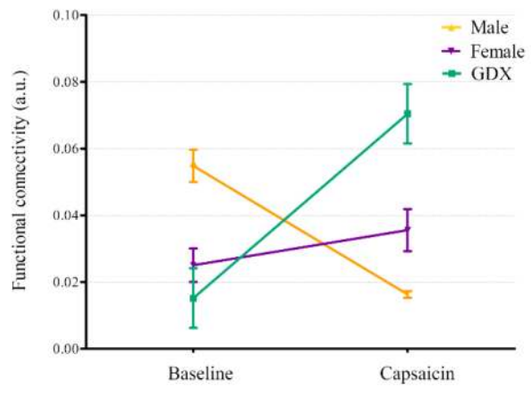


Figure 5. Right NAc seed region (yellow) with stronger connectivity with PrL, ACC, insula, thalamus and hippocampus in GDX males, compared to females and males. In contrast, male had decreased connectivity in the same areas after capsaicin injection. Plot shows extracted beta values (a.u.) from significant clusters (average ± S.E.M and p < .005) with cluster-forming thresholds at p < .05, .005, and .001 for each group and time-point.

Conclusions

Our findings suggest that testosterone plays a key role in reinforcing the endogenous pain inhibitory system, while circuitries related to reward and emotion are more strongly recruited in the absence of testosterone. Future work with larger sample sizes would be required to show reliability of the findings. Further investigation including more control groups to investigate opioid and dopaminergic receptors, pharmacological interventions and behavioral tests is needed to expand the mechanisms involved in sex-hormone related modulation of brain networks during DNIC.