Sex differences in diffuse noxious inhibitory control (DNIC) are mediated by the rostral anterior cingulate cortex (rACC) periaqueductal gray (PAG) circuit

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Introduction

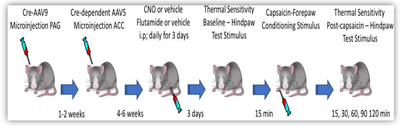
Diffuse noxious inhibitory controls (DNIC) serve as a prime example of experimental paradigms used to assess descending pain in rodents. In our previous research, we reported sex differences in DNIC in animal models. Males exhibited a more efficient DNIC response compared to females. Furthermore, we also found that greater DNIC responses in males were associated with stronger functional connectivity (FC) between the rostral anterior cingulate cortex (rACC) and the periaqueductal gray (PAG) when compared to females. In addition to these findings, we observed that DNIC is compromised in female rats under certain chronic pain conditions. The persistent phase of chronic pain responses in females was accompanied by weaker FC between PAG and rACC when compared to FC in males. In the current study, we delved into the nature of interactions between the rACC and PAG within the context of our DNIC paradigm. We utilized a capsaicin-induced DNIC behavioral assay, along with brain microinjections and chemogenetics to modulate the projections from rACC to PAG.

Methods

I - Animals: Age-matched adult male and female Sprague Dawley rats, 3-6 months old. The animals were named as young male (YM) and young female (YF).

II - DNIC-nociceptive threshold: Hind paw withdrawal thresholds to noxious thermal stimulation were measured before and 15, 30, 45, 60, 90 and 120 min after capsaicin injection (0,5% in 100µl) into the left forepaw. Thermal sensitivity of the left hind paw was assessed with the Hargreaves test.

III – Microinjections and Chemogenetics:
Naloxone microinjection: 5µg/0.5µL
Morphine microinjection: 20µg/0.5µL



Study design for chemogenetics experiments

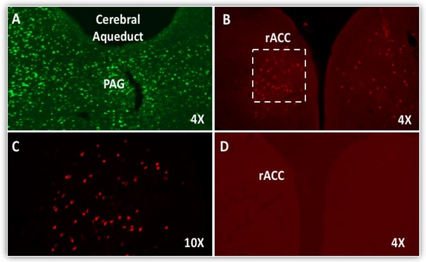


Figure 1. (A) PAG neurons transfected with ret-AAV-Cre. Green, GFP fused to Cre (B) rACC neurons transfected with AAV-DIO-DREADDi following the microinjection of ret-AAV-Cre in PAG. Red, mCherry fused to hM4Di. (C) The rACC region within the dotted box is shown in a higher magnification, (D) Injection of AAV-DIO-Cre in rACC without prior ret-AAV-Cre injection in PAG shows no mCherry expression in rACC.

Results

Systemic treatment with naloxone significantly attenuated the DNIC response in YM but not in YF (Fig 2A, B).

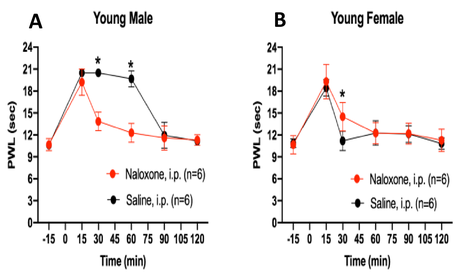


Figure 2. Naloxone i.p administration in young male and female rats. Treatment with naloxone before forepaw injection of capsaicin, significantly decreased thermal thresholds (i.e., DNIC) of the hind paw in male and female rats (A & B). (A) Males treated with naloxone had significantly less DNIC than male saline group (*p ≤ .05). Data are mean ± S.E.M., two-way ANOVA with Holm-Sidak method.

Naloxone injected directly into PAG of YM significantly attenuated the DNIC response in YM (Fig 3A). Morphine injected directly into the PAG of YF significantly enhanced the DNIC response to a similar extent to that observed in YM (Fig 3B).

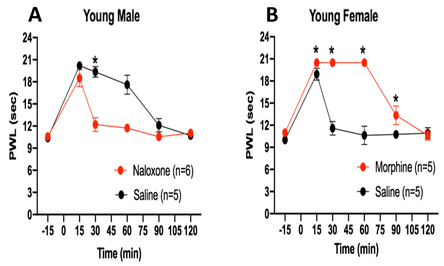


Figure 3. Naloxone and morphine significantly modulate DNIC. Naloxone (5ug/0.5uL) and morphine (20ug/0.5uL) were administrated in PAG of male and female rats, respectively. Saline was used as negative control. Forepaw injection of capsaicin, significantly decreased thermal thresholds (i.e., DNIC) of the hind paw in male and significantly increased in female rats. (A) naloxone and (B) morphine group had significantly difference in DNIC compared to saline (*p <.05). Data are mean ± S.E.M., two-way ANOVA with Holm-Sidak method.

Lidocaine significantly attenuated the DNIC response in YM in a concentration-dependent manner (Fig 4A). Morphine injected into the rACC of YF as a control failed to alter DNIC (Fig 4B).

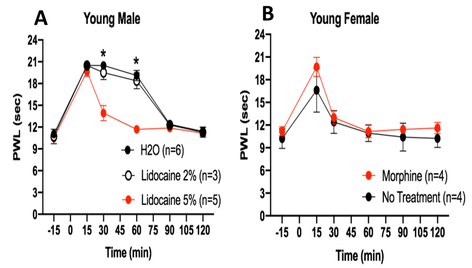


Figure 4. Lidocaine inhibits DNIC responses in YM and morphine does not modify DNIC in YF. Lidocaine 5% in the rACC, but not lidocaine 3%, significantly decreased thermal thresholds (i.e., DNIC) of the hind paw in male rats (A). Morphine in the rACC in female rats does not modulate DNIC response (B). (*p <.05). Data are mean ± S.E.M., two-way ANOVA with Holm-Sidak method.

Chemogenetic inhibition of rACC - PAG neurons led to significant reductions in DNIC in YM (Fig 5).

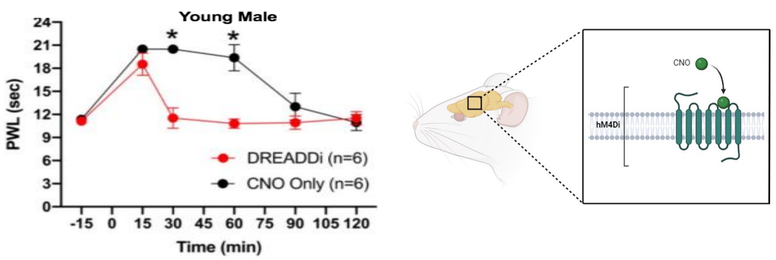
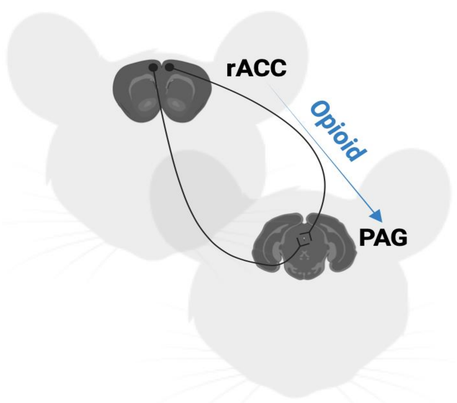


Figure 5. Inhibition of rACC neurons leads to significant reductions in DNIC in young male rats. DNIC responses were blocked in young males transfected with DREADDi and treated with CNO compared to naïve young males treated only with CNO. * p < 0.05.



Partial Conclusions	These results support our hypothesis that rACC to PAG connectivity, which involves opioid processing in PAG, is required for efficient diffuse noxious inhibitory control
Partial Conclusions	Sex differences in functional connectivity (FC) between rACC and PAG lead to sex differences in diffuse noxious inhibitory control
Future directions	Future studies will focus on detailed mechanisms involving rACC and PAG connectivity.

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