The Role of Median Preoptic Nucleus Astrocytes in Mediating Estradiol's (E2) Effects on Wake and NREM Sleep

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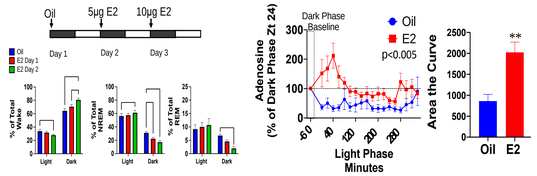
Introduction

Although 50-70 million Americans suffer from chronic sleep disorders, women are almost 2x as likely as men to experience sleep disruptions and insomnia. This discrepancy emerges at puberty and is strongly associated with fluctuations in the gonadal steroid, estrogen (E2), suggesting that gonadal hormones play a role in sleep homeostasis.

The presence of estrogen receptors in sleep active brain nuclei including the suprachiasmatic nucleus (SCN) and the median preoptic nucleus (MnPO) further suggests a role for E2 in regulating sleep-wake circuitry. Indeed, our lab has demonstrated that estradiol (E2) infusion in the MnPO increases Wake and decreases NREM sleep. However, the specific mechanism for E2 induced decrease in sleep remains unknown.

Previous work in the lab suggests a role for adenosine in mediating E2's sleep suppressive effects; E2 produced a 50% increase in MnPO extracellular adenosine and attenuates the action of specific agonists on the sleep promoting A2A-Receptor. Astrocytes represent a major source of adenosine in the brain and have been shown to play a role in the sleep-wake cycle. Thus, we hypothesize that E2 increases Wake and decreases NREM sleep by enhancing activity of MnPO astrocytes.



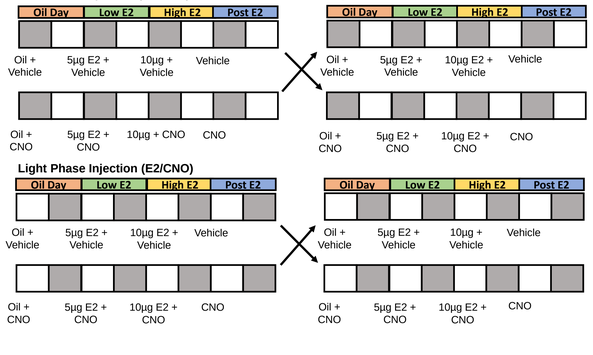
Hypothesis: E2 enhances MnPO astrocyte activity resulting in increased Wake and decreased NREM

Methods

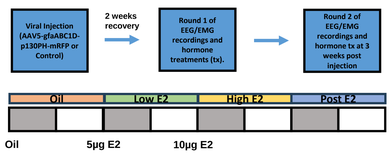
Gi-DREADD

Viral Injection (AAV-GFAP-hM4Di)

We expressed Gi-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in MnPO astrocytes to assess the role of MnPO astrocytes in mediating E2's Wake enhancing effects. Animals were then ovariectomized and implanted with transmitters to record EEG/EMG activity. Finally, animals were treated with Oil, and two consecutive injections of E2 at the start of the Dark Phase or Light Phase. Animals also received a Clozapine-N-oxide (CNO) (1.7mg/kg) injection at the time of their E2/Oil injections, and at the start of the Post Estrogen Day.



p130PH



We expressed p130PH in MnPO astrocytes to assess the role of intracellular Calcium (Ca^{2+}) signaling in MnPO astrocytes in mediating E2's Wake enhancing effects. Animals were ovariectomized and implanted with transmitters to record EEG/EMG activity and treated with Oil/E2 in the Dark Phase.

Results

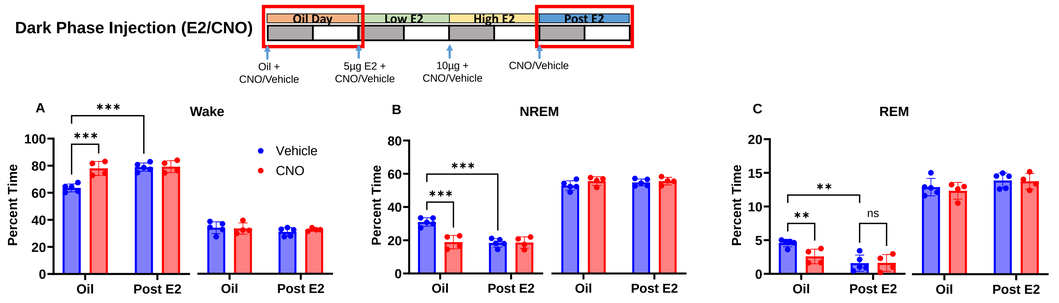


Fig 1. E2 increases Wake and decreases NREM and REM Sleep in the Dark Phase. CNO injected in the Dark Phase mimics E2's sleep enhancing effects. (A) E2 and CNO each enhance Wake in Dark Phase. Mixed Effects Analysis, Main Effect of E2 (F (1, 4) = 16.20, p=0.0158) and CNO (F (1, 4) = 24.08, p=0.008) followed by Uncorrected Fisher's LSD Oil: Vehicle vs CNO (95%CI -19.42 to -9.525, p=0.0004); Vehicle: Oil vs Post E2 (95%CI -21.06 to -9.575, p=0.0006) (B) E2 and CNO each decrease NREM in Dark Phase. Mixed Effects Analysis, Main Effect of E2 (F (1, 4) = 14.12, p=0.0198) and CNO (F (1, 4) = 27.34, p=0.0064) followed by Uncorrected Fisher's LSD Oil: Vehicle vs. CNO (95%CI8.172 to 16.09, p=0.0003); Vehicle: Oil vs Post E2 (95%CI 7.779 to 17.36, p=0.0007) (C) E2 and CNO each decrease REM in Dark Phase. Mixed Effects Analysis, Main Effect of E2 (F (1, 4) = 13.39, p=0.0216) followed by Uncorrected Fisher's LSD Oil: Vehicle vs CNO (95%CI 0.6898 to 3.254, p=0.0094); Vehicle" Oil vs Post E2 (95%CI 1.480 to 4.502, p=0.0029).

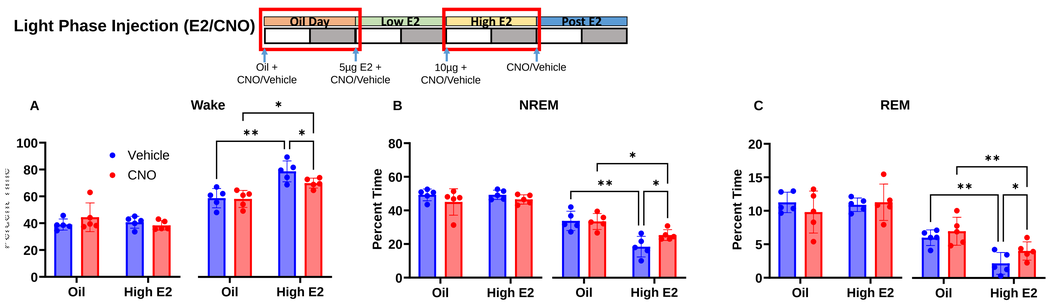
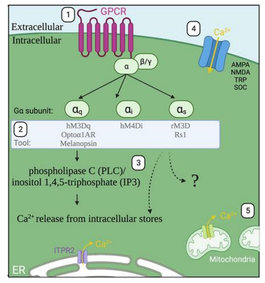
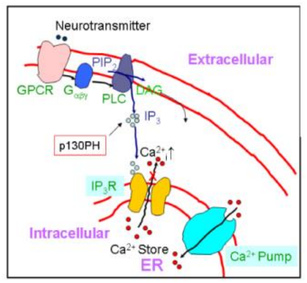


Fig 2. E2 injection in the Light Phase has no effect on Wake in the Light Phase but increases Wake and decreases NREM and REM in the Subsequent Dark Phase. CNO co-injected with E2 in the Light Phase has no effect in the Light Phase but attenuates E2's enhancement of Wake in the subsequent Dark Phase. (A) E2 increases Wake in Dark Phase, but E2's Wake enhancing effects are attenuated by CNO. 2-way ANOVA, Main effect of E2 (F (1, 4) = 29.54, p=0.0056) in Dark Phase, followed by Uncorrected Fisher's LSD High E2: Vehicle vs CNO (95%CI 0.2320 to 17.32, p=0.0463); Vehicle: Oil vs High E2 (95%CI -28.53 to -11.44, p=0.0029); CNO: Oil vs High E2 (95%CI -20.34 to -3.251, p=0.0186) (B) E2 decreases NREM in Dark Phase, but E2's effects are attenuated by CNO. 2-way ANOVA, Main effect of E2 (F (1, 4) = 28.10, p=0.0061) in Dark Phase, followed by Uncorrected Fisher's LSD High E2:Vehicle vs CNO (95%CI -13.90 to -0.1741, p=0.0465); Vehicle: Oil vs High E2 (95%CI 8.767 to 22.49, p=0.032; CNO: Oil vs High E2 (95%CI 1.396 to 15.12, p=0.0288) (C) E2 decreases REM in Dark Phase but E2's effects are attenuated by CNO. 2-way ANOVA, Main effect of E2 (F (1, 4) = 23.74, p=0.0082) and CNO (F (1, 4) = 11.75, p=0.0266) in Dark Phase, followed by Uncorrected Fisher's LSD High E2: Vehicle vs CNO (95%CI -3.601 to -0.1394, p=0.0399), Vehicle: Oil vs High E2 (95%CI 2.121 to 5.583, p=0.0035), CNO: Oil vs High E2 (95%CI 1.223 to 4.685, p=0.0091).



In astrocytes, Gi-DREADDs may act to increase Ca^{2+} release from intracellular stores.



p130PH prevents IP3 from binding to IP3-Receptor on Endoplasmic Reticulum and releasing intracellular Ca^{2+} stores.

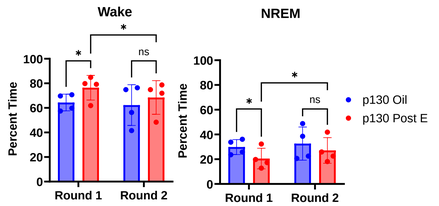
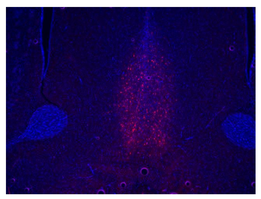
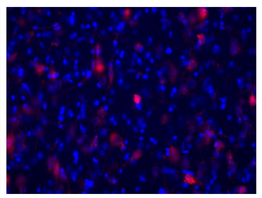


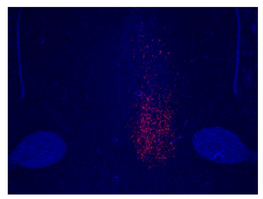
Fig 3. E2's Wake promoting effects are attenuated in p130PH expressing animals 3 weeks post viral injection, but not 2 weeks post viral injection. *Left:* E2 increases Wake in animals expressing p130PH 2 weeks after, but not 3 weeks after viral injection. 2-way RM ANOVA, No Effect of p130PH (% variation = 14.45, p=0.0511) followed by Uncorrected Fisher's LSD (Round 1: p130PH Oil vs p130PH Post E2 (95%CI -19.29 to -4.728, p=0.0135) and p130PH Post E: Round 1 vs. Round 2 (95%CI 0.6597 to 15.22, p=0.0403). *Right:* E2 decreases NREM Sleep 2 weeks after viral injection of p130PH, but not 3 weeks after viral injection. 2-way RM ANOVA, Main effect of p130PH (% Total variation = 14.58, p=0.0368) followed by Uncorrected Fisher's LSD (Round 1: p130PH Oil vs p130PH Post E2 (95%CI -2.958 to 15.63, p=0.0186) and p130PH Post E: Round 1 vs. Round 2 (95%CI -12.90 to -0.2263, p=0.0459).



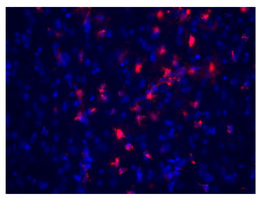
4x Image of AAV-GFAP-hM4DGi-mCherry mRNA (red) expression in MnPO with DAPI (blue).



40x image of AAV-GFAP-hM4DGi-mCherry mRNA (red) expression in MnPO with DAPI (blue).



4x Image of AAV-gfaABC1D-p130PH-mRFP (red) expression in MnPO with DAPI (blue).



40x Image of AAV-gfaABC1D-p130PH-mRFP (red) expression in MnPO with DAPI (blue).

Conclusions

E2 and CNO-activation of Gi-DREADD in MnPO astrocytes each modulate Dark Phase Wake but have no effect on Light Phase Wake.

In animals expressing Gi-DREADD in MnPO astrocytes, CNO injection in the ***Dark Phase*** increased Wake, mimicking E2's effects, but had no effect on Wake in the subsequent Light Phase. CNO injected in the Dark Phase also decreased NREM and REM sleep.

In animals expressing Gi-DREADD in MnPO astrocytes, CNO injection in the ***Light Phase*** had no effect on Light Phase Wake but attenuated E2's effects on Wake in the Dark Phase that follows. This suggests a circadian effect.

p130PH attenuates E2-mediated increase in Wake in the Dark Phase 3 weeks post viral injection but not 2 weeks post viral injection.

Future Directions

Increase sample size for p130PH experiments to further explore whether intracellular Ca signaling in MnPO astrocytes mediates E2's modulation of wake.

Bulk RNASeq in Astrocytes in Light and Dark Phase to determine whether circadian changes in astrocyte transcriptome and estrogen receptor expression underly E2/CNO enhancement of Wake in the Dark, but not the Light Phase.

Express Gq-DREADD in MnPO astrocytes to characterize role of MnPO astrocytes in Sleep-Wake behavior.

Fiber photometry to assess circadian fluctuations in Astrocytic Ca^{2+} signaling.

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