Different roles of T-type calcium channel isoforms in hypnosis induced by an endogenous neurosteroid epipregnanolone

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Background: Common general anesthetics that target $GABA_A$ and NMDA receptors are associated with developmental neurotoxicity in rodents and non-human primates. Hence, it is important to investigate new hypnotic agents with different mechanisms of action. Epipregnanolone [(3β,5β)-3-hydroxypregnan-20-one] is an endogenous neuroactive steroid that blocks T-type calcium channels but lacks any GABA-mimetic and NMDA receptor-blocking properties. Here, we utilized mouse genetics, behavioral experiments, and EEG analysis to investigate potential sedative/hypnotic and immobilizing properties of epipregnanolone (EpiP).

Methods:

Loss of Righting Reflex
- Flip mouse
- If mouse doesn't re-right in 30s = LORR

Loss of Withdrawal Reflex
- Pinch tail with alligator clip
- If no response for 30s = LOWR

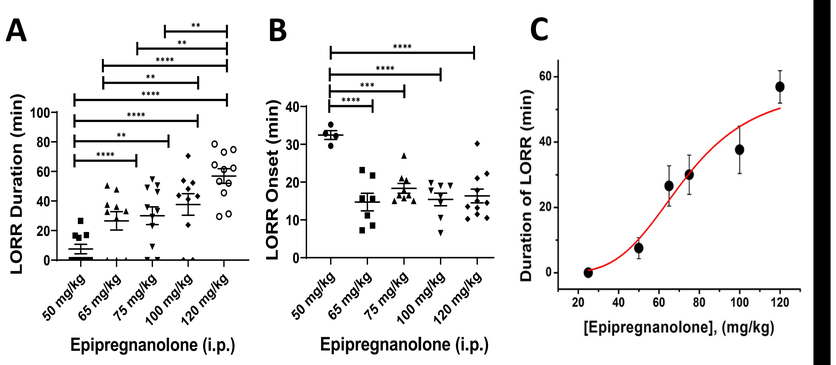

Figure 1: Epipregnanolone is a dose dependent hypnotic agent
A. Dose-dependent decrease in time to LORR with increasing concentration of epipregnanolone. **B.** Dose-dependent increase in LORR duration with epipregnanolone. **C.** The dose at which half of animals underwent LORR (ED_{50}) with epipregnanolone is 72.53 ± 4.00 mg/kg

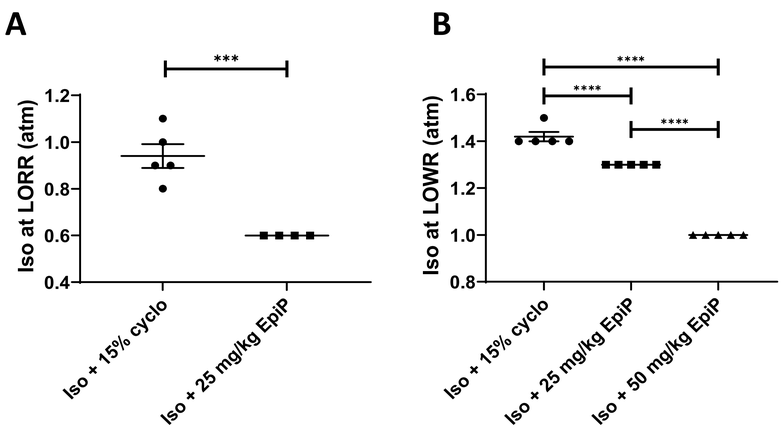

Figure 2: Epipregnanolone significantly lowers isoflurane concentration necessary to immobilize WT mice.
A. A low dose of epipregnanolone (EpiP) yielded a significant decrease in the isoflurane concentration necessary to induce LORR. B. Epipregnanolone lowered the concentration of isoflurane necessary to immobilize WT mice and inhibit LOWR

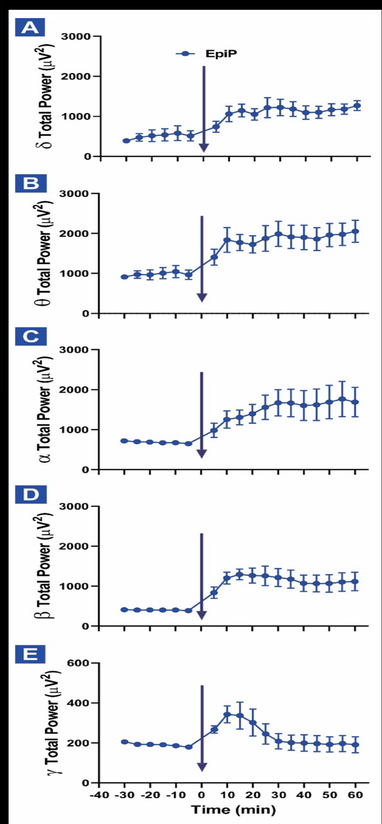

Figure 3 - Total EEG power is increased after EpiP injections
Analysis of recordings from 11 animals. Under EpiP: **A.** more absolute power in δ frequency range (0.5-4 Hz). **B.** more absolute power in θ frequency range (4-8 Hz). **C.** more absolute power in α frequency range (8-13 Hz). **D.** more absolute power in β frequency range (13-30 Hz). **E.** transient rise in absolute power in γ frequency range (30-50 Hz).

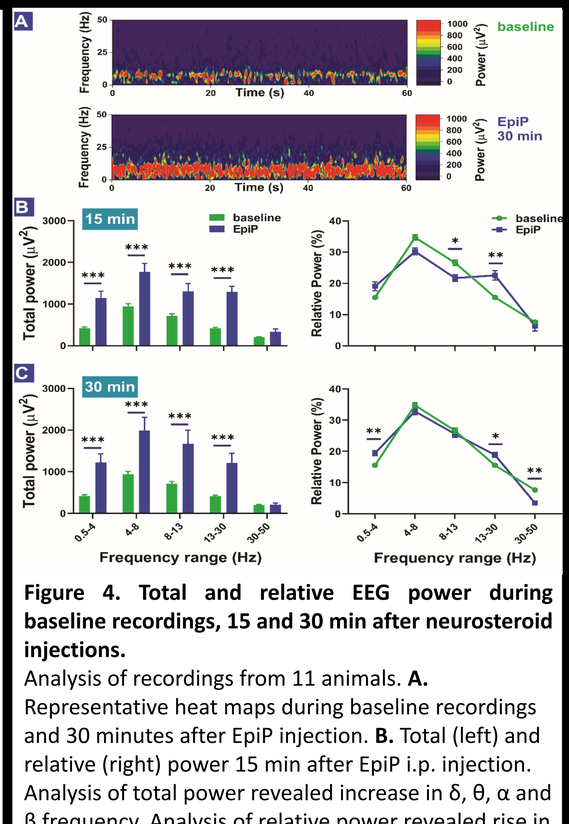

Figure 4. Total and relative EEG power during baseline recordings, 15 and 30 min after neurosteroid injections.
Analysis of recordings from 11 animals. **A.** Representative heat maps during baseline recordings and 30 minutes after EpiP injection. **B.** Total (left) and relative (right) power 15 min after EpiP i.p. injection. Analysis of total power revealed increase in δ, θ, α and β frequency. Analysis of relative power revealed rise in β and drop in α frequency range after EpiP. **C.** Total (left) and relative (right) power 30 min after i.p. injection of the neurosteroid. Analysis of total power revealed increase in δ, θ, α and β frequency. Analysis of relative power revealed rise in δ and β and drop in β relative.

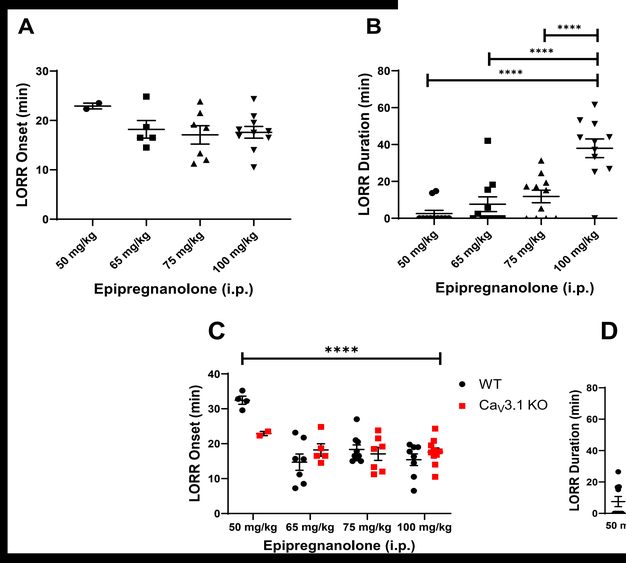

Figure 5 - Knockout of the $Ca_V3.1$ channel confers resistance to the hypnotic effects of epipregnanolone.
A No dose-dependent changes in LORR onset in $Ca_V3.1$ KO mice (p = 0.3661). **B.** Dose-dependent hypnosis in $Ca_V3.1$ KO mice (p < 0.0001). **C.** No difference in LORR onset between $Ca_V3.1$ KO and WT mice (p = 0.4379). **D.** Male $Ca_V3.1$ KO mice demonstrate an overall shorter LORR duration than WT males (p = 0.0032).

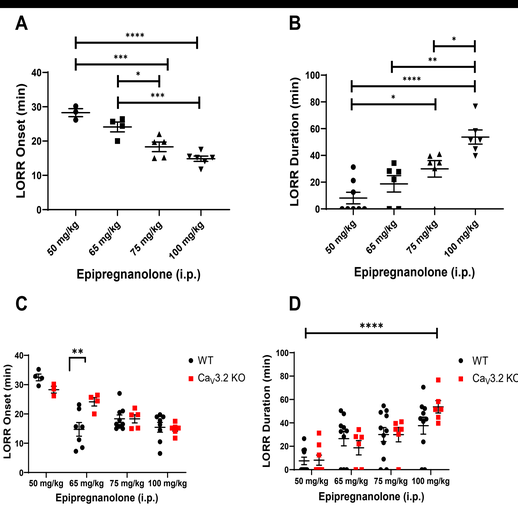

Figure 6 – EpiP exerts dose-dependent hypnosis in $Ca_V3.2$ KO mice with delayed induction but same duration when compared to WT mice.
A. $Ca_V3.2$ KO mice exhibited dose-dependent LORR onset in response to EpiP (p < 0.0001). **B.** EpiP generated a dose-dependent hypnosis in $Ca_V3.2$ KO mice (p < 0.0001). **C.** No difference in LORR onset between $Ca_V3.2$ KO and WT mice (p = 0.2939). **D.** LORR Duration in $Ca_V3.2$ KO male mice was not significantly different from WT male mice (p = 0.6134) and LORR duration was dose-dependent (p< 0.0001).

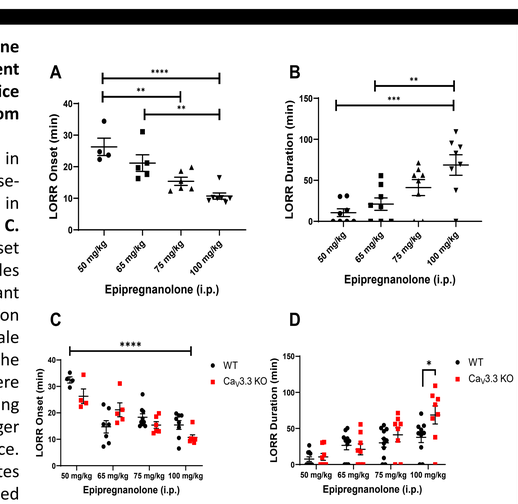

Figure 7 - Epipregnanolone induces dose-dependent hypnosis over $Ca_V3.3$ KO mice that is significantly longer from WT mice at a high dose.
A. Dose-dependent decrease in LORR onset (p < 0.0001). **B.** Dose-dependent hypnosis duration in response to EpiP (p = 0.0006). **C.** No difference in LORR onset between $Ca_V3.3$ KO and WT males (p = 0.2188). **D.** No significant difference in LORR duration between $Ca_V3.3$ KO and WT male mice. (p = 0.0617). Despite the insignificant finding, there appears to be a trend indicating that $Ca_V3.3$ KO mice show longer LORR duration than WT mice. Post-hoc analysis demonstrates that $Ca_V3.3$ KO mice exhibited longer LORR duration than WT at 100 mg/kg (p = 0.0171).

Conclusions:

- Epipregnanolone is an efficacious dose-dependent hypnotic in rodents.
- Epipregnanolone significantly lowers the required concentration of isoflurane needed to induce immobilization and loss of withdrawal to a painful stimulus.
- EEG changes are consistent with other sedative/hypnotic drugs.
- We noted differential response to epipregnanolone based on T-channel expression. WT mice ED_{50} 54.1mg/kg; $Ca_V3.1$ KO mice ED_{50} 67.1mg/kg; $Ca_V3.2$ KO mice ED_{50} 56.1mg/kg; $Ca_V3.3$ KO mice ED_{50} 51.1mg/kg

Future Directions:

- Investigate male to female differences in hypnotic response
- Consider other receptor targets of epipregnanolone in the brain

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