

# 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<sub>A</sub> 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 [(3 $\beta$ ,5 $\beta$ )-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).

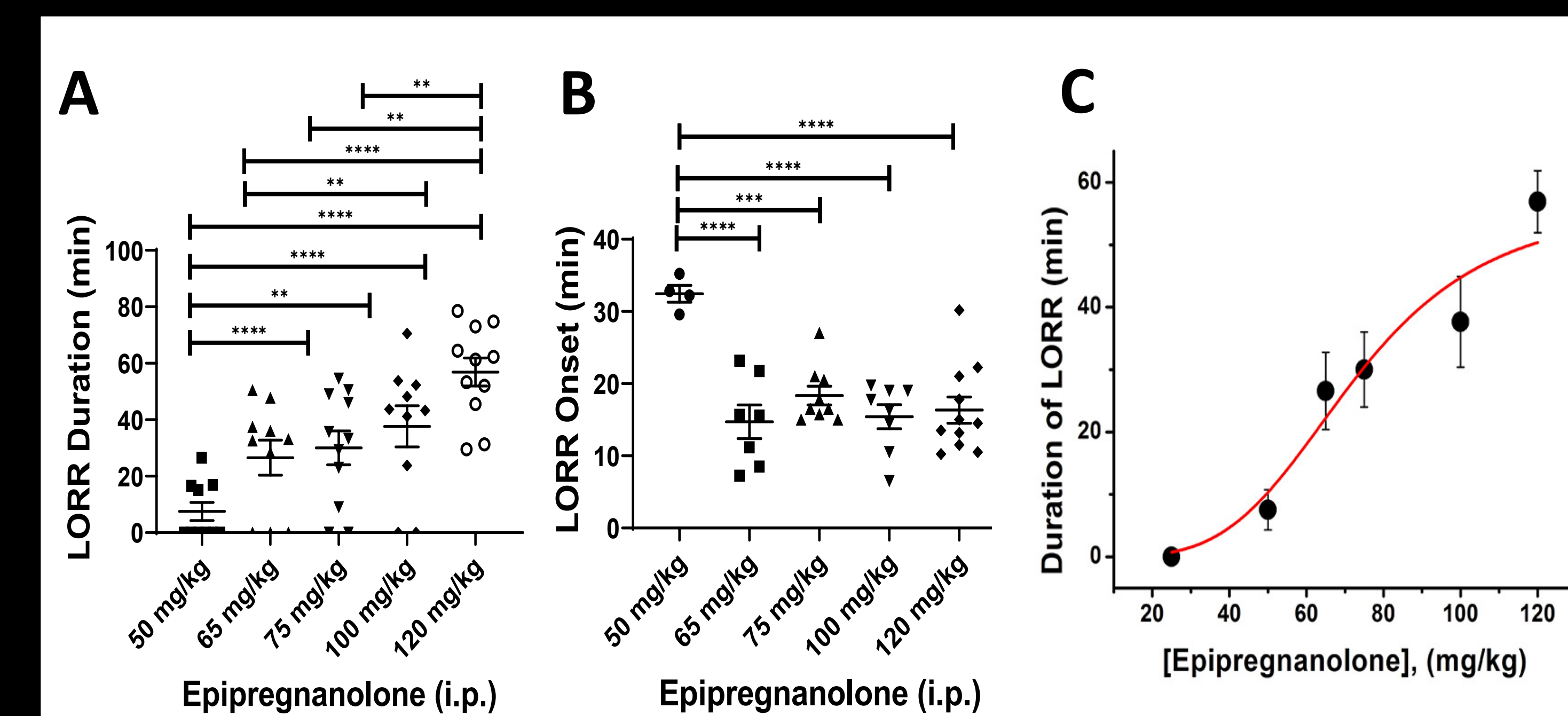
## Methods:

### Loss of Righting Reflex

- Flip mouse
- If mouse doesn't re-right in 30s = LORR

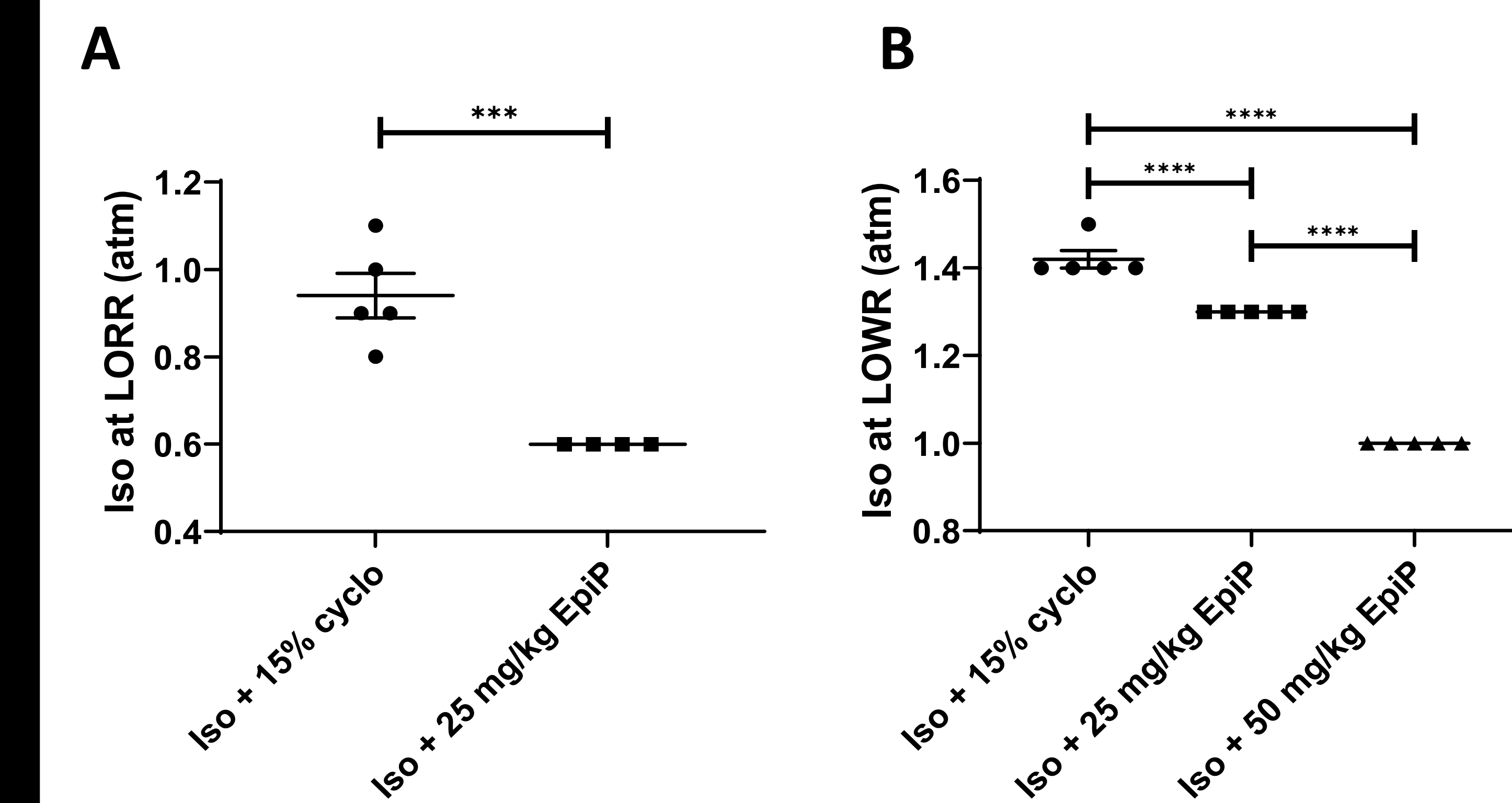
### Loss of Withdrawal Reflex

- Pinch tail with alligator clip
- If no response for 30s = LOWR



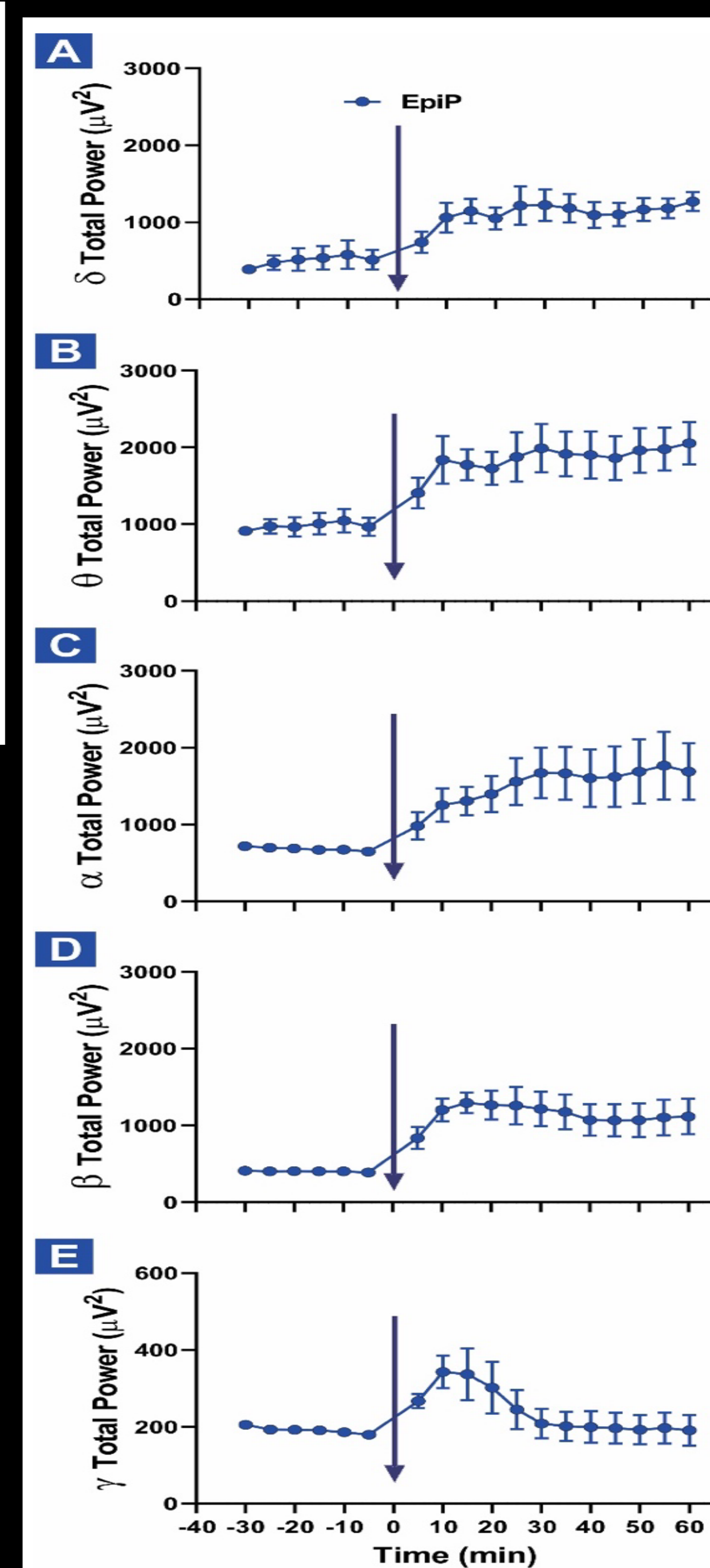
**Figure 1: Epipregnanolone is a dose dependent hypnotic agent**

**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<sub>50</sub>) with epipregnanolone is 72.53 ± 4.00 mg/kg



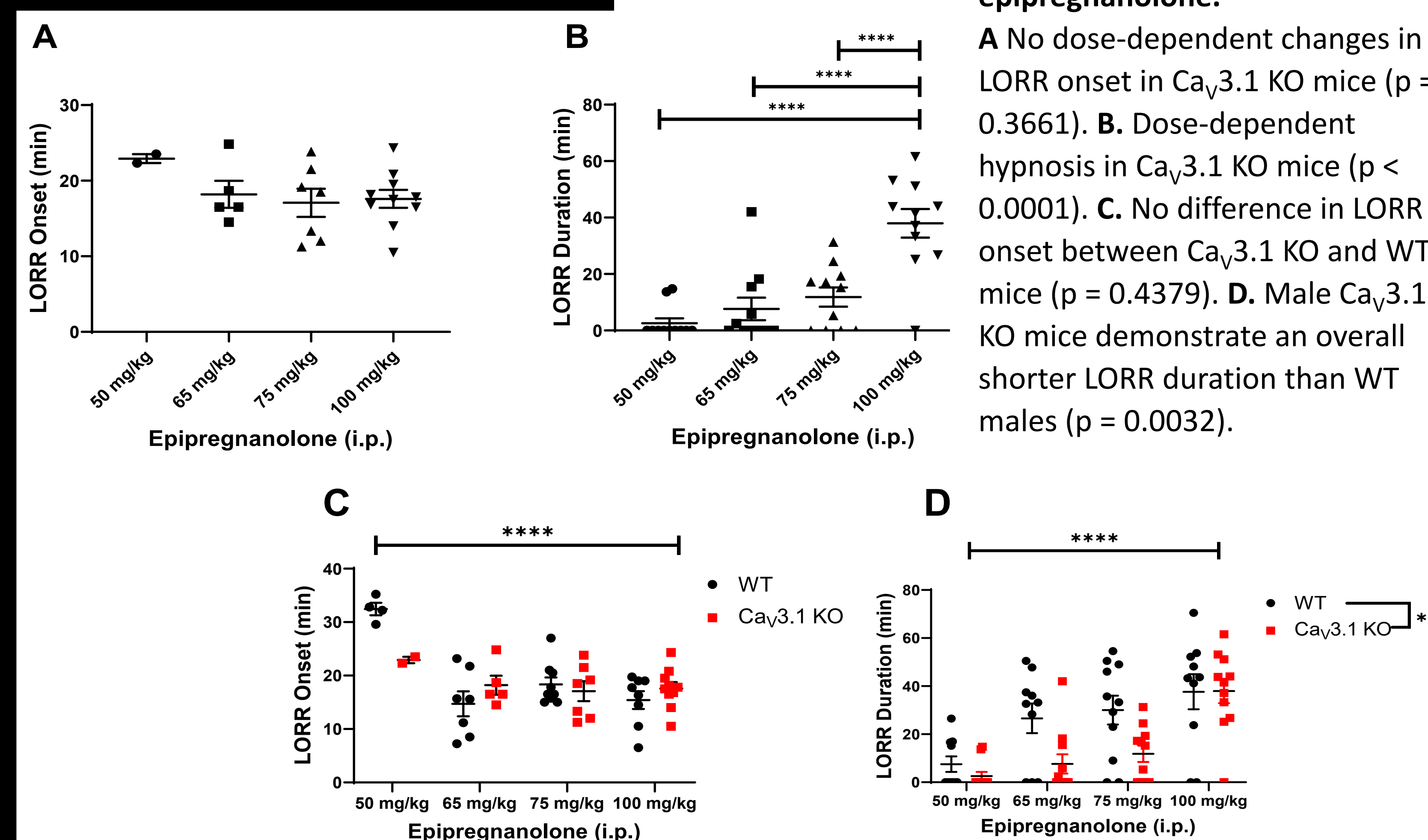
**Figure 2: Epipregnanolone significantly lowers isoflurane concentration necessary to immobilize WT mice.**

**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  $\delta$  frequency range (0.5-4 Hz). **B.** more absolute power in  $\theta$  frequency range (4-8 Hz). **C.** more absolute power in  $\alpha$  frequency range (8-13 Hz). **D.** more absolute power in  $\beta$  frequency range (13-30 Hz). **E.** transient rise in absolute power in  $\gamma$  frequency range (30-50 Hz).



**Figure 4. Total and relative EEG power during baseline recordings, 15 and 30 min after neurosteroid injections.**

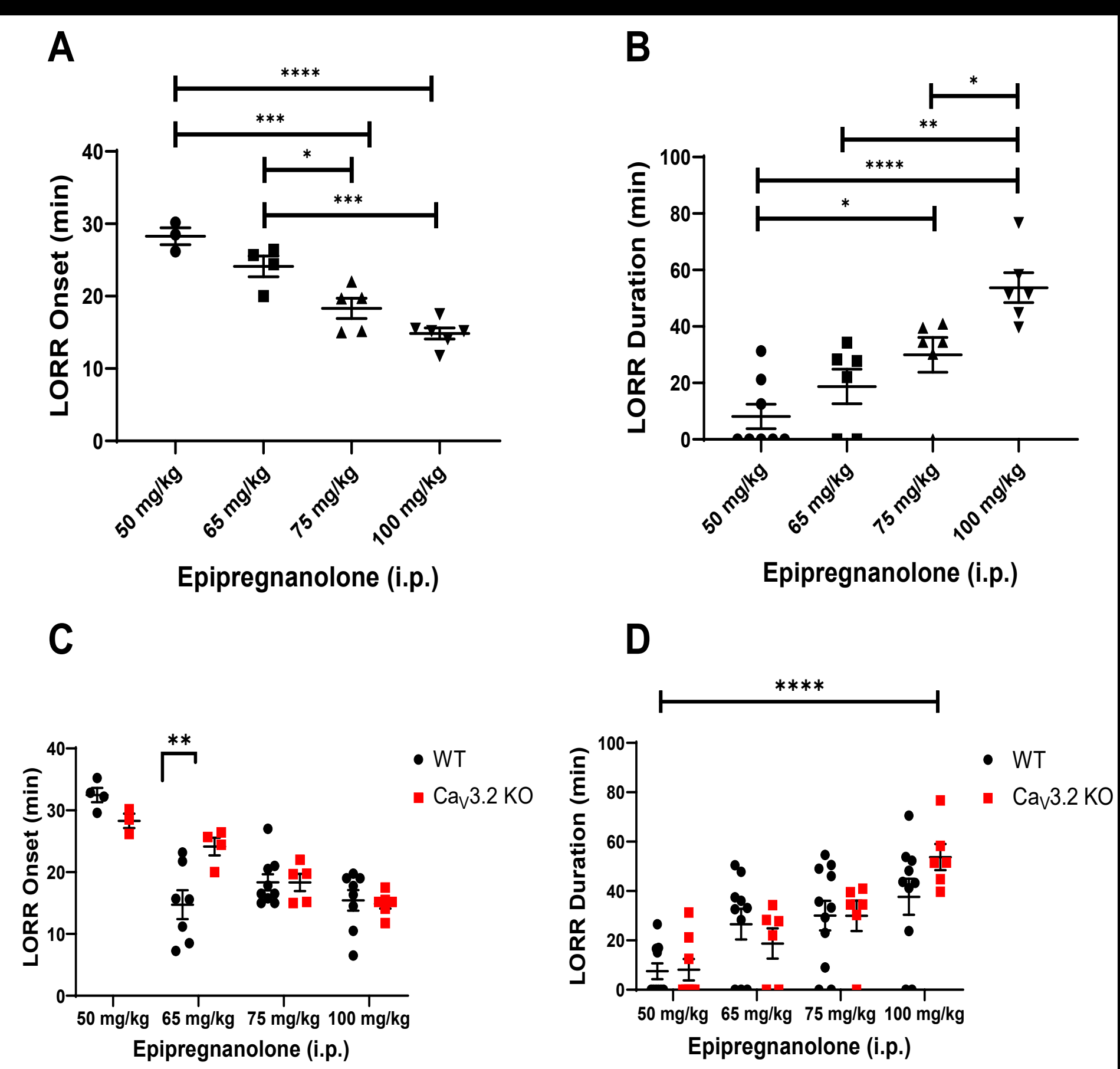
Analysis of recordings from 11 animals. **A.** Representative heat maps during baseline recordings and 30 minutes after EpiP injection. **B.** Total (left) and relative (right) power 15 min after EpiP i.p. injection. Analysis of total power revealed increase in  $\delta$ ,  $\theta$ ,  $\alpha$  and  $\beta$  frequency. Analysis of relative power revealed rise in  $\delta$  and  $\beta$  and drop in  $\alpha$  relative.

**Figure 5 - Knockout of the Ca<sub>v</sub>3.1 channel confers resistance to the hypnotic effects of epipregnanolone.**

**A** No dose-dependent changes in LORR onset in Ca<sub>v</sub>3.1 KO mice ( $p = 0.3661$ ). **B.** Dose-dependent hypnosis in Ca<sub>v</sub>3.1 KO mice ( $p < 0.0001$ ). **C.** No difference in LORR onset between Ca<sub>v</sub>3.1 KO and WT mice ( $p = 0.4379$ ). **D.** Male Ca<sub>v</sub>3.1 KO mice demonstrate an overall shorter LORR duration than WT males ( $p = 0.0032$ ).

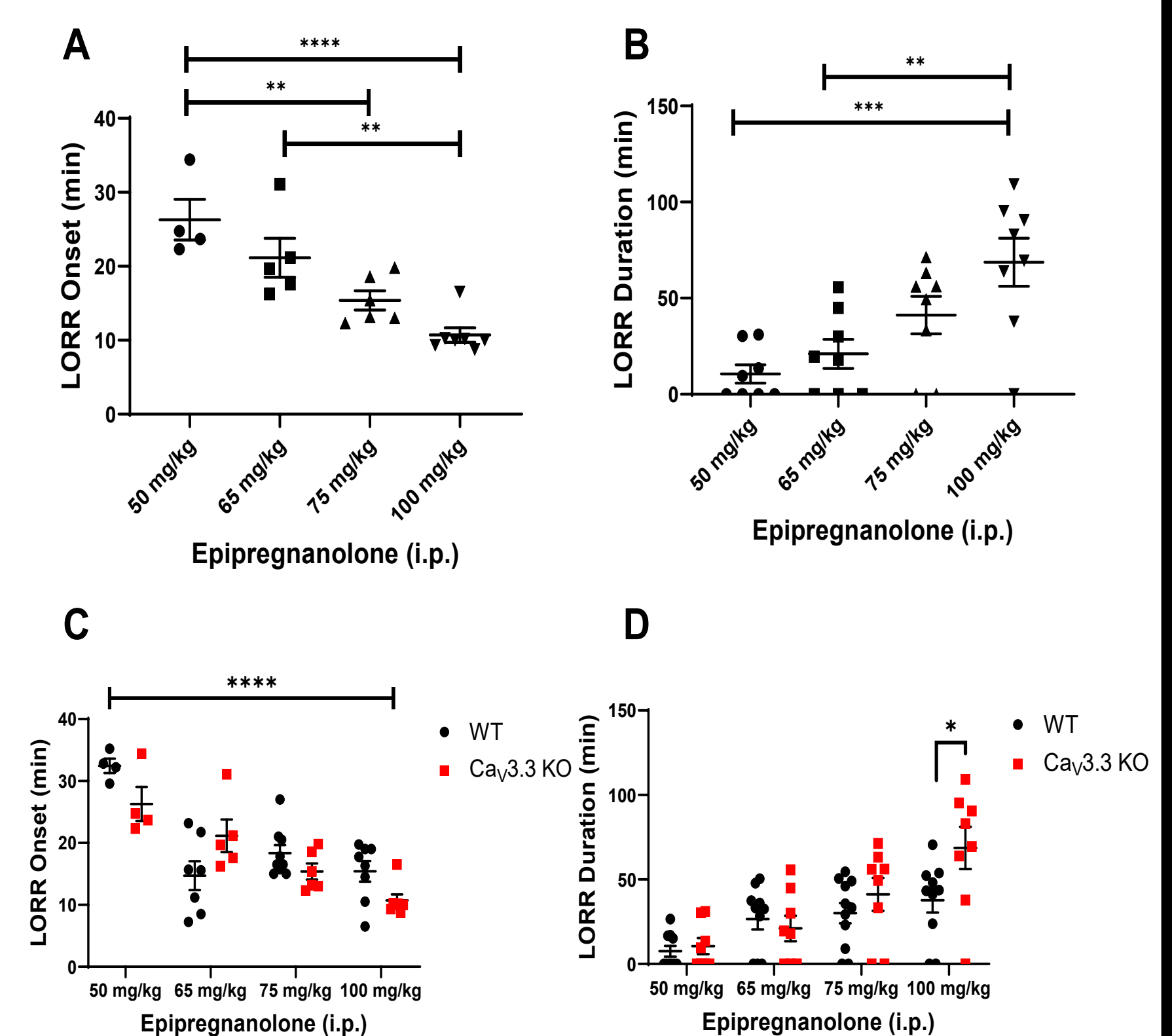
**Figure 6 – EpiP exerts dose-dependent hypnosis in Ca<sub>v</sub>3.2 KO mice with delayed induction but same duration when compared to WT mice.**

**A.** Ca<sub>v</sub>3.2 KO mice exhibited dose-dependent LORR onset in response to EpiP ( $p < 0.0001$ ). **B.** EpiP generated a dose-dependent hypnosis in Ca<sub>v</sub>3.2 KO mice ( $p < 0.0001$ ). **C.** No difference in LORR onset between Ca<sub>v</sub>3.2 KO and WT mice ( $p = 0.2939$ ). **D.** LORR Duration in Ca<sub>v</sub>3.2 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<sub>v</sub>3.3 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<sub>v</sub>3.3 KO and WT males ( $p = 0.2188$ ). **D.** No significant difference in LORR duration between Ca<sub>v</sub>3.3 KO and WT male mice. ( $p = 0.0617$ ). Despite the insignificant finding, there appears to be a trend indicating that Ca<sub>v</sub>3.3 KO mice show longer LORR duration than WT mice. Post-hoc analysis demonstrates that Ca<sub>v</sub>3.3 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<sub>50</sub> 54.1mg/kg; Ca<sub>v</sub>3.1 KO mice ED<sub>50</sub> 67.1mg/kg; Ca<sub>v</sub>3.2 KO mice ED<sub>50</sub> 56.1mg/kg; Ca<sub>v</sub>3.3 KO mice ED<sub>50</sub> 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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