Global genetic deletion of $Ca_V3.3$ channels facilitaites anaesthetic induction and enhances isoflurane-sparing effects of T-type calcium channel blockers

Simon Feseha[1], Tamara Timic Stamenić[1], Damon Wallace[1], Caesaré Tamag[1], Lingling Yang[4], Jen Q. Pan[4], Slobodan M. Todorovic[1,2,3]

1 Department of Anesthesiology, University of Colorado, Anschutz Medical Campus,
2 Neuroscience, University of Colorado, Anschutz Medical Campus
3 Pharmacology Graduate Programs, University of Colorado, Anschutz Medical Campus
4 Stanley Center of Psychiatric Research, Board Institute of Harvard and MIT

University of Colorado Anschutz Medical Campus

Background: It is known that general anesthetics (GAs) induce sedation/hypnosis by targeting neuronal $GABA_A$ and NMDA receptors, as well as voltage gated ion channels. the unique properties of T-type voltage-gated calcium channels (T-channels) are seemingly fitting for the regulation of neuronal excitability because they activate at low voltages, causing an influx of calcium ions. We have previously established that both native thalamic and recombinant $Ca_V3.3$ currents are inhibited by clinically relevant concentrations of volatile GAs including isoflurane, but studies to date have not specifically evaluated the role of $Ca_V3.3$ channels in anaesthetic mechanisms in vivo. Hence, we used mouse genetics and a selective pharmacological antagonist to investigate the role of $Ca_V3.3$ channels in isoflurane-induced hypnosis and underlying thalamocortical oscillations.

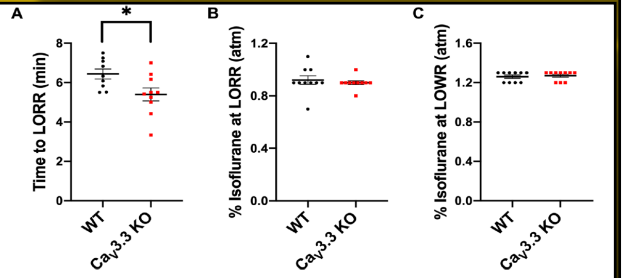

Figure 1. $Ca_V3.3$ KO mice display faster induction time, but have similar requirement for hypnotic and immobilizing effects of isoflurane when compared to the WT mice. (A) Time of induction at 1.2% isoflurane or the WT versus $Ca_V3.3$ KO mice with 15% cyclodextrin (vehicle). Mutant mice had a faster induction time in comparison to the WT mice (unpaired two-tailed t-test: t17 = 2.468, *p = 0.025). (B) Percent isoflurane at LORR for the WT versus $Ca_V3.3$ KO mice with 15% cyclodextrin. No significant difference was identified between two cohorts (unpaired two-tailed t-test: t18 = 0.557, p = 0.584). (C) Percent isoflurane at LOWR for the WT versus $Ca_V3.3$ KO mice with 15% cyclodextrin. There was no significant difference in % isoflurane required to reach LOWR between the WT and $Ca_V3.3$ KO mice (unpaired two-tailed t-test: t18 = 0.447, p = 0.660).

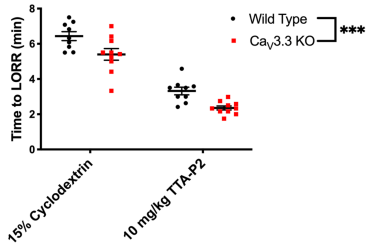

Figure 2. Selective pharmacological inhibition of T-channels with TTA-P2 facilitates anesthetic induction with isoflurane in the WT and mutant mice. Both WT and $Ca_V3.3$ KO mice were injected with vehicle first (data from Fig. 1A) or TTA-P2 on different day and placed in a chamber set at 1.2% isoflurane after a 30-min wait period. Successful induction was determined when a mouse failed to right within a 30-s period. Note that both cohorts demonstrated a significant treatment difference (two-way RM ANOVA: F1,17 = 127.40, p < 0.001). However, the $Ca_V3.3$ KO mice had overall faster induction times when compared to the WT group (two-way repeated measure (RM) ANOVA: F1,17 = 23.87, ***p < 0.001).

Methods: The hypnotic effects and surgical depth of an anesthesia was measured by the % of Isoflurane at Loss of Righting Reflex (LORR) and Loss of Withdrawal Reflex (LOWR) respectively (n=10-12 mice per group). Additionally, the quality of anesthesia was measured by the % isoflurane at which characteristic neuronal oscillations of 70% burst suppression Ratio (BSR) was elicited during electroencephalogram (EEG), which indicates disruption of thalamo-cortical information transfer.

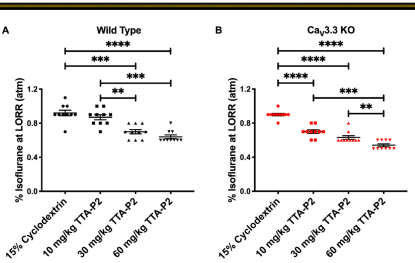

Figure 3. Dose-dependent sparing effect of TTA-P2 on isoflurane-induced hypnosis in the mutant and WT mice. (A) Percent isoflurane at LORR for WT mice. Data from WT mice injected with vehicle in Fig. 1B is used here as baseline. WT mice have a dose dependent decrease in the % isoflurane as the dose of TTA-P2 was escalated (one-way ANOVA: F3,36 = 29.14, p < 0.001). Bonferroni multiple comparison's test further elucidated significant differences between 15% cyclodextrin and 30 mg/kg TTA-P2 (p < 0.001), 15% cyclodextrin and 60 mg/kg TTA-P2 (p < 0.001), 10 mg/kg TTA-P2 and 30 mg/kg TTA-P2 (p = 0.007), and 10 mg/kg TTA-P2 and 60 mg/kg TTA-P2 (p = 0.001). (B) Percent isoflurane at LORR for $Ca_V3.3$ KO cohort. Data from mutant mice injected with vehicle in Fig. 1B is used here as control. Mutant mice had a dose dependent decrease in percent isoflurane for LORR as the dose of TTA-P2 increased (one-way ANOVA: F3,36 = 115.50, p < 0.001). Bonferroni multiple comparison's test further identified significant differences between 15% cyclodextrin and 10 mg/kg TTA-P2 (p < 0.001), 15% cyclodextrin and 30 mg/kg TTA-P2 (p < 0.001), 15% cyclodextrin and 60 mg/kg TTAP2 (p < 0.001), 10 mg/kg TTA-P2 and 60 mg/kg TTA-P2 (p < 0.001), 30 mg/kg TTA-P2 and 60 mg/kg TTA-P2 (p = 0.004).

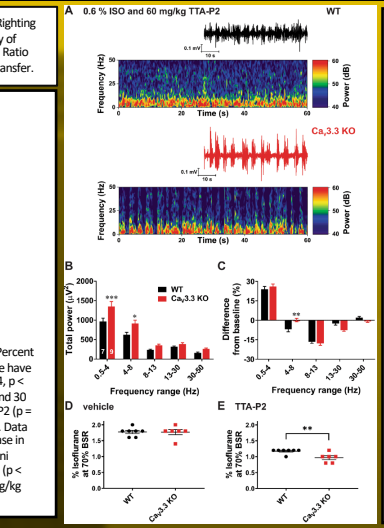

Figure 5. Oscillatory differences between the WT and $Ca_V3.3$ KO mice pretreated with TTA-P2 during administration of sub-hypnotic concentrations of isoflurane. (A) Representative EEG traces and heat maps from a WT mouse (upper panel) and a $Ca_V3.3$ KO mouse (lower panel) during administration of 0.6% isoflurane (ISO) following pretreatment with TTA-P2 at 60 mg/kg i.p. (B) Analysis of total power showed a rise in slow frequency range (δ and θ range) in the mutant mice in comparison with the WT group (two way RM ANOVA: Interaction F4,56 = 4.11, p = 0.005, Frequency F4,56 = 137.40, p < 0.001, Strain F1,14 = 6.89, p = 0.02; Bonferroni post hoc was presented on figure where ***p = 0.0007, and *p = 0.017). (C) Relative power during 0.6% isoflurane following pretreatment with TTA-P2 at 60 mg/kg i.p in WT and mutant mice relative to power during wakefulness (two-way RM ANOVA: Interaction F4,56 = 4.41, p = 0.004, Frequency F4,56 = 188.20, p < 0.001, Strain F1,14 = 0.26, p = 0.615; Bonferroni post hoc was presented on figure where **p = 0.004). (D) Animals from two cohorts pretreated with 15% cyclodextrin did not show difference in isoflurane requirements to achieve 70% BSR (unpaired two-tailed t-test: t11 = 0.051, p = 0.960). E) Mutant animals pretreated with 60 mg/kg TTA-P2 achieve 70% BSR with significantly lower isoflurane concentration than the WT group (unpaired two-tailed t-test: t11 = 3.177, **p = 0.009).

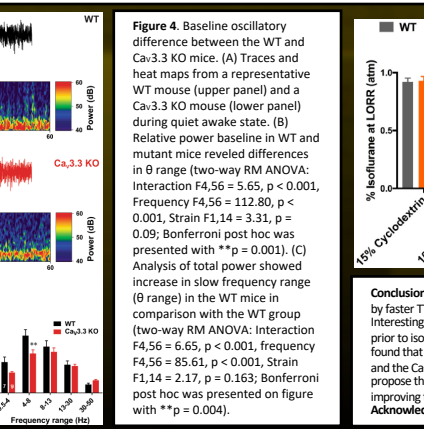

Figure 4. Baseline oscillatory difference between the WT and $Ca_V3.3$ KO mice. (A) Traces and heat maps from a representative WT mouse (upper panel) and a $Ca_V3.3$ KO mouse (lower panel) during quiet awake state. (B) Relative power baseline in WT and mutant mice reveled differences in θ range (two-way RM ANOVA: Interaction F4,56 = 5.65, p < 0.001, Frequency F4,56 = 112.80, p < 0.001, Strain F1,14 = 3.31, p = 0.09; Bonferroni post hoc was presented with **p = 0.001). (C) Analysis of total power showed increase in slow frequency range (θ range) in the WT mice in comparison with the WT group (two-way RM ANOVA: Interaction F4,56 = 6.65, p < 0.001, frequency F4,56 = 85.61, p < 0.001, Strain F1,14 = 2.17, p = 0.163; Bonferroni post hoc was presented on figure with **p = 0.004).

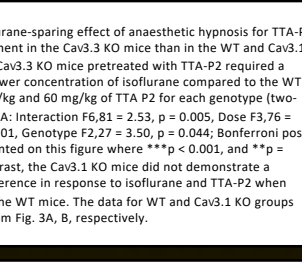

Figure 6. Isoflurane-sparing effect of anaesthetic hypnosis for TTA-P2 is more prominent in the $Ca_V3.3$ KO mice than in the WT and $Ca_V3.1$ KO mice. The $Ca_V3.3$ KO mice pretreated with TTA-P2 required a significantly lower concentration of isoflurane compared to the WT mice at 10 mg/kg and 60 mg/kg of TTA P2 for each genotype (two-way RM ANOVA: Interaction F6,81 = 2.53, p = 0.005, Dose F3,76 = 127.40, p < 0.001, Genotype F2,27 = 3.50, p = 0.044; Bonferroni post hoc was presented on this figure where ***p < 0.001, and **p = 0.004). In contrast, the $Ca_V3.1$ KO mice did not demonstrate a significant difference in response to isoflurane and TTA-P2 when compared to the WT mice. The data for WT and $Ca_V3.1$ KO groups were taken from Fig. 3A, B, respectively.

Conclusion: Our study demonstrates that global deletion of $Ca_V3.3$ channels facilitates induction with isoflurane as evidenced by faster TTLORR when compared to the WT mice, while the requirements of isoflurane for the LORR and LOWR were not affected. Interestingly, a low dose of TTA-P2 in WT mice also decreased TTLORR but did not affect LORR. However, when TTA-P2 was administered prior to isoflurane induction, it decreased TTLORR more prominently in the mutant mice when compared to the WT mice. Additionally, we found that TTA-P2 sparing effect for isoflurane-induced hypnosis measured with LORR were stronger in the $Ca_V3.3$ KO mice than in the WT and the $Ca_V3.1$ KO group. Our findings strongly suggest the specific value of the $Ca_V3.3$ channels in anaesthetic-induced hypnosis. We propose that T-channel blockers may be further explored as a valuable adjunct to reduce the usage of potent volatile anaesthetics, thereby improving their safety. We posit that the potential use of T-channel blockers in clinical anaesthesia warrants further investigation.

Acknowledgments: Supported by NIH grant GM 102525 to SMT. No conflicts of interest.