

Global genetic deletion of $Ca_v3.3$ channels facilitates anaesthetic induction and enhances isoflurane-sparing effects of T-type calcium channel blockers

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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 fitted 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.

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 (LWR) 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.

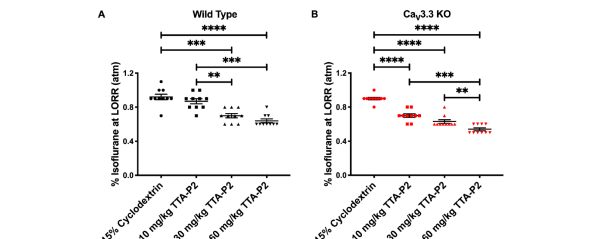


Figure 3. Dose-dependent sparing effect of TTA-P2 on isoflurane-induced hypnosis in the mutant and WT mice. (A) Percent isoflurane at LORR for WT mice. Data from WT mice injected with vehicle in Fig. 1B is used here as baseline. WT mice have a dose dependent decrease in the % isoflurane as the dose of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

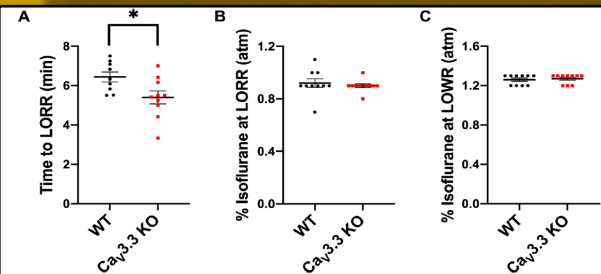


Figure 1. $Ca_v3.3$ KO mice display faster induction time, but have similar requirement for hypnotic and immobilizing effects of isoflurane when compared to the WT mice. (A) Time of induction at 1.2% isoflurane or the WT versus $Ca_v3.3$ KO mice with 15% cycloheximide (vehicle). Mutant mice had a faster induction time in comparison to the WT mice (unpaired two-tailed t-test: $t_{17} = 2.468$, $^*p = 0.025$). (B) Percent isoflurane at LORR for the WT versus $Ca_v3.3$ KO mice with 15% cycloheximide. No significant difference was identified between two cohorts (unpaired two-tailed t-test: $t_{18} = 0.557$, $p = 0.584$). (C) Percent isoflurane at LWR for the WT versus $Ca_v3.3$ KO mice with 15% cycloheximide. There was no significant difference in % isoflurane required to reach LWR between the WT and $Ca_v3.3$ KO mice (unpaired two-tailed t-test: $t_{18} = 0.447$, $p = 0.660$).

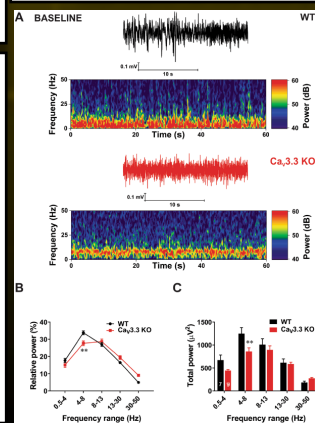
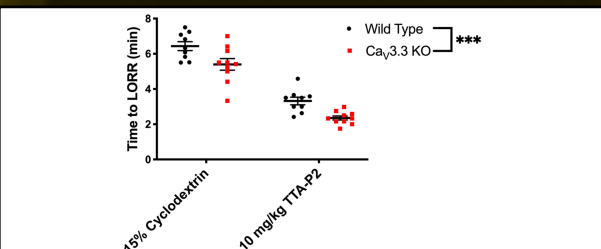


Figure 4. Baseline oscillatory difference between the WT and $Ca_v3.3$ KO mice. (A) Traces and heat maps from a representative WT mouse (upper panel) and a $Ca_v3.3$ KO mouse (lower panel) during quiet awake state. (B) Relative power baseline in WT and mutant mice revealed differences in theta range (two-way RM ANOVA: Interaction $F_{4,56} = 5.65$, $p < 0.001$, Strain $F_{1,14} = 3.31$, $p = 0.09$; Bonferroni post hoc was presented with $^{**}p = 0.001$). (C) Analysis of total power showed increase in slow frequency range (theta range) in the WT mice in comparison with the WT group (two-way RM ANOVA: Interaction $F_{4,56} = 6.65$, $p < 0.001$, Strain $F_{1,14} = 85.61$, $p < 0.001$, Strain $F_{1,14} = 2.17$, $p = 0.163$; Bonferroni post hoc was presented on figure with $^{**}p = 0.004$).

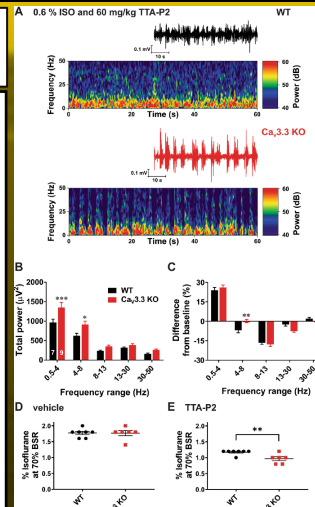


Figure 5. Oscillatory differences between the WT and $Ca_v3.3$ KO mice pretreated with TTA-P2 during administration of sub-hypnotic concentrations of isoflurane. (A) Representative EEG traces and heat maps from a WT mouse (upper panel) and a $Ca_v3.3$ KO mouse (lower panel) during administration of 0.6% 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 (delta and theta range) in the mutant mice in comparison with the WT group (two-way RM ANOVA: Interaction $F_{4,56} = 4.11$, $p = 0.005$, Frequency $F_{4,56} = 137.40$, $p < 0.001$, Strain $F_{1,14} = 6.89$, $p = 0.02$; 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 $F_{4,56} = 4.41$, $p = 0.004$, Frequency $F_{4,56} = 188.20$, $p < 0.001$, Strain $F_{1,14} = 0.26$, $p = 0.615$; Bonferroni post hoc was presented on figure where $^{**}p = 0.004$). (D) Animals from two cohorts pretreated with 15% cycloheximide did not show difference in isoflurane requirements to achieve 70% BSR (unpaired two-tailed t-test: $t_{11} = 0.051$, $p = 0.960$). (E) Mutant animals pretreated with 15% cycloheximide did not show difference in isoflurane requirements to achieve 70% BSR with significantly lower isoflurane concentration than the WT group (unpaired two-tailed t-test: $t_{11} = 3.177$, $^{**}p = 0.009$).

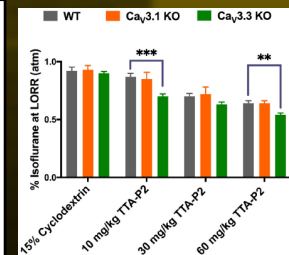


Figure 6. Isoflurane-sparing effect of anaesthetic hypnosis for TTA-P2 is more prominent in the $Ca_v3.3$ KO mice than in the WT and $Ca_v3.1$ KO mice. The $Ca_v3.3$ KO mice pretreated with TTA-P2 required a significantly lower concentration of isoflurane compared to the WT mice at 10 mg/kg and 60 mg/kg of TTA-P2 for each genotype (two-way RM ANOVA: Interaction $F_{6,81} = 2.53$, $p = 0.005$, Dose $F_{3,76} = 127.40$, $p < 0.001$, Genotype $F_{2,27} = 3.50$, $p = 0.044$; 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 TLLORR when compared to the WT mice, while the requirements of isoflurane for the LORR and LWR were not affected. Interestingly, a low dose of TTA-P2 in WT mice also decreased TLLORR but did not affect LORR. However, when TTA-P2 was administered prior to isoflurane induction, it decreased TLLORR 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 anesthetics, thereby improving their safety. We posit that the potential use of T-channel blockers in clinical anaesthesia warrants further investigation.

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