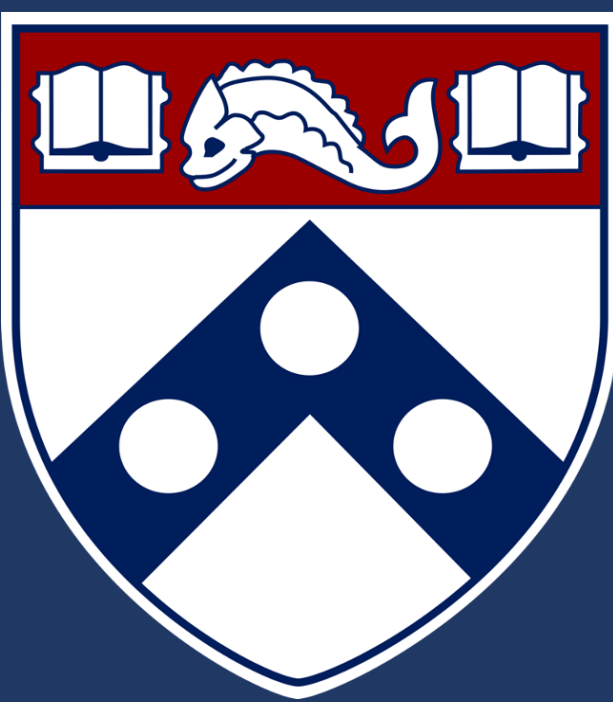
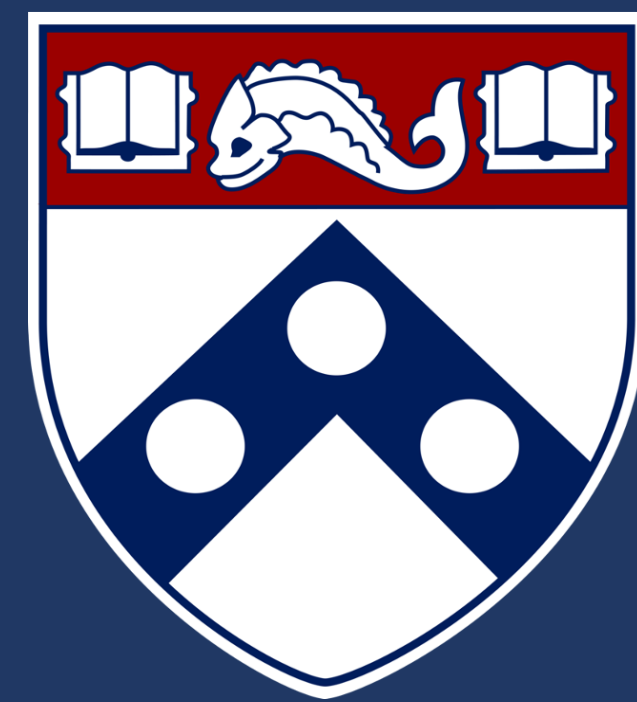




Influence of Anesthetic Antagonists on the Mandibular Movement Rate in Anesthetized Larval Zebrafish

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Introduction

- While modern anesthetics are widely well-tolerated and safe especially when administered by skilled providers, airway and respiratory complications remain the leading cause of anesthetic-related adverse events.
- Traditional mammalian respiratory depression models are limited by cost, technical complexity, and the morbidity of inducing respiratory depression.
- Zebrafish larvae (*Danio rerio*) provide an alternative for studying respiratory depression; by 12 days post-fertilization (dpf), they display a developed respiratory drive network with conserved neural circuitry but do not yet require aquatic respiration for survival due to their small size.
- This makes them an ideal model to observe respiratory rate (mandibular movement), including complete abolishment of respiration, without causing morbidity or mortality.
- Prior studies of respiratory depression in zebrafish have relied on restraints (such as capillary tubes) that can introduce stress, which can elevate respiratory rate.
- While originally established for studying opioid-induced respiratory depression, a “free-swimming” model is adapted here to explore the effects of co-administering caffeine and other novel antagonists to determine their ability to modulate respiratory depression and shift EC₅₀ values.

Methods

- Wildtype (Tübingen longfin, TLF) zebrafish at 12-14 dpf (5 fish x 3 biological replicates, n = 15) were placed into a freshly made solution of E3 medium containing anesthetic, anesthetic + antagonist, or vector control (1% DMSO).
- Four antagonists were tested for modulation of respiratory rate (RR) measured by mandibular movement: caffeine (5 µg/mL), propoflur (30 µM), PFAL 10 (30 µM), PEARL 6 (30 µM), or hydrocarbon control compound (30 µM).
- Each fish was transferred into custom-made, round, PVC wells (7 mm width x 2.8 mm depth) tailored to allow some freedom of movement to prevent significant stress.
- The fish were equilibrated for 15 minutes in the drug and under the lights of the microscope (for behavioral acclimation).
- The RR was then observed with an AmScope SM-1 Series Zoom Trinocular Stereo Microscope (25x magnification) and recorded in 4K high-definition video for 30-second segments using a Z CAM E2-M4 camera. Mandible movements were manually counted by video to quantify RR per minute.

Results

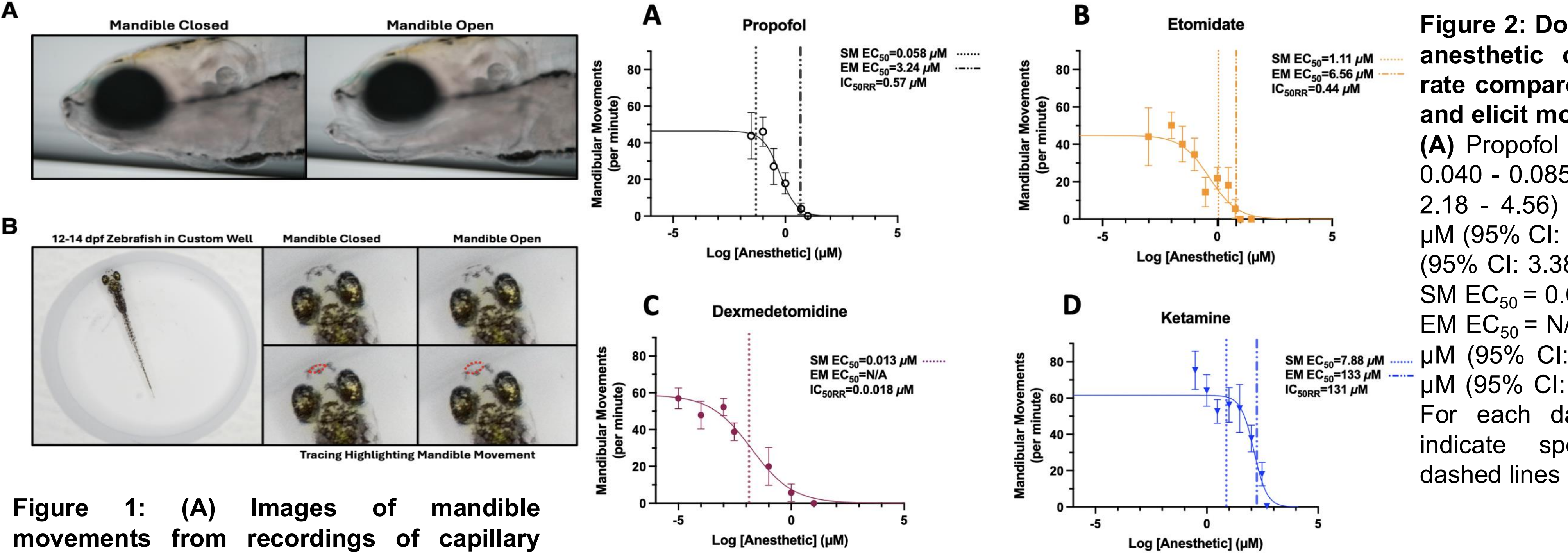


Figure 1: (A) Images of mandible movements from recordings of capillary tube method. Zebrafish respiratory rate reflected by mandibular movement in anesthetic solutions captured using 100x zoom with an inverted microscope. **(B) Images of mandible movements from free-swimming method.** Zebrafish respiratory rate reflected by mandibular movement in anesthetic solutions captured using 25x zoom with a stereo microscope.

Anesthetic only	SM EC ₅₀ , µM (95% CI)	EM EC ₅₀ , µM (95% CI)	RR EC ₅₀ , µM (95% CI)	Therapeutic Index (IC ₅₀ RR/EC ₅₀), SM	Therapeutic Index (IC ₅₀ RR/EC ₅₀), EM
Propofol	0.058 (0.040 - 0.085)	3.24 (2.18 - 4.56)	0.57 (0.31 - 1.10)	9.8	0.18
Etomidate	1.11 (0.65 - 1.77)	6.56 (3.38 - 11.5)	0.44 (0.20 - 1.00)	0.4	0.067
Dexmedetomidine	0.013 (0.007 - 0.022)	N/A	0.018 (0.008 - 0.039)	1.38	N/A
Ketamine	7.88 (5.30 - 11.5)	133 (? - 180)	131 (91 - 179)	16.6	0.98

Anesthetic + Antagonist	SM EC ₅₀ , µM (95% CI)	EM EC ₅₀ , µM (95% CI)	RR EC ₅₀ , µM (95% CI)	Therapeutic Index (IC ₅₀ RR/EC ₅₀), SM	Therapeutic Index (IC ₅₀ RR/EC ₅₀), EM
Caffeine	0.40 (0.29 - 0.55)	11.06 (5.43 - 14.8)	1.230 (0.93 - 1.59)	0.0101	0.111
PFAL 10	8.33 (? - ?)	N/A	1.31 (0.92 - 1.80)	0.157	N/A
Propoflur	8.37 (? - ?)	N/A	1.227 (0.93 - 1.57)	0.147	N/A
PEARL 6	8.18 (? - ?)	8.60 (5.51 - 12.2)	2.49 (1.83 - 3.26)	0.304	0.29

Figure 2: Dose-dependent relationships of anesthetic concentration on respiratory rate compared to spontaneous movement and elicit movement behavioral endpoints. (A) Propofol SM EC₅₀ = 0.058 µM (95% CI: 0.040 - 0.085), EM EC₅₀ = 3.24 µM (95% CI: 2.18 - 4.56) (B) Etomidate SM EC₅₀ = 1.11 µM (95% CI: 0.65 - 1.77), EM EC₅₀ = 6.56 µM (95% CI: 3.38 - 11.50) (C) Dexmedetomidine SM EC₅₀ = 0.013 µM (95% CI: 0.007 - 0.022), EM EC₅₀ = N/A (D) Ketamine SM EC₅₀ = 7.88 µM (95% CI: 5.30 - 11.47), EM EC₅₀ = 133 µM (95% CI: ? - 180). Error bars = 95% CI. For each data point, n=15. Dotted lines indicate spontaneous movement (SM), dashed lines indicate elicit movement (EM).

Table 1: Anesthetic EC₅₀ values for spontaneous movement (SM), elicited movement (EM), IC₅₀ values for respiratory rate, and calculated therapeutic indices. The therapeutic index is calculated as the ratio of IC₅₀ for respiratory rate to EC₅₀. EM EC₅₀ for dexmedetomidine, PFAL 10, and propoflur were unable to be determined. 95% CI ranges for PFAL 10, propoflur, and PEARL 6 were unable to be calculated due to the steepness of their respective hill slopes.

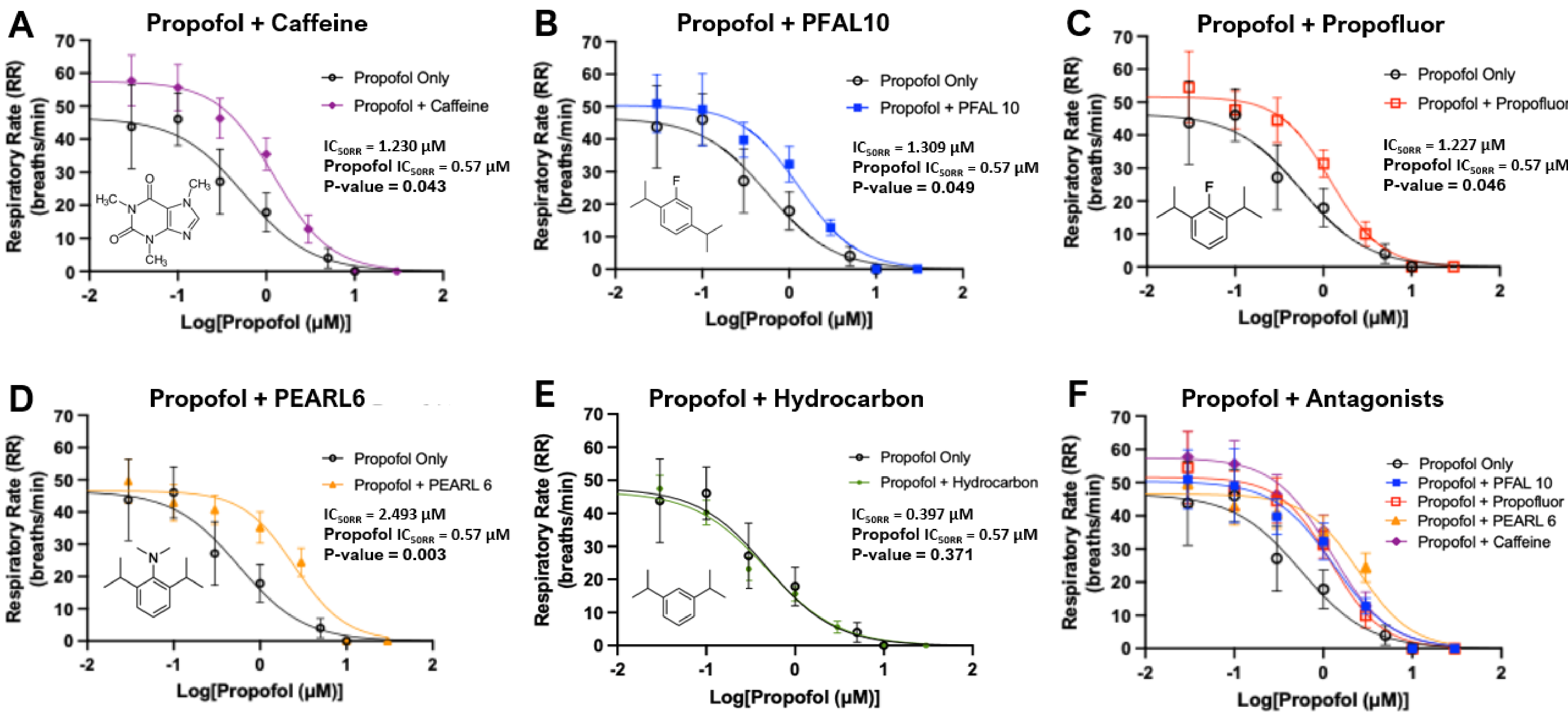


Figure 3: Modulation of propofol-induced respiratory depression with antagonists. (A) Propofol + Caffeine (5 µg/mL) IC₅₀ = 1.230 µM (95% CI: 0.929 - 1.589). (B) Propofol + PAL10 (30 µM) IC₅₀ = 1.309 µM (95% CI: 0.9319 - 1.795). (C) Propofol + Propoflur (30 µM) IC₅₀ = 1.227 µM (95% CI: 0.932 - 1.570). (D)) Propofol + PEARL 6 (30 µM) IC₅₀ = 2.49 µM (95% CI: 1.831 - 3.261). (E) Propofol + Hydrocarbon (30 µM) IC₅₀ = 0.379 µM (95% CI: 0.308 - 0.469) (F) Combined graph with tested antagonists, hydrocarbon control, and propofol. Error bars = 95% CI. For each data point, n=15.

Discussion

- All four anesthetics produced dose-dependent decreases in mandibular movement, allowing calculation of therapeutic indices (IC₅₀/EC₅₀) for anesthetic endpoints and respiratory depression.
- Propofol:** the SM EC₅₀ coincided with the first signs of respiratory depression and progressed to complete apnea at the EM EC₅₀, similar to clinically observed sedative vs. general anesthetic dosing.
- Etomidate:** produced respiratory depression at an earlier dose than movement endpoints, which is a deviation from clinical observations.
- Dexmedetomidine:** exhibited dose-dependent respiratory depression at doses similar to its inhibition of SM.
- Ketamine:** showed the highest therapeutic indices, consistent with its utility as a sedative that preserves respiratory drive in settings like awake airway management or sedation without airway protection.
- Our early evidence indicates that multiple antagonists effectively reversed anesthetic-induced endpoints in zebrafish at 12–14 dpf.
- Caffeine:** antagonized all three endpoints, supporting both its role as a nonspecific respiratory stimulant and its potential for anesthetic antagonism.
- PFAL10 and Propoflur:** both exhibited a modest rightward shift of the EC₅₀ of RR, but not EM which is also seen in 5 dpf.
- PEARL6:** was the most potent antagonist for all endpoints: RR, SM, and EM.
- These findings support antagonism of anesthetic-induced respiratory depression in 12-14 dpf zebrafish, consistent with previous findings with movement-based endpoints of anesthetic antagonism in 5 dpf zebrafish where PEARL 6 had similar potency.

Future Work

- Looking forward, our priorities include automating data collection for this model, further development of antagonists, using these antagonists to probe anesthetic mechanisms, and eventual translation to mammalian models.
- Ideally, this will lead to clinically viable anesthetic reversal agents that restore consciousness and respiratory function safely.

References & Acknowledgements

