Inhibition of the Locus Coeruleus Impedes Emergence from Alkylphenol Hypnosis (note: 2 page abstract)

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Introduction: Despite extensive use over the past thirty years both to induce and maintain states of anesthesia, the neuronal mechanisms by which the prototype alkylphenol anesthetic, propofol, works to produce hypnosis remain unknown. The locus coeruleus (LC) is a wake-active adrenergic center with widespread ascending projections to cortex and thalamus. The LC is known to modulate the hypnotic effects of alpha-2 agonists, GABAergic, and volatile agents, and LC neurons are inhibited by clinically-relevant concentrations of propofol. We here investigate the contribution of the LC to alkylphenol hypnosis using the photoadductible propofol analog, azi-propofol-m (aziPm), which in the presence of UVA light, is converted to a reactive carbene which covalently binds nearby targets.

Methods: LC neurons were identified using morphologic characteristics in intact brain slices continuously bathed in aCSF, taken from adult B6/C57 mice. After patching on and establishing baseline characteristics of a cell, the slice was bathed in 10 µM aziPm for 3 minutes followed by a washout. After returning to a baseline firing rate, cells were reexposed to 10 µM aziPm for 3 minutes with UVA illumination for the final minute. 12-20 week B6/C57 male mice were implanted with 5-lead EEG and EMG, and chronically indwelling bilateral cannulae stereotactically targeted to the LC. After 2 weeks recovery, systemic aziPm was administered intravenously (IV) by bolus (100 mg/kg) and infusion (10 mg/kg/min over 10 minutes) with or without fiberoptically-delivered 375 nm laser light (UVA) at 5 mW/mm2 via the cannulae. After a 1-week recovery the animals were group switched and re-exposed. After another week recovery, mice were given an IV bolus and infusion of propofol (25 mg/kg followed by 2.5 g/kg/min for 10 minutes) with and without laser exposure and then group switched after another week recovery. For all experiments EEG was continuously recorded and time to return of righting reflex noted. Temperature was maintained within 1.0 degrees of baseline using a heating pad and lamp, monitored via chronic subcutaneous implant. Cannula placement was confirmed histologically post-mortem.

Results: AziPm exposure to LC neurons in slice caused a significant decrease in firing rate (38±10 % of baseline) with subsequent recovery within 5 minutes of washout (Figs 1A, B.) When neurons were reexposed to aziPm in the presence of UVA, they exhibited long-term inhibition without recovery over 35 minutes of recording. Following within-subjects randomization, our preliminary data demonstrates that mice with cannulae that accurately targeted the LC exhibited a significant doubling in the duration of hypnosis upon photoadduction (aziPm + UVA: 902 ± 17 seconds) as compared to the duration of hypnosis in the same animals exposed to the anesthetic without photoadduction (aziPm – UVA: 457 ± 17 seconds), as well as a significant increase in hypnotic duration over placement control mice with photoadduction (aziPm + UVA: 389 ± 36, Fig 1C.) In those same nearby placement control mice with cannulae that missed the LC, there was no effect of UVA photoillumination on the duration of hypnosis. Control studies using propofol±UVA targeting the LC, reveal no effect of the laser itself (Fig 1D).

Discussion: Increased time to return of righting following alkylphenol photoadduction in the LC suggests that the LC reactivation is one mechanism necessary for prompt emergence. These studies are also consistent with inhibition of LC permitting entry into a state of anesthesia.

References:

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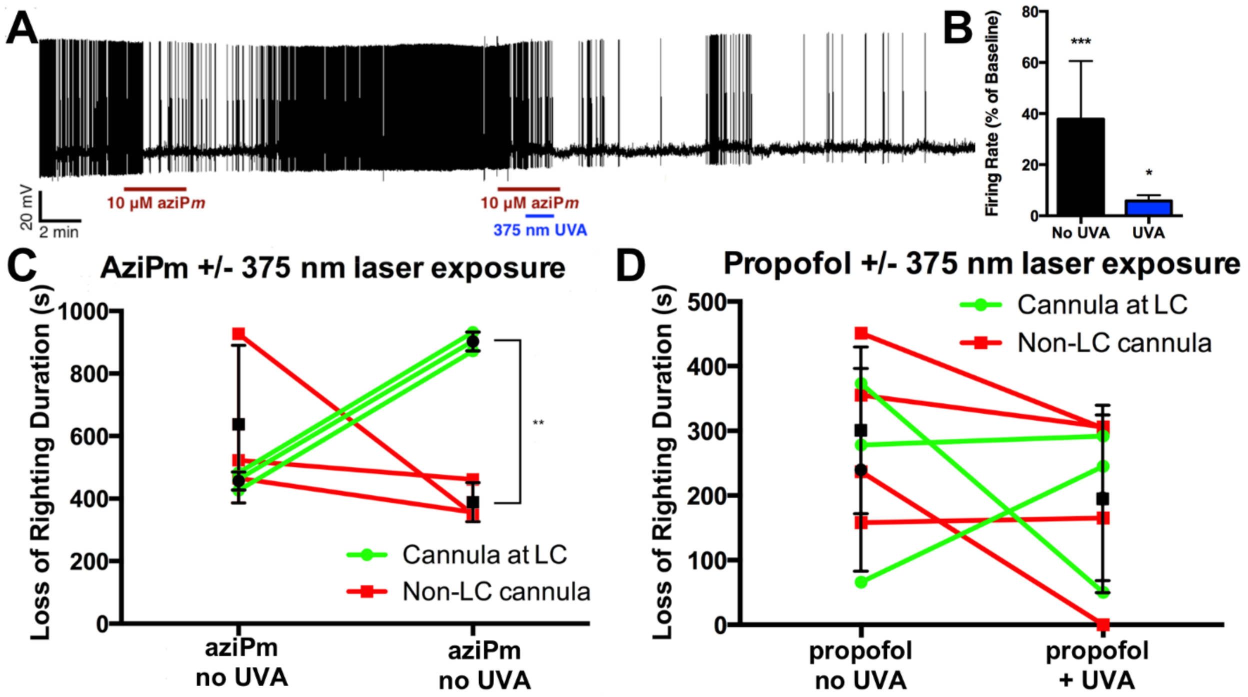

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Figure 1. Prolonged inhibition of the LC by UVA light + alkylphenols delays emergence. A Spontaneously active LC neuron is slowed by a 3 min bath application of 10µM aziPm. In the absence of UVA light, aziPm effects washout within 5 min. Re-exposure to 10µM aziPm plus UVA light causes a long-lasting inhibition of LC activity that does not recover over 35 min washout. B Summary of firing changes at LC with aziPm +/- UVA. C&D: In vivo OptoAnesthesia C Two-way ANOVA showed a significant interaction ($p<0.05$) between cannula location and UVA exposure in mice exposed to aziPm, and Sidak's multiple comparisons test demonstrated a significant difference ($p < 0.01$) in LoRR duration between cannula targeted to LC versus nearby placement controls. D There was no significant effect of placement nor UVA exposure in mice receiving propofol infusions.