Journal Club

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Disinhibition of Histaminergic Neurons: Lack of Effect on Arousal Switch Following Propofol Hypnosis

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Review of Zecharia et al.

Transitions between wake and sleep are a routine and essential part of our lives. The bistable "switch" model of consciousness considers these two mutually exclusive states to be governed by interconnected subcortical nuclei (Saper et al., 2010). Wake- or sleep-promoting nuclei enforce state stabilization and transitions, and impaired signaling in these networks can cause sleep disturbances varying from insomnia to narcolepsy. Recent work on the circuitry underlying arousal state has also provided insights into the enigmatic phenomena of general anesthetic-induced hypnosis and subsequent emergence.

General anesthetics are a diverse class of injectable and inhaled drugs that likely cause unconsciousness through multiple molecular and systematic mechanisms. Similarities between non-rapid eye movement sleep and anesthetic hypnosis have led to the hypothesis that conserved neuronal pathways may underlie both states (Franks, 2008). Thus, during anesthesia, one might anticipate enhanced signaling from sleep-active centers [e.g., ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus (MnPO)] or decreased signaling from wake-active centers [e.g., tuberomammillary nucleus (TMN), locus coeruleus, perifornical hypothalamus, and ventral periaqueductal gray]. Pharmacologic inhibition of glutamatergic or cholinergic neuronal populations within the ascending reticular activating system and/or basal forebrain may also promote cortical depression and hypothalamic network activity that parallels sleep throughout general anesthetic-induced unconsciousness (Saper et al., 2010).

The unique specificities of different anesthetic ligands for their molecular targets and the distinct anatomic expression of these substrates suggest that anesthetics influence sleep/wake circuitry in a variety of ways. Further, the common endpoint of anesthetic hypnosis is achieved by targeting pathways that differentially contribute to the overall state of consciousness. Experimenters often manipulate candidate molecular and systems substrates with the aim of selectively shifting general anesthetic sensitivity. Though not yet achieved, complete resistance to a general anesthetic through genetic or pharmacologic manipulation would reveal necessary or sufficient targets for drug efficacy.

Propofol (2,6-diisopropylphenol) is a widely used injectable anesthetic and a potent positive allosteric modulator of select $GABA_A$ receptor subtypes. Potentiation of $GABA_A$ receptor signaling was proven to be a major mechanism by which propofol hypnosis occurs (Jurd et al., 2003). Sleep-active VLPO and MnPO neurons send inhibitory GABAergic and galaninergic signals to wake-active monoaminergic and orexinergic nuclei, many of which reciprocate inhibitory (non-GABAergic) projections. Potentiation of inhibitory postsynaptic currents (IPSCs) by propofol on these wake-active nuclei should encourage the consciousness equilibrium to "switch" toward hypnosis. Upon drug clearance, and the resumption of endogenous signaling mediated by homeostatic and circadian drives, the switch back to consciousness (i.e., emergence) can occur.

Zecharia et al. (2012) recently investigated whether pharmacologic potentiation of synaptic TMN $GABA_A$ receptors contributes to propofol hypnosis in a continuation of previous work (Nelson et al., 2002; Zecharia et al., 2009). This was performed by selectively floxing out the $GABA_A$ $\gamma 2$ subunit, which is required for the postsynaptic localization of $GABA_A$ receptors (Schweizer et al., 2003), in histamine decarboxylase-expressing neurons. Thus, $GABA_A$ receptors in histaminergic TMN neurons were rendered insensitive to propofol-potentiated fast synaptic inhibition. TMN neurons from mutated mice were found to be resistant to propofol-induced hyperpolarization and to be more excitable than wild-type neurons *ex vivo*. The mutant mice habituated more slowly to new environments, an "active-waking" phenotype, yet retained the sleep–wake cycle of wild types. Surprisingly, the duration of loss of righting reflex (LORR), a surrogate measure of hypnosis (Franks, 2008), was

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unaffected in mutants following bolus injections of propofol. This led the authors to conclude that the TMN does not appear to contribute to propofol-induced hypnosis. Moreover, "... inhibition of histaminergic neurons is probably coincident with, rather than necessary for, the behavioral effects of propofol."

This interpretation requires discussion as it contradicts previous work that identified a role of the TMN in the efficacy of GABAergic agents. Direct microinjection of the $GABA_A$ antagonist gabazine into the TMN was shown to attenuate propofol sedation, while similarly targeted microinjection of the $GABA_A$ agonist muscimol caused sedation (Nelson et al., 2002). Zecharia and others also demonstrated a resistance of propofol-enhanced IPSCs *ex vivo* in the TMN of $GABA_A$ β3(N265M) mice (Zecharia et al., 2009), a knock-in strain significantly resistant to propofol anesthesia (Jurd et al., 2003). The observed disparity may rest in the LORR assay (i.e., measurement of hypnotic duration following drug dosing). Additional consideration of this assay and the recently explored genetic paradigm is essential before excluding histaminergic neurons as relevant substrates of propofol.

The timed LORR assay as presented investigates the return of righting reflex, as it effectively characterizes the emergence from, but not the induction of, propofol-induced hypnosis. The physiologic processes underlying the induction of anesthetic hypnosis are not simply reversed during emergence, and genetic or pharmacologic manipulations can differentially influence either process (Friedman et al., 2010). The induction EC_{50} and additional measures (e.g., latency to LORR following a bolus) are necessary to assign relevance to hypnotic contributors. Further, genetic exclusion of $GABA_A$ receptors from synapses results in a form of disinhibition; thus, in mutated mice, wake-active histaminergic TMN neurons should be more resistant to the pharmacologic effects of propofol in awake animals. In other words, the ability of MnPO and VLPO to inhibit the TMN is significantly diminished in mutants, yet the TMN can still inhibit these sleep-active nuclei that also project to other wake-active sites; therefore, one might expect the switch to be most affected where VLPO inhibits arousal circuitry, which is at the time of induction, and not vice versa.

We commend the elegant experimental design put forth by the authors, and agree that data suggest histaminergic neurons are not involved in emergence from propofol hypnosis. On the other hand, the potential that TMN $GABA_A$ receptors play a role in propofol induction of hypnosis must still be addressed, and this information could be readily garnered from the experimental model that is already in place. Additionally, while not extensively discussed here, the finding that $GABA_A$ receptors on histaminergic neurons are important for habituation to a new environment is a finely nuanced confirmation that reinforces our understanding of the specific roles of the TMN. In conclusion, this work creates a fine model for systematically dissecting potential general anesthetic targets in the sleep–wake circuitry, and leaves opportunities to refine our understanding of how histaminergic TMN neurons participate in active waking and anesthetic induction.

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