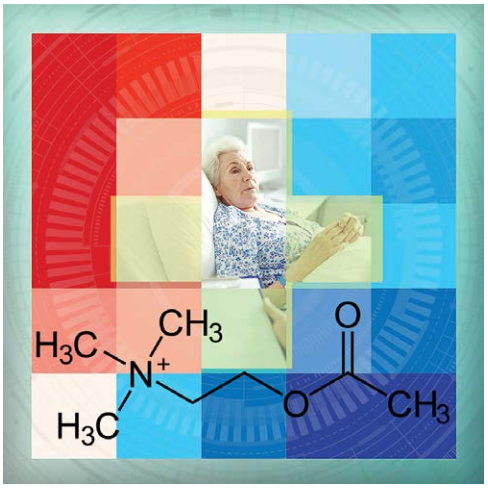
Consciousness, Anesthesia, and Acetylcholine

Dinesh Pal, Ph.D., George A. Mashour, M.D., Ph.D.

Although the precise neural correlates of consciousness have not yet been identified, there is one neurochemical marker that appears to track with our capacity to experience something: acetylcholine concentration in the cerebral cortex. Cortical acetylcholine concentration is high during wakefulness, decreases during slow-wave sleep, and increases again during rapid eye movement sleep, when we can have the conscious experience of dreaming.[1] Based on the neuropharmacology of the particular anesthetic drug we might choose, the state of general anesthesia shows parallel changes. For example, the effects of the γ-aminobutyric acid–mediated (GABAergic) anesthetics propofol and sevoflurane are similar to slow-wave sleep and include low acetylcholine concentration, slower electroencephalographic frequency, and a low probability of experience.[2,3] By contrast, the effects of the non-GABAergic anesthetics ketamine and nitrous oxide are similar to rapid eye movement sleep and include high acetylcholine concentration, faster electroencephalographic frequency, and a higher probability of experience in the form of dreams or hallucinations.[2,4] In both humans[5,6] and rodent models,[7,8] interventions that raise acetylcholine concentration in the brain are associated with a reversal of anesthetic traits or a reduction in anesthetic potency. Conversely, lesions of cholinergic neurons in the basal forebrain—the main source of acetylcholine for the cortex—reduce anesthetic requirements for commonly used drugs such as isoflurane and propofol.[8,9]



"…acetylcholine could potentially play a more prominent role in emergence from anesthesia compared to induction."

In this issue of Anesthesiology, Leung *et al.*[10] leverage genetic mice models to probe the role of acetylcholine in general anesthesia by testing the hypothesis that deficiency of forebrain acetylcholine would enhance anesthetic sensitivity to isoflurane and ketamine and decrease the spectral power in the gamma frequency (*i.e.*, faster frequencies in the electroencephalogram). The authors used mice that were genetically modified to alter the expression of the vesicular acetylcholine transporter by either reducing it centrally and peripherally (heterozygous knockdown mice) or completely eliminating it from the basal forebrain (forebrain knockout mice). Vesicular acetylcholine transporter is a transmembrane protein that moves acetylcholine from the cytoplasm to the synaptic vesicles. Upon neuronal depolarization, these synaptic vesicles are released from nerve terminals into the synaptic cleft, initiating the cascade of chemical neurotransmission. Decreased expression of vesicular acetylcholine transporter would be predicted to reduce the amount of acetylcholine available for synaptic signaling. Indeed, the heterozygous knockdown mice were characterized by a generalized decrease in acetylcholine concentration (~45%) across the brain, whereas forebrain knockout mice were characterized by complete elimination of acetylcholine in the brain regions innervated by the basal forebrain.

Using these genetic models of acetylcholine deficiency, the authors first showed that, compared to the wild-type control mice, the forebrain knockout mice required significantly less isoflurane (~8.8% difference) and ketamine (~15% difference) to lose the righting reflex, a surrogate for loss of consciousness in rodents. Estimation of the half-maximal effective dose (ED_{50}) as a function of acetylcholine deficiency revealed a dose-dependent pattern. The ED_{50} for isoflurane was highest in the wild-type mice (0.76%), followed by heterozygous knockdown mice (0.72%), and decreased further in forebrain knockout mice (0.69%). A similar pattern was observed for ketamine, where an ED_{50} gradient paralleled the graded acetylcholine deficiency, *i.e.*, 160 mg/kg for wild-type mice *versus* 150 mg/kg for heterozygous knockdown mice *versus* 124 mg/kg for forebrain knockout mice. These data demonstrate that a deficiency of forebrain acetylcholine enhances anesthetic sensitivity,

Image: A. Johnson, Vivo Visuals.

This editorial accompanies the article on p. 588.

Accepted for publication January 4, 2021. From the Center for Consciousness Science, Department of Anesthesiology, Neuroscience Graduate Program, University of Michigan Medical School, Ann Arbor, Michigan.

 Anesthesiology 2021; 134:515–7. DOI: 10.1097/ALN.0000000000003696

<zdoi;. DOI: 10.1097/ALN.0000000000003696>

which is consistent with previous findings showing a direct causal role of the cholinergic system in reversing anesthesia[7] and increasing spontaneous wakefulness.[11] The authors also showed that hippocampal high-gamma (63 to 100 Hz) power in acetylcholine-deficient mice was attenuated during isoflurane and ketamine anesthesia as compared to that observed under anesthesia in wild-type mice. However, a similar effect was not observed for high-gamma power in frontal cortex. Of note, it has been shown that electroencephalographic features, cortical acetylcholine concentrations, and anesthetic effects do not always correlate well.[3,12] Thus, these relationships are complex, and it is not entirely surprising that there could be differences between the hippocampus and the cortex.

One limitation of these mice models is the lack of site specificity for regions innervated by the target sites. For example, given that both the frontal cortex and the hippocampus are innervated by basal forebrain, the mice models used in this study do not allow dissection of the individual roles of either of these areas. Furthermore, analysis of recovery could have been informative and revealed asymmetries relative to induction. Such asymmetry has been noted for orexin, a neurotransmitter synthesized in neurons of the hypothalamus, which has been shown to play a preferential role in anesthetic emergence.[13] Of relevance to the study by Leung *et al.*,[10] activation of orexin nerve terminals in the basal forebrain, which would increase cortical acetylcholine, accelerated emergence from isoflurane anesthesia in mice.[14] Thus, acetylcholine could potentially play a more prominent role in emergence from anesthesia compared to induction. This will be a key question to answer for clinicians, with the ultimate goal of better controlling the emergence process. Drugs within our armamentarium such as physostigmine, ketamine, and caffeine are all known to increase cortical acetylcholine concentrations and have been explored in preclinical models or human studies as tools to accelerate emergence.

Despite these limitations, the manipulation of cholinergic system *via* genetic downregulation of acetylcholine release, as was done in this study,[10] offers a model that overcomes the potential confounds associated with drug infusions (solubility, specificity, concentration) and nonspecific effects in lesion studies. Another advantage of these mice models is that only the acetylcholine transmission is affected, without impacting the corelease of other neurotransmitters, which will occur after cellular lesions. An interesting application of this model could be to understand the effect of endogenous acetylcholine modulation on sleep–wake states. This will be particularly important because it has been shown that specific lesions of basal forebrain cholinergic neurons do not produce any significant changes in sleep–wake states.[15]

Although significant advances have been made in our understanding of the relationship between cortical acetylcholine and states of consciousness, including from the current study, there is much that remains to be explored. First, these data make it clear that, despite robust correlation with different levels of consciousness, acetylcholine makes only partial contributions to arousal and anesthesia, consistent with recent data highlighting the complexity of neurochemical changes during the anesthetized state.[16] There is a need for a more comprehensive cartography of neurochemical changes associated with general anesthesia. Second, the ultimate clinical relevance of acetylcholine in the perioperative period requires further study, with a particular focus on reversal of general anesthesia and perioperative neurocognitive disorders. The current study by Leung *et al.*[10] provides an important foundation for these future scientific and clinical investigations.

Research Support

Supported by grant No. R01GM111293 from the National Institutes of Health, Bethesda, Maryland.

Competing Interests

The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

Correspondence

Address correspondence to Dr. Mashour: gmashour@umich.edu

References

1. Lydic R, Baghdoyan HA: Sleep, anesthesiology, and the neurobiology of arousal state control. Anesthesiology 2005; 103:1268–95
2. Shichino T, Murakawa M, Adachi T, Arai T, Miyazaki Y, Mori K: Effects of inhalation anaesthetics on the release of acetylcholine in the rat cerebral cortex *in vivo*. Br J Anaesth 1998; 80:365–70
3. Pal D, Silverstein BH, Lee H, Mashour GA: Neural correlates of wakefulness, sleep, and general anesthesia: An experimental study in rat. Anesthesiology 2016; 125:929–42
4. Pal D, Hambrecht-Wiedbusch VS, Silverstein BH, Mashour GA: Electroencephalographic coherence and cortical acetylcholine during ketamine-induced unconsciousness. Br J Anaesth 2015; 114:979–89
5. Meuret P, Backman SB, Bonhomme V, Plourde G, Fiset P: Physostigmine reverses propofol-induced unconsciousness and attenuation of the auditory steady state response and bispectral index in human volunteers. Anesthesiology 2000; 93:708–17
6. Plourde G, Chartrand D, Fiset P, Font S, Backman SB: Antagonism of sevoflurane anaesthesia by physostigmine: Effects on the auditory steady-state response and bispectral index. Br J Anaesth 2003; 91:583–6

7. Pal D, Dean JG, Liu T, Li D, Watson CJ, Hudetz AG, Mashour GA: Differential role of prefrontal and parietal cortices in controlling level of consciousness. Curr Biol 2018; 28:2145–52.e5
8. Luo TY, Cai S, Qin ZX, Yang SC, Shu Y, Liu CX, Zhang Y, Zhang L, Zhou L, Yu T, Yu SY: Basal forebrain cholinergic activity modulates isoflurane and propofol anesthesia. Front Neurosci 2020; 14:559077
9. Leung LS, Luo T, Ma J, Herrick I: Brain areas that influence general anesthesia. Prog Neurobiol 2014; 122:24–44
10. Leung LS, Chu L, Prado MAM, Prado VF: Forebrain acetylcholine modulates isoflurane and ketamine anesthesia in adult mice. Anesthesiology 2021; 134:588–606
11. Parkar A, Fedrigon DC, Alam F, Vanini G, Mashour GA, Pal D: Carbachol and nicotine in prefrontal cortex have differential effects on sleep–wake states. Front Neurosci 2020; 14:567849
12. Pal D, Li D, Dean JG, Brito MA, Liu T, Fryzel AM, Hudetz AG, Mashour GA: Level of consciousness is dissociable from electroencephalographic measures of cortical connectivity, slow oscillations, and complexity. J Neurosci 2020; 40:605–18
13. Kelz MB, Sun Y, Chen J, Cheng Meng Q, Moore JT, Veasey SC, Dixon S, Thornton M, Funato H, Yanagisawa M: An essential role for orexins in emergence from general anesthesia. Proc Natl Acad Sci USA 2008; 105:1309–14
14. Wang D, Guo Y, Li H, Li J, Ran M, Guo J, Yin L, Zhao S, Yang Q, Dong H: Selective optogenetic activation of orexinergic terminals in the basal forebrain and locus coeruleus promotes emergence from isoflurane anaesthesia in rats. Br J Anaesth 2021; 126:279–92
15. Blanco-Centurion C, Xu M, Murillo-Rodriguez E, Gerashchenko D, Shiromani AM, Salin-Pascual RJ, Hof PR, Shiromani PJ: Adenosine and sleep homeostasis in the basal forebrain. J Neurosci 2006; 26:8092–100
16. Zhang X, Baer AG, Price JM, Jones PC, Garcia BJ, Romero J, Cliff AM, Mi W, Brown JB, Jacobson DA, Lydic R, Baghdoyan HA: Neurotransmitter networks in mouse prefrontal cortex are reconfigured by isoflurane anesthesia. J Neurophysiol 2020; 123:2285–96