

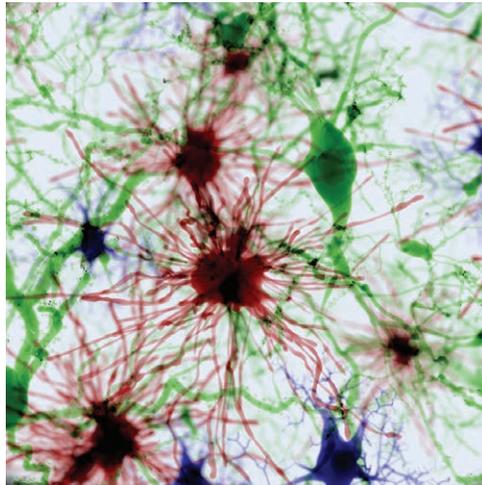
# Targeting Microglia

## A New Avenue for Anesthesia Neuroprotection after Brain Injury?

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**T**HE concept of pharmacologic neuroprotection by general anesthetics has attracted a considerable amount of interest during the past few decades.<sup>1</sup> *Via* their actions on neuronal metabolism and signaling, general anesthetics are plausible candidates to protect the brain after injury. In line with this possibility, laboratory studies have revealed some neuroprotective properties of anesthetic agents in experimental models of both ischemic and traumatic brain lesions. However, clinical evidence of the relevance or benefit of these observations is lacking. Despite years of laboratory investigations and clinical trials, neither anesthetics nor any other drugs have been shown to improve outcome after brain injury in humans.<sup>2</sup> Hence, preserving neuronal networks, or enhancing their functional recovery after deleterious insults to the central nervous system (CNS), remains a major unsolved challenge in clinical medicine. Among the numerous variables hindering extrapolation of laboratory results to clinical practice in this domain is the extraordinary complexity of molecular and cellular events initiated by either ischemic or traumatic insults. Research aimed to better understand how these lesion-induced pathologic cascades are linked to each other over time is a prerequisite for understanding how future clinical trials should be designed to target neuroprotection.

In this issue of the journal, Peters *et al.* provide us with a thought-provoking, new discovery on the protective role of ketamine after traumatic brain injury.<sup>3</sup> The authors studied the effect of a 1-week-long postinjury ketamine exposure at subanesthetic concentrations on hippocampal cell proliferation and memory function in a well-established rodent model of brain trauma. In this model, a cortical impact produces a local lesion, but also decreases neuronal proliferation in the hippocampus. Through a series of pulse-chase approaches



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using the cell proliferation marker bromodeoxyuridine, they found that ketamine both induced cell proliferation and increased the long-term survival of newly generated cells in the hippocampus. Immunohistochemical characterization of these proliferating cells revealed unexpected results. Ketamine did not change the cortical lesion-induced decrease in hippocampal neurogenesis. It did inhibit the generation of astrocytes, and most surprisingly, the authors found that the ketamine-induced increase in cell proliferation was due to the generation of microglia, a glial cell type that constitutes the main form of active immune defense in the CNS. Finally, by performing behavioral testing, they demonstrated that the ketamine-induced increase in microgliogenesis was associated with functional improvement after traumatic brain injury.

The experimental design and novel observations made in this study pave the way for new pharmacologic strategies that target neuroprotection by general anesthetics (and possibly other classes of drugs). In contrast to most previously published preclinical approaches where drugs have been administered for a limited duration extending up to a few hours after the insult, here the authors continuously delivered ketamine, *via* a subcutaneously implanted osmotic pump, for a whole week after brain injury. This homeostatic approach to delivery means that they have created a continuous pharmacologically modified environment during the early, and probably most active, phase of the postinjury period that is characterized by marked neuronal excitability and excitotoxicity. The ketamine-induced sustained attenuation of neuronal activity during this period of vulnerability could conceivably be a mechanism for neuroprotection, although this was not shown in the investigation. In this context, it is also important to note that while some

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clinical trials were conducted to administer *N*-methyl-D-aspartate (NMDA) receptor antagonists during a prolonged period after traumatic brain injury, they could not demonstrate a therapeutic benefit.<sup>4</sup> Several factors might account for this apparent discrepancy. First, in the clinical settings, these drugs were administered in daily bolus dosages which, unlike in the experimental model from Peters *et al.*, does not result in constant plasma concentrations. Second, the extent of the dose-dependent blockade of NMDA receptors may be different between the current experimental and the previous clinical studies. These two issues are of utmost relevance, since one should aim for blocking excessive NMDA receptor activation to alleviate excitotoxicity while leaving normal receptor function preserved to avoid side effects. Finding this appropriate balance may be extremely difficult in the ever-changing postlesion environment. To add further complexity, NMDA receptor hypofunction also occurs after brain trauma and enhanced functional recovery has been demonstrated in a mouse model of closed head injury in the presence of pharmacologic NMDA receptor agonism.<sup>5</sup>

A third possibility to explain the lack of detectable neuroprotective effects of sustained NMDA receptor antagonism in humans is related to the inherently greater complexity of real life neurotrauma when compared to highly standardized experimental models. In line with this possibility, despite adequately powered sample size, the effect size of behavioral improvement in the study of Peters *et al.* is relatively modest and might be difficult to detect in human trials. Last but not least, a fourth possibility is that the protective effects of ketamine are independent of NMDA receptor blockade.

An additional interesting aspect of the study design is that, instead of evaluating ketamine-related brain protection on the primary lesion site, *per se*, the authors focused their interest on the hippocampus, a structure that is not directly affected by the cortical injury applied in this experimental model. The rationale behind this approach is that functional recovery after brain trauma may involve reorganization of remaining, uninjured or, at least, less injured brain circuitry. In line with this hypothesis, the improved functional recovery after cortical impact in the ketamine group was correlated with cellular changes in the hippocampus, where neuronal loss also occurs in this experimental model, despite the absence of direct tissue injury, but did not decrease the size of the cortical lesion when compared to the control group. These results do not provide direct proof that functional neuroprotection is causally related to ketamine-induced changes in the hippocampus; however, they make a compelling case for pharmacologic modulation of plasticity in the remaining neuronal circuitry around the lesion as a way forward for research in neuroprotection. This approach is fundamentally different from most studies in the field, where a decrease in lesion size is often considered as the primary outcome.

One of the most surprising and counterintuitive aspects of the study is the effect of ketamine on microglia in the context of traumatic brain injury. Earlier, in various experimental

models, ketamine has been shown to exert antiinflammatory properties.<sup>6</sup> Although, the explanation and mechanism for this discrepancy remains unclear, enhanced microgliogenesis by ketamine, after CNS injury, may be an important factor to promote recovery.<sup>7</sup> Indeed, an increasing number of studies demonstrate a role for microglia in CNS repair. These cells are rapidly activated in response to CNS damage, even in sites far remote from focal injuries. Dependent on their highly complex activation state, microglia can exert context-dependent neuroprotective or neurotoxic effects. Understanding how these activation states can translate into protection is a major challenge and would give rise to potential new therapeutic approaches. Therefore, the present model is of important fundamental value since it is exceptionally well-suited to study how pharmacologic manipulations could prime microglia toward a beneficial restorative phenotype.

The mechanisms through which ketamine exerts its protective action after traumatic brain injury remains unknown. While there is a correlation between microglial proliferation and functional recovery in the presence of ketamine, a causal link has not been shown. The intriguing observations of Peters *et al.* produce a clear call for future studies to decipher the molecular pathways linking ketamine to microglial proliferation and activation. For example, the role of NMDA receptors in mediating these effects is an important question. In fact, while ketamine primarily exerts an inhibitory action on NMDA receptors, it also acts as positive allosteric modulator of  $\gamma$ -aminobutyric acid type A receptors, and as a potential inhibitor of both cholinergic and serotonergic receptors.<sup>8</sup> Moreover, recent studies in the context of major depressive disorders revealed that it is not ketamine itself, but rather its metabolite norketamine, that drives the therapeutic effects *via* the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptors.<sup>9</sup> The dose response and the duration of administration of ketamine are additional important therapeutic issues to answer. Last but not least, whether other anesthetics have similar actions on microglia in the context of brain traumatism is also an exciting question, with possible therapeutic applications. Answering these important unknowns may not only reignite research on anesthesia neuroprotection, but will also help us to gain further fundamental insights into the pathomechanisms underlying brain injury.

### 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.

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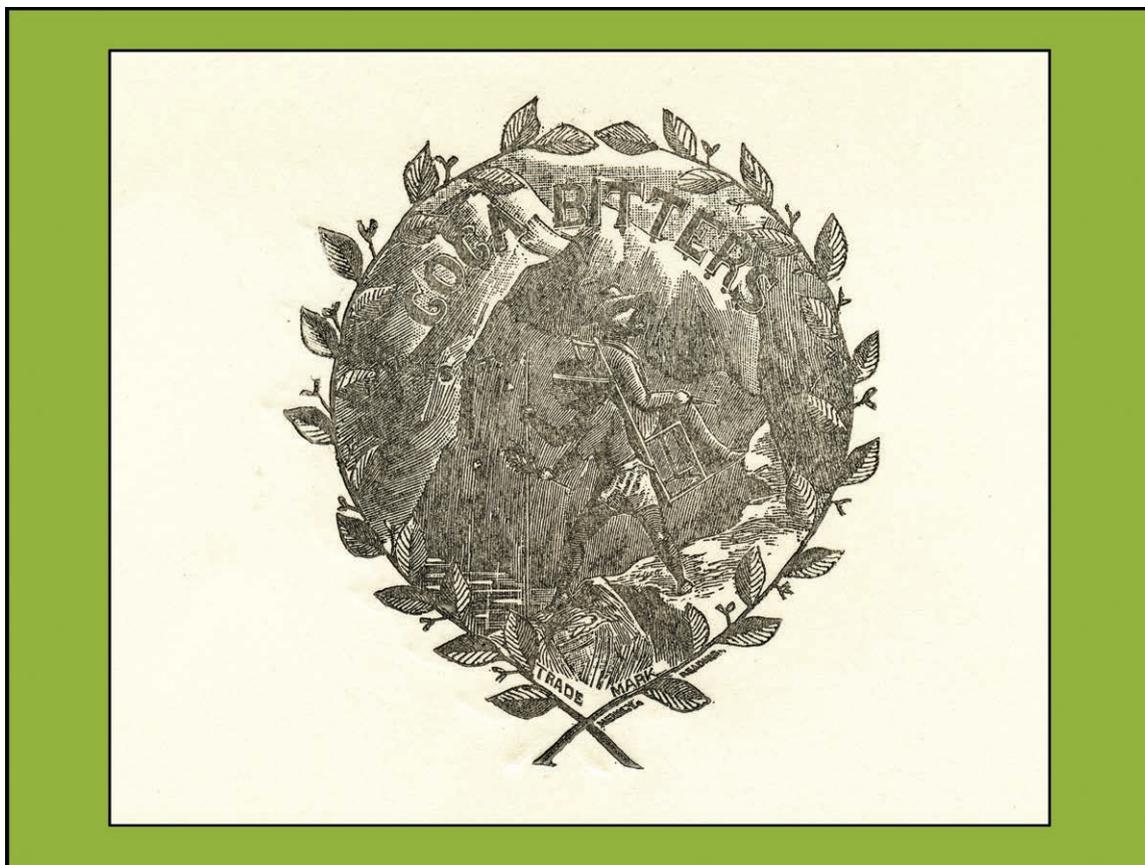
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## ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

### Coca Bitters—Numbing the Fatigue Rather Than the Pain



Historically, the debilitating, recurring fevers of malaria were remedied with cinchona (quinine vs. shivering) and coca (cocaine vs. fatigue)—botanically, a bark and a leaf, respectively. However, quinine has direct antimalarial properties, which coca lacks. This reality did not prevent New York City's Quichua [*sic*] Coca Company from falsely advertising the malaria-fighting powers of pharmaceuticals and beverages mixed with the company's Coca Bitters. The printer's proof (*above*) of the logo for those bitters trademarked a presumably cocaine-driven Quechuan Amerindian hiking through Peruvian jungle carting a seated man whose chair was lashed to the tireless porter's forehead and waist. So, 5 yr before Karl Koller's research on the numbing properties of topical cocaine, Coca Bitters were peddled in 1879 as socially acceptable stimulants for numbing the effects of fatigue. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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