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At the Sharp End of Spines

Anesthetic Effects on Synaptic Remodeling in the Developing Brain

ONCE considered unthinkable, the alarming possibility that general anesthetics might have lasting effects on the developing brain has gained traction with anesthesiologists, the Food and Drug Administration, the popular media, and parents. The basis for concern comes mainly from animal studies showing that exposure to a variety of commonly used intravenous and volatile anesthetics produces cognitive and social behavioral deficits that last into adulthood.[1,2] Although cause–effect relationships have not been established, these cognitive and behavioral dysfunctions have been attributed to the transient suppression of neurogenesis or widespread neuroapoptosis that are well documented in animals after anesthetic exposure in the early postnatal period.[1,3] At least in the case of neuroapoptosis, the developing brain is most vulnerable during the period of rapid synaptogenesis (*i.e.*, the brain growth spurt),[1,4] putting synapse development at center stage in the story of anesthetic neurotoxicity. In this issue of ANESTHESIOLOGY, Briner *et al.*[5] take the research in this area a step further. In a series of elegant *in vitro* and *in vivo* experiments, they demonstrate in rodents that during the early stage of postnatal brain development synapse development itself is altered by exposure to volatile anesthetics. As such, this work adds synaptic remodeling to the list of potential mechanisms by which anesthetics might produce long-lasting changes in the developing rodent brain.

By using iontophoretic labeling of cortical neurons with the fluorescent dye lucifer yellow and sophisticated imaging techniques, Briner *et al.*[5] examined the effects of three volatile anesthetics on the number and morphology of dendritic spines, which are tiny (0.5–1 μm) but highly dynamic structural specializations that protrude from the postsynaptic neuron. Spines compartmentalize all the essential molecules involved in postsynaptic signaling and plasticity, including neurotransmitter receptors, cytoskeletal and scaffolding proteins, and signaling molecules.[6–8] Most excitatory glutamatergic connections are made on dendritic spines; therefore, measurements of spine density provide an estimate of the density of excitatory synapses.[8] For example, in the hippocampus, there is almost a one-to-one relationship between spines and excitatory synapses.[9] What Briner *et al.* found is that rodent pups exposed to clinically relevant concentrations of isoflurane, sevoflurane, or desflurane for 30–120 min on postnatal day (PND) 16 had a significantly increased density of dendritic spines in the apical and basal dendrites of layer 5 pyramidal neurons of the prefrontal cortex. In addition, almost all the new spines were of a specific morphologic type, namely, thin spines. There were potency differences among the volatile agents in that the duration of anesthetic exposure required for a statistically significant increase in spine density was 30 min for sevoflurane, 60 min for isoflurane and 120 min for desflurane. Importantly, the basic structure of the dendritic arbor was not perturbed and, unlike when the brain is exposed at an earlier stage of development (PND 7), the anesthetic agents did not cause neuronal apoptosis. Neither the persistence nor the function of these new spines was studied, and behavioral correlates are not reported. However, the same group recently reported that midazolam, propofol, and ketamine evoke a similar increase in spine density in cortical neurons of PND 15 and 20 but not PND 30 mice; the newly generated spines were thin and lasted for at least 5 days after exposure, and a significant fraction became functional and integrated into neuronal networks.[10] Thus, in aggregate, these results strongly suggest that commonly used anesthetics and sedatives produce synaptic remodeling that is both dependent on developmental age and persistent.

These results seem to be at odds with a recent study demonstrating a substantial loss of dendritic spines and a reduction in synapses in cultured neurons and the hippocampus of PND 7 rodents exposed to isoflurane.[11] However, there are important differences between the studies. In the investigation by Head *et al.*, anesthetic exposure occurred at PND 7, and neuronal apoptosis was a prominent feature. In contrast, Briner *et al.* studied PND 16 mice and did not observe neuronal loss. This shift from vulnerability to neuroapoptosis (peak at approximately PND 7) to resistance (approximately PND 14) in response to anesthetics has previously been established[1,11] Therefore, it is possible that a similar shift in dendritic spine vulnerability might also occur with increasing age. Experimental data support this notion. In the former

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investigation, neuroapoptosis was caused in large measure by proBDNF (brain-derived neurotrophic factor) signaling *via* $p75^{NTR}$,[11] a receptor whose expression is developmentally regulated such that it is high during the period of vulnerability to anesthesia-induced apoptosis and reduced by the time the brain is no longer vulnerable at approximately PND 14.[12,13] In addition, as the authors point out, mainly due to differences in the expression of the potassium chloride cotransporter, γ-aminobutyric acid is excitatory at PND 7 but inhibitory at PND 16. Therefore, rather than being discrepant, these studies reinforce the concept that the response of the brain to anesthesia is critically dependent on postnatal age.

The data of Briner *et al.* have important implications for the debate about lasting effects of anesthetics on the developing brain because dendritic spine number and morphology are developmentally regulated, potently influenced by sensory experience, and provide a structural basis for encoding of experience. In animals, the rate of spine turnover is high in the first few weeks of life when synaptogenesis is particularly active[8,10] but declines gradually thereafter primarily because the rate of spine elimination starts to exceed that of formation. By 1–2 months of age (adolescence to early adulthood in the rodent), approximately 55% of spines in the somatosensory cortex and 70% in the visual cortex are stable and, by 4–5 months of age, this increases to 70% and 90%, respectively.[14–16] This is an important point because spine morphology correlates strongly with the stability and strength of the synapse.[6] Mushroom-shaped spines, which have large heads and narrow necks, are stable for long periods and are proposed to be "memory spines." These are the type that predominate in the mature central nervous system. In contrast, thin spines with small heads are highly motile structures that typically grow or change shape over minutes to days. These thin spines with small head diameter are dominated by glutamate receptors (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate and *N*-methyl-D-aspartic acid), meaning that they have greater capacity for activity-dependent plasticity and, as such, are proposed to be "learning spines."[6,7] It is noteworthy, therefore, that the majority of the newly formed spines that develop after exposure to anesthetics and sedatives are of the thin variety.[5,10] This is not too surprising. One of the key drivers of spine plasticity is sensory experience or neuronal activity. Pertinent to this discussion, antagonists of excitatory neurotransmitter receptors,[17,18] transient sensory deprivation,[19,20] and loss of function due to injury[21,22] increase production of spines with small head diameter. This proliferation of new thin spines is presumably a compensatory response to decreased sensory stimulation and is believed to be essential for preservation or restoration of function. However, under some conditions, synaptic remodeling can go wrong, inducing formation of aberrant neural connections and potentially neurodevelopmental cognitive abnormalities.[23] Therefore, the results of Briner *et al.* must be interpreted cautiously. Increased production of spines with small head diameter after exposure to anesthetics and sedatives could be an adaptive response to reduced sensory experience or blockade of specific excitatory neurotransmitter receptors or it could be maladaptive by promoting development of abnormal neural circuits. Additional work is needed to sort this out. The key point is that synaptic remodeling in and of itself is not evidence of toxicity. In fact, such remodeling is paramount for normal, lifelong activity-dependent synaptic plasticity and learning. Therefore, pending work on functional consequences, the study of Briner *et al.*, is best viewed according to a conceptual framework of altered synaptic plasticity rather than toxicity.

Like most good science, this work raises many questions. Chief among them is whether these anesthetic and sedative effects extend beyond synapses to circuits. Spine density significantly increased in the prefrontal cortex, somatosensory cortex, and hippocampus[5,10] when animals were exposed to the anesthetic or sedative between PND 15 and 20. This is an age range that is well within the so-called critical period of a number of neuronal networks, including those involving vision, tone perception, cerebellar function, and stress and anxiety.[24] The critical period is the developmental age of greatest sensitivity to environmental stimuli that impact, often irreversibly, the construction of neural circuits. Circuits are particularly receptive to experience-driven sculpting during the critical period and become consolidated or "hard wired" when it closes. This is relevant here because the onset, duration, and closure of the critical period are profoundly affected by γ-aminobutyric acid type A agonists.[24] Thus, given that volatile anesthetics and many sedatives are potent agonists at γ-aminobutyric acid type A receptors, is it possible that they close the critical period prematurely and effectively hard-wire changes into the developing brain? Newer methods of spine imaging, such as two-photon microscopy, allow a given set of spines to be monitored *in vivo* for months (although anesthesia is required). This type of imaging might provide answers as to the important question of whether an episode of anesthesia during a critical period of development induces not only remodeling of synapses but also hard wiring of the changes at the circuit level.

The work of Briner *et al.* is important for a number of reasons, but mainly because it moves the field beyond cell level changes (apoptosis and neurogenesis) to even more subtle subcellular events that could lead to a fundamental rewiring of the developing brain by anesthetics and sedatives. One implication of this research is that it suggests the developing brain might be vulnerable to these drugs for longer than previously thought. On the basis of neuroapoptosis, the first week of postnatal life has been considered the period of greatest vulnerability to anesthetic neurotoxicity in rodents. If anesthetic exposure at PND 15–20 causes synaptic remodeling, then the window for a morphologic effect of anesthetics on the developing brain will have opened significantly. Not a sanguine thought. Another consideration is that the anesthetic effects described here might apply to circumstances besides development. For example, dendritic spine turnover and plasticity are high after brain injury and during neuro-

pathic pain.[21,22,25] Therefore, much as during neurodevelopment, these drugs may have unexpected consequences when the nervous system is attempting to restore function after an injury. Ultimately, however, determining whether sedative and anesthetic-induced changes in dendritic spine dynamics and morphology are an interesting scientific curiosity or cause for clinical concern must await information about circuit development and functional or behavioral correlates as well as evidence that these events occur in higher species. That said, the concept of anesthetic-induced neuroplasticity, as distinct from neurotoxicity, adds an important new dimension to the debate around potential long-term effects of these drugs and reminds us that pharmacologic agents can change neurons without killing them. That insight points us in a fascinating new direction.

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