Wake Up, Neurons! Astrocytes Calling

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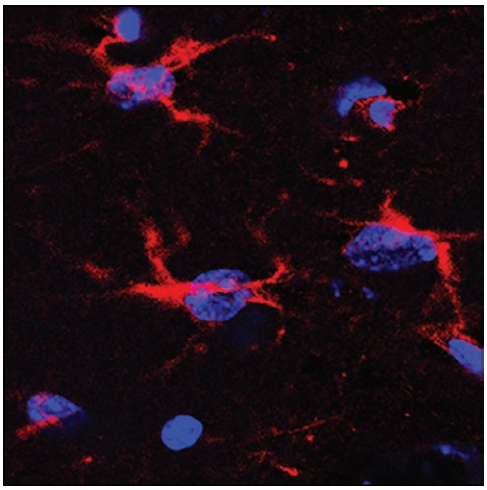
Roughly 20 yr ago, the concept of the "tripartite synapse" was introduced. It indicated that neuroglia might need to be promoted from a structurally supportive "neuronal putty" to an equal partnership with neurons. Since then, glial cells have constantly expanded their reach into every aspect of brain pathology and physiology. In addition to forming an integral part of the neuronal-glial-vascular unit, controlling local blood flow and supplying neurons with energy substrates and antioxidants, glia organize and coordinate the defense of the brain from intruders. Glia also now emerge as surprising actors in anesthetic mechanisms. As reported by Ramadasan-Nair *et al.* in this issue,[1] introducing a mutation that interferes with energy production in glia leads to delayed emergence from anesthesia. This phenotype is complementary to the heightened sensitivity to induction of anesthesia that occurs when the very same mutation is introduced into the glutamatergic neurons. This finding thus adds an intriguing layer to our understanding of the mechanisms by which volatile anesthetics suppress central nervous system (CNS) function.



"Glia…now emerge as surprising actors in anesthetic mechanisms…[interfering] with energy production in glia leads to delayed emergence from anesthesia."

Neuroglial cells were first recognized as a separate population of cells in the CNS at about the same time as the first successful public demonstration of anesthesia. Contrary to popular opinion, *glia* is not rooted in the Greek term for glue (κολλα) but, as the German *Kitt* indicates, in the Greek word for putty or gum (itself derived from the Protoindoeuropean *glei*, which means "clay"). The great German pathologist Rudolf Virchow formally coined the term "neuroglia" around 1858. In the subsequent decades, leading anatomists described a variety of nonneuronal cells within nervous tissue; by the 1920s, three principal types were recognized: astroglia, oligodendrocytes (myelin producing), and microglia (of hematopoietic origin). We now know that this classification was overly simplistic, as new types (*e.g.*, the NG-2 glia) and new subtypes (*e.g.*, morphologically and functionally distinct astrocytes) are still being discovered. Although more functions have been attributed to glial cells, estimates of their numbers relative to neurons have decreased; the current consensus is that there are approximately equal numbers of glia and neurons, with some heterogeneity between brain areas. Also, contrary to previous teaching, astrocytes are not the most numerous nonneuronal cell type in the brain; they account for only 20 to 40% of glial cells.

One of the many essential roles played by astrocytes is that of "supplier," providing neurons with energetic and metabolic substrates. Neuronal activity is costly, especially when it involves excitatory neurotransmission. The energy that is needed depends heavily on firing rate. For projection neurons, glutamatergic neurotransmission accounts for an estimated 80% of the energy needs, while maintenance of the resting potential consumes only about 13%. Neurons entirely depend on oxidative phosphorylation, but they do not store glycogen, which, in other cells, serves as a reserve to meet changing demand. Where, then, does the fuel for the mitochondrial electron transport chain originate? One important source is astrocytes, *via* the astrocyte–neuron lactate shuttle. Lactate is a product of glycolysis, the predominant energy-producing pathway in astrocytes. It serves both as fuel for basal neuronal oxidative phosphorylation and as a signaling molecule to modulate neuronal excitability. However, lactate is not the primary source during increased demand. As their firing rate increases, neurons also rapidly increase their uptake of glucose, resulting in aerobic glycolysis. For this to

Image: Adapted from Ramadasan-Nair R, Hui J, Itsara LS, Morgan PG, Sedensky MM: Mitochondrial function in astrocytes is essential for normal emergence from anesthesia in mice. ANESTHESIOLOGY 2019; 130:423–34.

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occur, the supply of glucose must be increased—a task that is regulated by astrocytes, whose endfeet provide a virtually complete coverage of brain microvessels and thereby control the microscopic distribution of energy substrates. Astrocytes also rapidly resupply neurons with the stock of glutamine that they use to resynthesize glutamate to replenish their synaptic vesicles. Thus, astrocytes contribute both directly and indirectly to meeting the energetic and metabolic needs of neurons.

Research from the anesthetic genetics research group led by Drs. Morgan and Sedensky takes us into the middle of this heavily trafficked intersection between energy supply, excitatory neurotransmission, glutamate trafficking, and regulation of blood supply. Previously, this group found that interference with oxidative phosphorylation by globally knocking out the Ndufs4 subunit of Complex I of the mitochondrial electron transport chain profoundly increased sensitivity to volatile anesthetics, mimicking the anesthetic sensitivity of patients with Leigh syndrome, who carry hypomorphic mutations affecting oxidative phosphorylation.[2] They then showed that interfering with mitochondrial function, specifically in glutamatergic neurons, was necessary and sufficient to create the anesthetic-sensitive phenotype.[3]

The intriguing new finding reported here, that targeting oxidative phosphorylation *via* Ndufs4 gene knockout in astrocytes, but not neurons, produced a completely different anesthetic phenotype—delayed emergence from anesthesia—is unexpected. The mechanism proposed by the authors—that the mutation produces an energetic bottleneck in astrocytes that impedes the resumption of neuronal glutamatergic signaling—is plausible, it builds upon their prior results, and it is amenable to focused tests in future studies. It also adds a new dimension to previous work that has focused on presynaptic suppression of glutamate-mediated excitatory neurotransmission. Multiple molecular mechanisms within the presynaptic terminal, including voltage-dependent sodium channels, calcium entry, and direct modulation of vesicle fusion, have been implicated by *in vitro* studies. The current findings expand our view to include astrocytes. Also, they further support the possibility that cellular energetics play an equal or even more important role than direct action at neurotransmitter receptors or synaptic release machinery in the cascade of effects leading to anesthesia and possibly toxic collateral effects produced by the currently used volatile agents.

The results presented here raise some interesting questions. Does the exaggerated hysteresis seen in the astrocyte-Ndufs4 knockout mice arise by the same mechanism as described by the Kelz laboratory[4] in wild-type mice? Is this hysteresis in the setting of astrocyte-Ndufs4 knockout a general property of all glutamatergic synapses, or are there some brain regions that are particularly sensitive and might be responsible for this effect? If so, targeting specific brain regions using virus injection or other genetic means may reveal what brain regions drive hysteresis. Do other glial cells or neuronal populations materially contribute to some anesthetic endpoints? Recent research indicates that oligodendrocytes may play a role similar to that of astrocytes in the energetic supply of myelinated axons. Would compromised oxidative phosphorylation in this cell type produce an anesthetic phenotype? Is depression of oxidative phosphorylation in glia or other cells linked to any aspect of anesthetic neurotoxicity?

It should be noted that the studies reported here, which were carried out in mammals, would not have been possible without the previous decades-long work with invertebrate model organisms. Experiments in the worm *Caenorhabditis elegans* led to the discovery of a critical role of oxidative phosphorylation, and made it possible to conduct research in mammals targeting specific components of the mitochondrial electron transport chain in the context of anesthetic action. The glutamatergic transmission studied here is only one of many targets of volatile general anesthetics that might contribute to their effects on CNS function. We still do not know to what extent these targets matter—but as the current research demonstrates, the availability of ever more sophisticated genetic tools has the potential for surprising discoveries.

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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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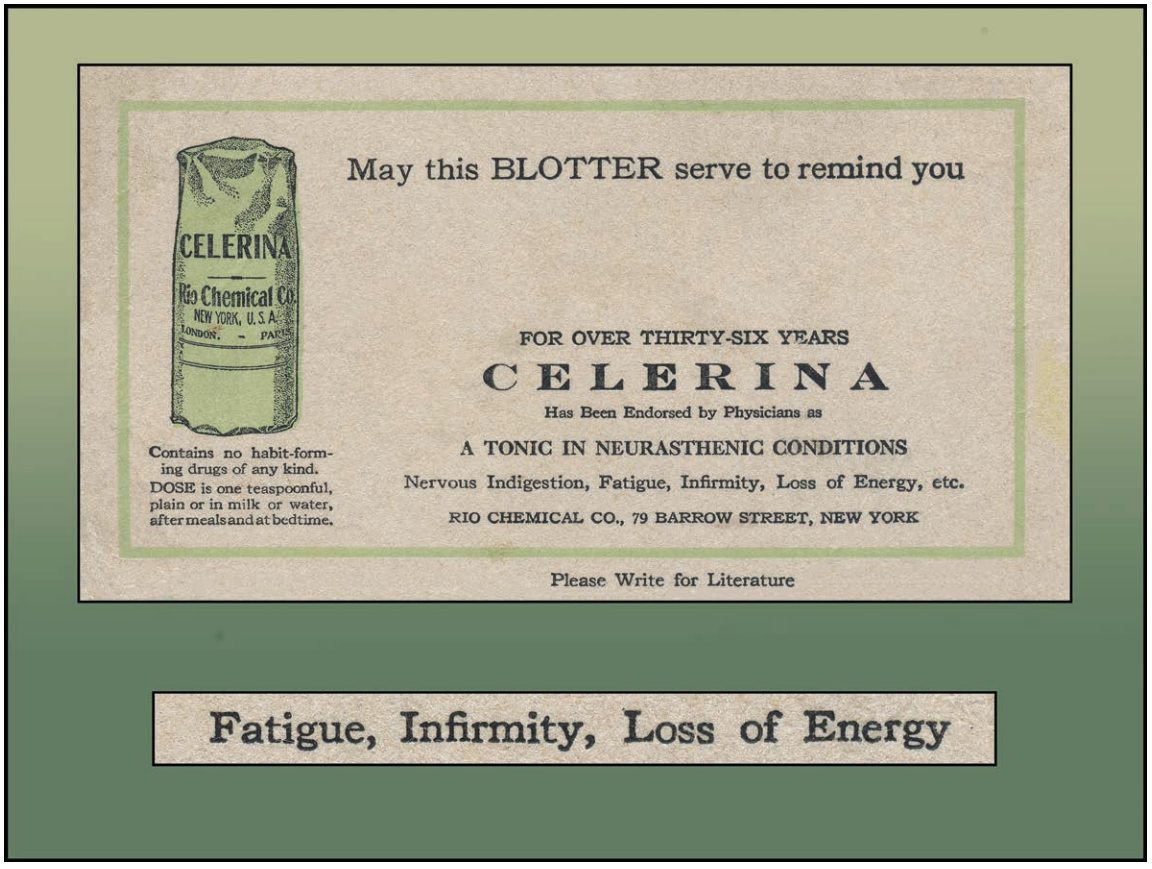

Advertising its previously cocaine-laced panacea, Celerina, the Rio Chemical Company of St. Louis (and then New York City) released this ink blotter (*upper panel*) by 1916, promising that Celerina could still treat (*lower panel*) fatigue, infirmity, loss of energy, etc. The company may also have been reacting to the American Medical Association's blistering 1915 critique of Celerina's vegetable cocktail (Cola, Viburnum, Celery, Ladyslipper, and Prickly Ash) as lacking "any recognizable activity." As for treating "dipsomaniacs" with "84-proof" Celerina, the AMA exclaimed, "Think of prescribing an alcoholic nostrum four times a day to promote recovery from alcoholic excess!" (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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