COMMENTARY

Exploitation of the spreading depolarization-induced cytotoxic edema for high-resolution, 3D mapping of its heterogeneous propagation paths

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Spreading depolarization (SD) is among the most archaic pathological phenomena of the central nervous system and already occurs in comparably primitive animals, such as locusts and cockroaches (1). SD describes a regenerative, all-or-none type of depolarization wave in gray matter of the central nervous system characterized by the abrupt, near-complete breakdown of the transneuronal ion gradients. It is assumed that SD is perceived as migraine aura when it invades a perceptual and eloquent brain region, where it induces spreading depression of the normal brain activity. However, SD also occurs in various conditions other than migraine, including stroke and traumatic brain injury (TBI). Although there is unequivocal electrophysiological evidence that SD often induces spreading depression of activity in stroke and TBI, computational dysfunction before SD migration through the tissue usually precludes the patient percept of a migraine aura in these injurious conditions (2). Initiation, recovery, pharmacology, depression patterns, and toxicity may vary dramatically along the propagation path of a single SD wave, dependent on the local conditions of the tissue. However, all SDs—no matter whether they occur in well-nourished, traumatized, or severely ischemic tissue—share the same phenomenology, including the same magnitude of neuronal depolarization and the principal ion changes involved, a similar release of free energy (free-energy starving) and spread in the tissue. SDs also share influx of water into neurons driven by the ionic changes across the cellular membrane. In other words, SD is the principal mechanism of the cytotoxic edema in many gray matter structures of the brain. Using two-photon microscopy, the cytotoxic edema is observed as SD-induced dendritic beading (3). Beaded morphology allows a larger volume to be encompassed within an equivalent surface area. Beading-induced changes in cell membrane morphology are sufficient to significantly hinder intracellular water mobility (4). Using diffusion-weighted MRI (DW-MRI), this translates into a characteristic drop in the apparent diffusion coefficient when SD is moving in the tissue, no matter whether or not the tissue is ischemic (5) (Fig. 1). In PNAS, Cain et al. elegantly exploit this characteristic trait to trace SD's propagation path in the naïve mouse brain in a 3D, whole-brain perspective with high-temporal resolution

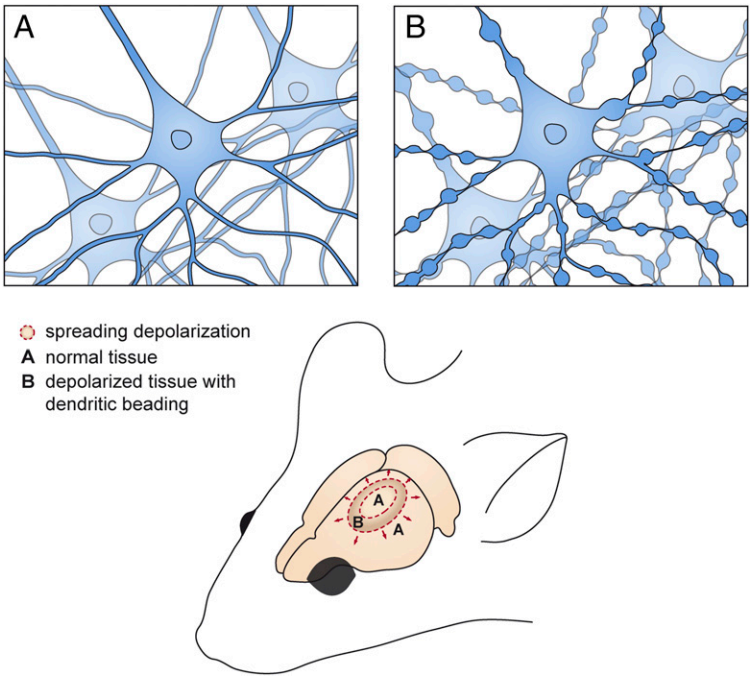

Fig. 1. SD is associated with near-complete breakdown of the transcellular ion concentration gradients, which causes intracellular hyperosmolality. The resulting water influx into neurons leads to cytotoxic edema, which causes abrupt dendritic beading. Beaded morphology allows a larger volume to be encompassed within an equivalent surface area. In normal neurites, water mobility is highly restricted by the cell membrane perpendicular to the main axis, whereas water molecules diffusing along the main axis of the neurite encounter few barriers on the timescale of DW-MRI measurements. However, undulation of the cell membrane induced by neurite beading causes reduction in the mobility of intracellular water along the main axis of each neurite. This intracellular diffusion restriction can be visualized with DW-MRI and was used by Cain et al. (6) for high-resolution, 3D mapping of SD's propagation path in the cortex and subcortical gray matter. (*A*) Normal neurons. (*B*) Depolarized neurons with dendritic beading.

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using DW-MRI (6). To further enhance visualization and quantitative comparisons, Cain et al. designed a custom Matlab script to automatically detect the wave's front and to represent it in each slice as a heatmap.

Cain et al.'s (6) study also includes familial hemiplegic migraine type 1 (FHM-1) mutant mice expressing human mutations (R129Q and S218L) in the $Ca_v2.1$ (P/Q)-type voltage-gated calcium channel (VGCC) subunit. FHM is clinically characterized by complicated forms of migraine aura. It was previously found that mice expressing these mutations have a lower electrical threshold for SD than WT animals and the wave's velocity is significantly higher (7, 8). Cain et al.'s (6) paper almost entirely confirms these results, but the threshold of R129Q mice compared with WT mice did not differ. This discrepancy may be explained by the use of isoflurane anesthesia in the present study, whereas urethane was applied in previous studies. Isoflurane is known to increase the threshold for SD in various models through its antagonistic effect on NMDA receptors (9). This aspect could have partially masked the effect of the R192Q mutation.

A different question is whether electrical stimulation is a good model for the natural conditions that lead to SD associated with the patient percept of migraine aura. The caveat is that electrical stimulation sufficient for SD induction in animals is of several magnitudes more intense than even the most pathological brain activity, and an anecdotal report mentioned histopathological evidence of a tiny zone of injury at the stimulation site (2). A decreased electrical threshold in mutant mice may thus indicate increased vulnerability to electrical injury rather than a purely functional network anomaly. This notion is further supported by the observation that cerebral blood flow required for tissue survival after middle cerebral artery occlusion was higher in FHM-1 mutant mice compared with WT mice, leading to infarction with milder ischemia (10). Accordingly, mutant mice showed a shorter delay between onset of ischemia and the first SD; subsequent SDs were more frequent. In consequence, the mutants developed larger infarcts and worse neurological outcomes after ischemic stroke.

The reason why clearly noninjurious triggers for SD are not applied in experiments is that none are currently available. At first glance, this notion agrees much better with the unequivocal electrophysiological evidence of SD in patients with stroke and TBI (2, 11) than with its role as pathophysiological correlate of the mostly harmless clinical condition of migraine aura. However, there is ample evidence that SD also occurs during migraine aura (12), and the toxicity of a single SD wave is not sufficient to induce lasting neuronal damage when it runs in normal, well-nourished tissue. Hence, there is need for the search for triggers more congruent with the hypothesis that SD associated with migraine aura does not usually emerge from injury but from episodic disruptions of the excitation/inhibition balance and hyperactivity of cortical circuits as a result of excessive recurrent excitation (13).

However, increased vulnerability of FHM-1 mutant mice to electrical and vascular disruptions is no less remarkable than would be a threshold shift of a trigger closer to the realities of migraine aura. This is because increased vulnerability sheds light on another clinical aspect of outstanding interest, namely the ever-increasing epidemiological evidence that the neuronal network anomalies underlying migraine with aura represent a weak but significant risk factor for cerebral injuries (14). This applies even more so to FHM, which can show rare courses up to life-threatening febrile coma (15) and stroke (16). In view of the DW-MRI findings of Cain et al. (6), Leão's original hypothesis that migraine aura and cerebral ischemia are linked through SD (17, 18) may then be reformulated: if certain features render a given neuronal network more likely to develop SD as the principal mechanism of cytotoxic edema, the likelihood that the network develops a cytotoxic edema under conditions inducing a cytotoxic edema will be higher. Whether or not cytotoxic edema then shows progression toward cellular injury may be determined by the local tissue conditions and their repercussions on mechanisms involved in the SD process. The term "SD continuum" describes these changing characteristics of the wave determined by the local tissue conditions (2, 11).

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Currently, propagation path and velocity in naïve brain may be the aspects of a migraine aura that can be modeled most accurately in animal experiments. Cain et al. (6) provide a powerful neuroimaging tool for this purpose. The authors combine this with a pharmacological approach to exemplify possible applications. The chosen drug, pregabalin, logically followed the fact that FHM-1's gain-of-function mutations are located in the gene of the $Ca_v2.1$ (P/Q)-type VGCC subunit because pregabalin is a P/Q-type VGCC inhibitor. Accordingly, the drug slowed the propagation speed in both R192Q and S218L strains but not in WT mice. Absence of the effect in WT mice corresponded well with previous findings in naïve rats that the propagation speed of pinprick-induced SD was not altered by the P/Q VGCC inhibitor ω-agatoxin IVA (19). Acute systemic administration of pregabalin in vivo also selectively prevented the invasion of SD into subcortical striatal and hippocampal regions in the R192Q strain that exhibits a milder phenotype and gain of $Ca_v2.1$ VGCC function than the S218L strain. Nonetheless, it was a counterintuitive finding that pregabalin treatment significantly increased the electrical SD threshold only in WT mice, whereas the mutant mice merely showed a statistical trend. In our opinion, absence of the effect in the mutant mice may be reinvestigated with higher statistical power. Isoflurane may be replaced with urethane anesthesia in such experiments to exclude its possible interference. However, it was less surprising that the SD threshold was reduced in the WT mice. This finding could be related to previous observations in naïve rats that a KCl crystal, another artificial SD trigger associated with local injury (20), elicited only one or very few SDs and their repetition rate was dramatically reduced once ω-agatoxin IVA was applied to the brain surface (19).

The fact that the SD hypothesis can gather an enormous bandwidth of partially harmless, partially deleterious clinical conditions under one roof renders a suitable match between a given experimental model and the appropriate clinical condition a challenge. This necessitates a holistic approach beyond the focus on a single disease when it comes to the translation of pharmacological strategies from bench to bedside. However, the present case of a well-characterized Mendelian

model disease may be somewhat easier because genetic testing allows individual treatment stratification in both animals and clinical studies. In our opinion, the results of the present animal study particularly justify a clinical trial of pregabalin in carriers of both mild and more severe FHM-1 mutations, although the findings suggest that less-affected patients profit more. Such a trial should thus include a subgroup analysis to clarify whether a possible pharmacological benefit is mutation-dependent. Furthermore, we would like to call on the clinical community to search for diffusion anomalies in patients acutely undergoing migraine aura, although it certainly represents a logistical challenge.

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